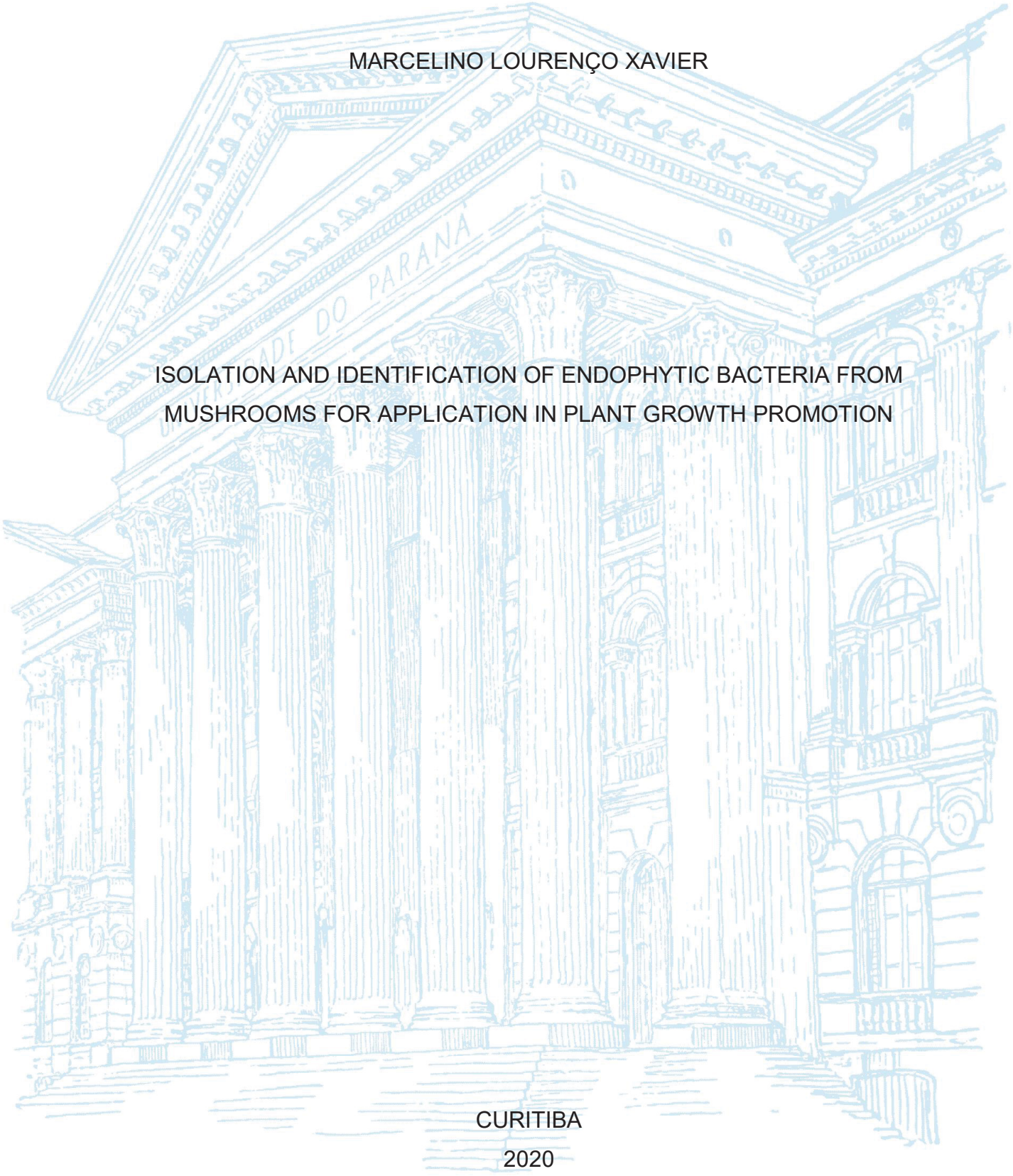
UNIVERSIDADE FEDERAL DO PARANÁ

MARCELINO LOURENÇO XAVIER



ISOLATION AND IDENTIFICATION OF ENDOPHYTIC BACTERIA FROM MUSHROOMS FOR APPLICATION IN PLANT GROWTH PROMOTION

CURITIBA

2020

MARCELINO LOURENÇO XAVIER

ISOLATION AND IDENTIFICATION OF ENDOPHYTIC BACTERIA FROM MUSHROOMS FOR APPLICATION IN PLANT GROWTH PROMOTION

Tese apresentada ao curso de Pós-Graduação em Engenharia de Bioprocessos e Biotecnologia, Setor de Tecnologia, Universidade Federal do Paraná, como requisito parcial à obtenção do título de Doutor em Engenharia de Bioprocessos e Biotecnologia.

Orientador: Prof. Dr. Carlos Ricardo Soccol

Coorientadora: Prof.ª Dr.ª Susan Grace Karp

CURITIBA

2020

Catalogação na Fonte: Sistema de Bibliotecas, UFPR
Biblioteca de Ciência e Tecnologia

X3i Xavier, Marcelino Lourenço
Isolation and identification of endophytic bacteria from mushrooms for application in plant growth promotion [recurso eletrônico] / Marcelino Lourenço Xavier. – Curitiba, 2020.

Tese - Universidade Federal do Paraná, Setor de Tecnologia, Programa de Pós-Graduação em Engenharia de Bioprocessos e Biotecnologia, 2020.

Orientador: Carlos Ricardo Soccol – Coorientador: Susan Grace Karp

1. Bactérias. 2. Bactérias Endofíticas. 3. Ácido acético. 4. Cogumelos. 5. Metagenômica. 6. Crescimento (Plantas) . I. Universidade Federal do Paraná. II. Soccol, Carlos Ricardo. III. Karp, Susan Grace. IV. Título.

CDD: 572.8293

Bibliotecário: Elias Barbosa da Silva CRB-9/1894

MINISTÉRIO DA EDUCAÇÃO
SETOR DE TECNOLOGIA
UNIVERSIDADE FEDERAL DO PARANÁ
PRÓ-REITORIA DE PESQUISA E PÓS-GRADUAÇÃO
PROGRAMA DE PÓS-GRADUAÇÃO ENGENHARIA DE BIOPROCESSOS E BIOTECNOLOGIA - 40001016036P8

TERMO DE APROVAÇÃO

Os membros da Banca Examinadora designada pelo Colegiado do Programa de Pos-Graduação em ENGENHARIA DE BIOPROCESSOS E BIOTECNOLOGIA da Universidade Federal do Parana foram convocados para realizar a arguição da tese de Doutorado de **MARCELINO LOURENÇO XAVIER** intitulada: **ISOLATION AND IDENTIFICATION OF ENDOPHYTIC BACTERIA FROM MUSHROOMS FOR APPLICATION IN PLANT GROWTH PROMOTION**, sob orientação do Prof. Dr. CARLOS RICARDO SOCCOL, que apos terem inquirido o aluno e realizada a avaliação do trabalho, são de parecer pela sua APROVAÇÃO no rito de defesa.

A outorga do titulo de doutor esta sujeita a homologação pelo colegiado, ao atendimento de todas as indicações e correções solicitadas pela banca e ao pleno atendimento das demandas regimentais do Programa de Pos-Graduação.

CURITIBA, 21 de Agosto de 2020.

Assinatura Eletrônica
28/08/2020 10:58:04.0
CARLOS RICARDO SOCCOL
Presidente da Banca Examinadora

Assinatura Eletrônica
27/08/2020 10:58:57.0
SUSAN GRACE KARP
Avaliador Interno (UNIVERSIDADE FEDERAL DO PARANÁ)

Assinatura Eletrônica
28/08/2020 11:23:22.0
ADENISE LORENCI WOICIECHOWSKI
Avaliador Interno (UNIVERSIDADE FEDERAL DO PARANÁ)

Assinatura Eletrônica
26/08/2020 17:19:42.0
JOSÉ DARCY DOS SANTOS
Avaliador Externo (BAIC)

Assinatura Eletrônica
26/08/2020 17:18:39.0
ANDRÉ LUIZ GOLLO
Avaliador Externo (BAIC)

This thesis is dedicated to the memory of my brothers António, Águida and Lourenço Júnior, and to the living brothers Xavier and Xandinha; to my wife Lúcia and my three children Nayra, Aren and Ezry.

ACKNOWLEDGEMENTS

The conclusion of this Doctoral thesis was made possible thanks to the support of many people. I would like to take this opportunity to express my sincere gratitude and thanks to all of those who made this Doctoral thesis possible.

First of all, I would like to express my special thanks to Prof. Dr. Carlos Ricardo Soccol for giving me the wonderful opportunity to carry out my Doctoral thesis under his supervision, it is truly an honor. I would like to thank you for allowing me to access the laboratory facilities and for your valuable support and guidance.

I would also like to express my deepest gratitude to my research co-supervisor, Prof. Dr. Susan Grace Karp, for her valuable contributions and scientific guidance.

I would like to sincerely thank all the professors of the Post-Graduate Program in Bioprocess Engineering and Biotechnology (PPGEBB). Special thanks to Prof. Dr. Vanete Soccol, for allowing me to use the facilities available at the Molecular Biology Laboratory to identify bacterial strains.

I also thank the laboratory team for their kind support. I thank Carlos, Otacilio, Ronei and Mitiyo who were always ready to dedicate their precious time to help whenever necessary.

I would like to thank the laboratory colleagues Gisela, Walter, Luiz, Jéssica, Dão, Alex, Luisa, Denise, Diego, Manuel and many others who somehow contributed to the work, for their friendship and companionship. I am immensely grateful!

A special mention of thanks to the Mozambican companions: Mateus Manharage, Idalecio Comundela, Caisse Amisse, Jaime Macuacua, Verônica Uamusse, Domingos Lusitaneo and many others for their invaluable support and friendship. My special thanks to Mateus Manharage, for being both roommate and friend. I appreciate your constant support. Your help and friendship should always be remembered.

I owe my deepest gratitude to my wife Lúcia for her unconditional love, patience and support. I want to extend my special thanks to my three wonderful children, Nayra, Aren and Ezry, for giving me happiness and inspiration. You have been my strength and motivation!

I am also very grateful to all members of my family for their constant inspiration and encouragement. Special thanks to my brother Xavier and my sister Xandinha, for their great love and support.

I would like to especially thank my older brothers who passed away, António, Águida and Lourenço Júnior, who inspired my personal and academic life.

I am grateful to CAPES (Coordenação de Aperfeiçoamento de Pessoal de Nível Superior) for providing me with financial support for the completion of this Doctoral thesis.

Finally, I thank God for giving me the wisdom, health and strength necessary to carry out this research task and allow me to complete it.

Education is the most powerful weapon which you can use to change the world! (Nelson Mandela, 1918-2013)

RESUMO

Este trabalho de pesquisa teve como objetivo isolar novas bactérias endofíticas de cogumelos, selecionar melhores produtores de ácido indol-3-acético (IAA) com outras caracteristicas de promoção de crescimento de plantas, como solubilização de fosfato e atividade de fixação de nitrogênio, e otimizar as condições de produção de IAA, para aplicação na agricultura como promotores de crescimento de plantas. Para atingir esse objetivo, duas cepas bacterianas endofíticas, identificadas pelo sequenciamento parcial do gene 16S rRNA como *Providencia* sp. e *Enterobacter* sp., foram escolhidas com base no potencial de produção de IAA na presença de L-triptofano, de setenta e uma (71) bactérias endofíticas isoladas de vários cogumelos silvestres. A natureza endófita destas duas estirpes bacterianas foi confirmada por análise metagenômica do respectivo corpo de frutificação dos cogumelos a partir do qual foram isoladas (*Lactarius deliciosus* e *Boletus edulis*). O potencial de produção IAA foi detectado a partir de filtrados de cultura usando o método colorimétrico e confirmado por HPLC. A produção do IAA por esses dois isolados foi otimizada com sucesso usando a combinação de métodos clássicos (Um-Fator-De-Cada-Vez) e estatísticos (Planejamento Fatorial Completo de dois-níveis, Planejamento de Plackett-Burman e Metodologia de Superfície de Resposta). A produção de IAA pela cepa *Providencia* sp. foi afetada apenas pela concentração de L-triptofano, para cada unidade de aumento na concentração de L-triptofano foi observado um aumento de 244,29 unidades de IAA (244,29µg/mL de IAA). A cepa bacteriana *Providencia* sp. atingiu o pico de alta produção de IAA de 2880±11 µg/mL às 15 horas de incubação, o que significa uma produção horária de 192±0,7 µg/mL.h. Por outro lado, a produção de IAA pela cepa *Enterobacter* sp. foi significativamente afetado pelas concentrações de amido e L-triptofano. O pico de alta produção de IAA pela cepa *Enterobacter* sp. foi observado às 168 horas de incubação, com produção horária de IAA de 13,8±0,9 µg/mL.h (2311,0±155,7 µg/mL). A suspensão bacteriana de cada cepa (*Providencia* sp. e *Enterobacter* sp.) contendo IAA, foi avaliado na cultura da soja em casa de vegetação e observou-se que ambas as suspensões bacterianas contendo IAA promoveram positivamente o crescimento (germinação e crescimento de plantas). Além disso, ambas as cepas bacterianas foram positivas para quase todas as características de promoção de crescimento de plantas testadas (solubilização de fosfato, fixação de N_2 atmosférico, produção de HCN, actividade antifúngica contra *Aspergillus niger*, *Fusarium moniliforme* and *Gibberella* sp.).

Palavras-chave: Bactérias Endofíticas. Ácido Indol-3-Acético. Auxinas. Cogumelos. Análise Metagenômica. Promoção do Crescimento de Plantas

ABSTRACT

This research work aimed at the isolation of novel endophytic bacteria from mushrooms, the selection of better indol-3-acetic acid (IAA) producers with other plant growth promotion traits such as phosphate solubilization and nitrogen fixation activity, and the optimization of the conditions of IAA production, for application in agriculture as plant growth promoters. To achieve this goal, two endophytic bacterial strains, identified by partial 16S rRNA gene sequencing as *Providencia* sp. and *Enterobacter* sp., were chosen based on the production potential of IAA in the presence of L-tryptophan, from seventy-one (71) endophytic bacteria isolated from several wild mushrooms. The endophyte nature of these two bacterial strains was confirmed by metagenomic analysis of the respective mushrooms fruiting body from which they were isolated (*Lactarius deliciosus* and *Boletus edulis*). The IAA production potential was detected from culture filtrates using the colorimetric method and confirmed by HPLC. The IAA production by these two isolates has been successfully optimized using the combination of classic (One-Factor-At-Time) and statistical methods (Two-level Full Factorial Design, Plackett-Burman Design and Response Surface Methodology). The production of IAA by the strain *Providencia* sp. was affected only by the concentration of L-tryptophan, for each unit of increase in the concentration of L-tryptophan generated an increase of 244.29 units of IAA (244.29µg/mL of IAA). The bacterial strain *Providencia* sp. reached the peak of high IAA production of 2880±11 µg/mL at 15 hours of incubation, which means an hourly production of 192±0.7 µg/mL.h. On the other hand, the production of IAA by the strain *Enterobacter* sp. was significantly affected by starch and L-tryptophan concentrations. The peak of high IAA production by the strain *Enterobacter* sp. was observed at 168 hours of incubation, with hourly IAA production of 13.8±0.9 µg/mL.h (2311.0±155.7 µg/mL). The bacterial suspension of each strain (*Providencia* sp. and *Enterobacter* sp.) containing IAA, was evaluated in the soybean culture in a greenhouse and it was observed that both bacterial suspensions containing IAA positively promoted the growth (germination and plant growth). In addition, both bacterial strains were positive for almost all tested plant growth promotion characteristics (phosphate solubilization, atmospheric N_2 fixation, ammonia production, HCN production, antifungal activity against *Aspergillus niger*, *Fusarium moniliforme* and *Gibberella* sp.).

Keywords: Endophytic Bacteria. Indol-3-Acetic Acid. Auxins. Mushrooms. Metagenomic Analysis. Plant Growth Promotion

LIST OF FIGURES

LIST OF TABLES

LIST OF ABBREVIATIONS OR ACRONYMS

ANOVA	- Analysis of variance
ATP	- Adenosine triphosphate
BBD	- Box Bohnken Design
BNF	- Biological Nitrogen fixation
CCD	- Central Composite Design
gDNA	- Genomic DNA
HPLC	- High-performance liquid chromatography
IAA	- Indol-3-Acetic Acid
NA	- Nutrient agar
NB	- Nutrient broth
NBRIP	- National Botanical Research Institute´s Phosphate
NCBI	- National Center of Biotecnology Information
NfB	- Nitrogen-free bromothymol blue
NGS	- Next-generation sequencing
OFAT	- One-Factor-At-Time
PBD	- Plackett-Burman Design
PCR	- Polymerase chain reaction
PGP	- Plant growth promotion
PGPB	- Plant growth promoting bacteria
rpm	- Revolutions per minute
rRNA	- Ribosomal ribonucleic acid
RSM	- Response Surface Methodology
Try	- Tryptophan
UV-Vis	- Ultraviolet – Visible

LIST OF SYMBOLS

% – Percent

µL – Microliter

µg – Microgram

mL – Milliliter

min - minute

mg – Milligram

^{o}C – Degree Celsius

cm – Centimeter

g – Gram

L – Liter

CONTENTS

1 INTRODUCTION

Auxin was the first identified plant hormone (phytohormone). It plays a fundamental role in plant growth and development (Fu et al. 2015; Pařízková et al. 2017) and is responsible for regulation of a number of biological processes such as cell division, cell expansion, root initiation, phototropism, geotropism, apical dominance, ethylene production, and fruit development (Rincón et al. 2003). Among all naturally occurring endogenous auxins in plants, Indol-3-Acetic Acid (IAA) is the most abundant and has been well characterized so far (Zhao 2010). IAA is not only produced by plants, it is also produced by several microorganisms including endophyte bacteria. Due to its importance in plants, mediated production of IAA by endophyte bacteria has gained a great deal of attention (Aswathy et al. 2013). The use of endophytic bacteria in agricultural practices has increased significantly in recent years, both for promotion of plant growth and for biological control of pests and plant diseases, among other applications, and they are seen as potential substitutes for chemical products (Erica 2015). As well, the application of bacterial IAA has been reported in several studies to promote plant growth (length, fresh and dry mass of roots and shoots) (Rincón et al. 2003; Ali et al. 2008; Malik and Sindhu 2011). Alam et al. (2020) reported that foliar application of IAA to salt stressed tomato plants rescued the plants and had significantive positive effects on growth and yield of tomato plants. Likewise, Datta et al. (1997) reported that pre-treatment of *Triticum aestivum L.* seeds with IAA alleviated the growth inhibiting effect due to salt stress.

In addition, Fernández-Falcón et al. (2003) reported that exogenous application of IAA to banana plants induces resistance against *Fusarium wilt* of banana (Panama disease). Similarly, Sharaf and Farrag (2004) reported that IAA exerts disease suppression in tomato plants. In their study, the IAA was tested *in vitro* on growth of tomato wilt pathogen *Fusarium oxysporum lycopersici*, and observed reduced spore germination, mycelial dry weight and protein content. And, *in vivo* application of IAA to soil of the uninoculated plants (controls), Sharaf and Farrag (2004) observed improved growth and yielded longer shoot and root, particularly at low concentrations.

1.1 OBJECTIVE

1.1.1 General objective

The general objective of this work was to isolate endophytic bacteria from wild mushrooms, select better IAA producers, optimize conditions for IAA production, evaluate growth promotion traits like nitrogen fixation and phosphate solubilization and evaluate the effectiveness of the produced IAA in promoting plant growth.

1.1.2 Specific objectives

- Isolate endophytic bacteria from wild mushrooms;
- Select most efficient IAA producers;
- Conduct molecular identification of the selected endophytic bacteria;
- Conduct metagenomic analysis of mushrooms to confirm the endophytic nature of the isolated bacteria;
- Conduct optimization study for IAA production by selected endophytic bacteria;
- Assess other plant growth promotion traits of the selected endophytic bacteria;
- Evaluate, under greenhouse condition, the effect of the bacterial suspension containing IAA in improving plant growth, particularly of the soybean plant.

2 LITERATURE REVIEW

2.1 ENDOPHYTES

The term endophyte is derived from the Greek words “endon” which means within and “phyte” which means plant (Wilson 2020). Endophytes are microorganisms such as bacteria, archaea, fungi, and protists (Kandel et al. 2017) that spend the whole or part of their life cycle colonizing the interior of health living tissues (Weyens et al. 2009; Dutta et al. 2014), seeds, roots, petioles, stems (Weyens et al. 2009), fruit, buds, and leaves (Gouda et al. 2016; Santoyo et al. 2016), without causing any visible damage to plant host (Bandara et al. 2006; Gautam et al. 2016), being distinguished from other plant-associated microbes based on the isolation from surface-sterilized plant materials (Hurst et al. 2016).

Endophytes are classified based on their life style as obligate or facultative (Egamberdieva and Ahmad 2018). Obligate endophytes spend the entire life cycle inside the host, unable to live outside plant tissues, depend on the host plant to survive, and disseminate through seed or vegetative plant tissue (Frank et al. 2017; Kumar and Khapre 2017). The obligate endophytes are not culturable or are hardest to cultivate on the laboratory since they require more specific conditions for their growth (Maela and Serepa-dlamini 2019). On other hand, facultative endophytes are free-living. They colonize plants opportunistically, this is, exist outside of the plant for a part of their life span, and for the rest, they inhabit inside the plant (Singh et al., 2016). They also may enter in plants through germinated tissue, roots, stomata, and damaged tissue (Herlina et al. 2017), remaining there or becoming systemic by transport or active migration through the conducting elements (Lodewyckx et al. 2015).

Plant and endophyte microorganisms interact in mutualistic manner. Endophyte microorganisms profit from the nutrients made available by the plants including photosynthates (Guo et al. 2017) and residency (Weyens et al. 2009), which in return, the bacteria improve plant nutrient acquisition from the environment such as nitrogen, iron and phosphorus (Kandel et al. 2017), protect the host plant from pathogen and pests (Gunatilaka 2006), help the phytohormone synthesis and nitrogen fixation (Suhandono et al. 2016).

2.1.1 Isolation of endophytic bacteria

In plants, the isolation of endophyte bacteria generally consist on washing of a freshly collected plant tissue, to remove dusty, soil particles and epiphytic microorganisms adhered to surface, followed by immersion in sterilizing agents such as sodium hypochlorite, ethanol, sodium bicarbonate, hydrogen peroxide, or mercuric chloride to ensure effective removal of surface microbial flora (Miche and Balandreau 2001; Puri et al. 2017).Being ethanol, sodium hypochlorite and sodium bicarbonate the most used (Muzzamal et al. 2012).

There is no common protocol for tissue surface sterilization. The concentration of sterilizing agents, combination treatment, and immersion period for sterilization should be adapted according to the plant species, and type of plant tissue (Varma and Kharkwal 2009; Puri et al. 2017). Sterilization efficiency may be achieved by the use of one sterilizing agent or by the combination of two or more.

In order to increase the isolation efficiency of endophyte microorganisms, the surface sterilization procedure must be sensitive enough in order to, on the one hand, eliminate epiphytic microorganisms from the plant surface and, on the other hand, allow the recovery of high number of endophytic microorganisms (Amaresan, Velusamy, & Thajuddin, 2014). The immersion period of plant tissue on the solution of sterilizing agent should not last too much, because they may penetrate in the plant tissue, thus being lethal to both epiphytic and endophyte microorganism (Anjum and Chandra 2015). In addition, the residual sterilizing agent should be removed totally by rinsing the plant tissue in sterile distilled water after treatment with sterilizing agent, as it may likely affect growth of microorganism or even induce mutagenesis (Rosenblueth and Martínez-Romero 2006).

2.1.2 Identification of endophytic bacteria

Classical methods for bacterial strain identification are based on observation of phenotypic characteristics such as Gram staining, colony morphologies, culture requirements, biochemical activity, and sensitivity to antimicrobial agents. However, these methods are only applicable to organisms that can be cultured *in vitro,* and the identification is based on comparison of the observed characteristics with the known bacterial strains (Petti 2007). Therefore, when a bacterial strain exhibits unique

characteristics that do not fit the pattern of any known genus and species, accurate identification by phenotypes method could be compromised (Woo et al. 2008).

Genotypic identification of bacteria by partial 16S rRNA gene sequencing has emerged as a preferred technique, and is widely considered more accurate and faster method to identify a wide variety of aerobic and anaerobic bacteria, with the added capability of defining taxonomical relationships among bacterial strains (Petti et al. 2005; Park et al. 2011). 16S rRNA region is universally distributed among bacterial species and is species-specific (Senthilraj et al. 2016), and pursue two regions (conserved and variable regions) very important for differentiation purposes, and enables to draw conclusions regarding evolution and phylogeny (Toldrá 2008; Vetrovsky and Baldrian 2013).

The procedure for identification of bacterial strains involves the isolation of genomic DNA, 16S rRNA amplification by Polymerase Chain Reaction (PCR), purification and direct sequencing of the PCR products, and the obtained sequences are then compared with available sequence databases such as GeneBank.

The comparison of the 16S rRNA gene sequences allows differentiation between organisms at the genus, species and subspecies levels (Clarridge, 2004). The occasional exceptions to the usefulness of 16S rRNA gene sequencing is usually related to the inability to identify bacteria at species level when there is high sequence similarities between some species (Petti 2007; Peker et al. 2019). Therefore, bacteria that are indistinguishable by 16S rDNA sequences, i.e., those that share complete sequence identity, could be better identified by use of other bacterial gene targets such as *rpoB*, *tuf*, and *sodA* (Petti 2007; Sentausa and Fournier 2013).

2.1.3 Metagenomic analysis – endophyte bacteria diversity

Metagenomic analyses of bacterial diversity are often performed using the prokaryotic 16S ribosomal RNA (rRNA) gene through ‘next-generation’ sequencing (NGS) technologies such as Illumina, 454/Roche and Ion Torrent/Ion Proton platforms (Garza and Dutilh 2015; Deurenberg et al. 2017). These technologies enable to sequence microbial communities directly from the sample, without the necessity to obtain the pure cultures. This is important as only around 1-2% of bacteria can be cultured in the laboratory (Wade 2002). Moreover, metagenomic

technologies enable a large number of reads in a single run, and detection of low abundant genera (Capobianchi et al. 2013).

2.2 PLANT GROWTH PROMOTION MECHANISM

2.2.1 Atmospheric N_2 fixation

Nitrogen is the most important macronutrient for plant growth and development. It is a necessary component of many biomolecules including proteins, nucleic acids, chlorophyll, and other important organic compounds (Ohyama 2010). Nitrogen is an unlimited resource and is highly abundant in atmosphere, accounting for approximately 78% of the atmospheric gases, and mainly present in the diatomic form (N_2).

Plants cannot use directly atmospheric N_2, they only uptake nitrate (NO_3^-) or ammonium (NH_4^+) as inorganic nitrogen sources and amino acids under particular conditions of soil composition. Therefore, regardless of huge abundance of atmospheric N_2, the plant-available nitrogen is commonly deficient in agricultural soils.

Some bacteria, either symbiotic or non-symbiotic (free-living), possess *nif* genes that encode nitrogenase enzyme involved in Biological Nitrogen Fixation (BNF). BNF consist in conversion of atmospheric nitrogen (N_2) into ammonia (NH_3). The *nif* genes are found only in some prokaryotes, termed diazotrophs (Poza-Carrión et al. 2014; Santi et al. 2015). Legumes are the most well-known class of plant that utilizes atmospheric N_2 through symbiotic association with diazotrophs.

2.2.2 Phosphate solubilization

Phosphorus is the second most important macronutrient for plant growth and development, next to nitrogen, and makes up about 0.2% to 0.8% of plant's dry weight (Alori et al. 2017). Phosphorus is an essential component of biological molecules such as nucleic acids, adenosine triphosphate (ATP), and phospholipids (Sharon et al. 2016; Kalayu 2019). In plant, phosphorus is involved in process development of roots, strengthening of stalk and stem, formation of flowers and seeds, and promotion of crop maturity (Sharon et al. 2016; Bononi et al. 2020).

Phosphorus level in the soil is about 0.05% (w/w), but a considerable part of which, 95 to 99%, is present in the form of insoluble phosphates, and hence only 0.1% of this phosphorus is available for plant use (Zhu et al. 2011; Alori et al. 2017). Plants are only able to uptake in considerable amount the orthophosphatic ions ($H_2PO_4^-$ or HPO_4^{2-} and PO_4^{3-}) from soil solution.

The challenge of phosphorus deficiency in agricultural soil is usually compensated by application of phosphorus fertilizers. However, nearly 70 to 90% of phosphorus fertilizers applied is rapidly immobilized by cations (Ca^{2+}, Al^{3+} and Fe^{3+}). The speciation of phosphurus is mainly governed by the pH value of soil, in neutral to alkaline soils there are predominance of cation Ca^{2+} which form a complex calcium phosphate ($Ca_3(PO_4)_2$), whereas in acidic soils there is predominance of cations Al^{3+} and Fe^{3+} which rapidly immobilize phosphorus to form aluminum phosphate ($AlPO_4$) and ferrous phosphate ($FePO_4$).

Phosphorus availability can also be augmented by the usage of phosphate-solubilizing microorganisms, which are capable to release phosphorus in phosphorus-containing mineral (e.g. apatite) though soil acidification, and release of complexing or mineral dissolving compounds such as organic acid anions, siderophores and protons (Nannipieri et al. 2011; Sharon et al. 2016).

2.2.3 Phytohormones

Phytohormones (plant hormones) are organic substances synthesized in any organ of plants, then transported to other organs (Aziz et al. 2015). Phytohormones do not have nutritional function, they act as signal molecules to influence or control some physical, biochemical and morphological characteristic and responses at different stages of plant development, at very low concentrations (Gaspar et al. 2003; Sauer et al. 2013).

The synthesis of phytohormones may occur everywhere in plant, and their action in plant growth or development can be at distance from their sites of synthesis or even at the same organ where they were synthesized (Gaspar et al. 2003). They are not only synthesized by plants, they can also be synthesized by some associated microorganisms such as bacteria and fungi (Costacurta and Vanderleyden 1995; Shi et al. 2017).

Phytohormones are commonly divided in five categories: auxins, cytokinins, abscisic acid (ABA), gibberellins (GAs), and ethylene (Halmann 1990). However, some other categories of phytohormones may also be included such as brassinosteroids (BRs), jasmonic acid (JA), and salicylates (Halmann 1990; Gray 2004; Sauer et al. 2013; Shi et al. 2017). Each category comprises both naturally occurring hormones and synthetic substances (Gana and A 2011; Wanderley et al. 2014).

2.2.4 Antifungal activity

Endophyte bacteria have been reported to indirectly promote plant growth through suppression of phytopathogen fungi (Lacava et al. 2007; Ryan et al. 2008; Afzal et al. 2019).

The mechanisms responsible for antagonistic activity against phytophatogen fungi include production of antibiotics such as HCN, phenazines, pyrrolnitrin, 2,4-diacetyl phloroglucinol, pyoluteorin, viscosinamide and tensin (Bloemberg and Lugtenberg 2001; Ganeshan and Kumar 2007; Beneduzi et al. 2012), production of fungal cell wall-degrading enzymes such as chitinases, cellulases, β-1,3-glucanases, and proteases (Glick 2012; Palaniyandi et al. 2013; Figueiredo et al. 2016) and production of volatile metabolites such as mesityl oxide, acetic acid, 2-methylpropyl ester, 4-methyldecane (Alijani et al. 2019), α-pinene and limonene (Tenorio-salgado et al. 2013).

The antifungal potential of endophytic bacteria is often accessed *in vitro* through dual culture antagonism method, as well by production of volatile HCN, siderophore (Alijani et al. 2019) and extracellular enzymes such as chitinases, since chitinases hydrolyze chitin, which is the major structural component of fungal cell walls (Aktuganov et al. 2008; Caulier et al. 2019).

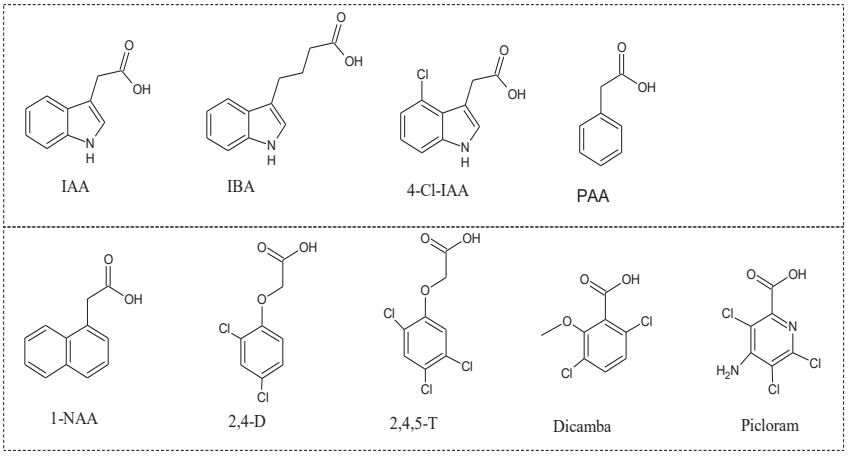
2.3 AUXINS

Auxins can be defined as weak organic acids with low molecular weight, containing an indole or an aromatic ring, and a carboxyl group, which to be active, the aromatic ring and the carboxyl acid side need to be separated at distance of 0.55Å (Sauer et al. 2013). Auxins are crystalline, soluble in organic solvents (ethanol,

methanol, acetone, diethyl ether and dimethyl sulphoxide) but slightly soluble in water (Machackova et al. 2008). They are stable with the exception of indol-3-acetic acid (IAA), which is particularly sensitive to oxidants (Machackova et al. 2008) and to light illumination, particularly UV (Yamakawa et al. 1979).

The naturally occurring auxins in plants include IAA, indole-3-butyric acid (IBA), 4-chloroindole-3-acetic acid (4-Cl-IAA), and phenylacetic acid (PAA) (Cao et al. 2019). Along with endogenous auxins, there are many synthetic auxin analogues which include 1-naphthaleneacetic acid (NAA), 2,4-dichrophenoxyacetic acid (2,4-D), 2,4,5-trichlorophenoxacetic acid (2,4,5,-T), 3,6-dichloro-2-methoxybenzoic acid (dicamba), 4-amino-3,5,6-trichloropicolinic acid (picloram), quinclorac and many others (FIGURE 1).

FIGURE 1 – Auxins



SOURCE: Sauer et al. (2013)

Among all naturally occurring endogenous auxin in plants, IAA is the most abundant and has been well characterized so far (Zhao 2010). The term auxin refers to IAA in plants by strict definition, is an important signal molecule in the regulation of plant growth and development (Patten and Glick 2002b; Donoso et al. 2016). Its central role include stimulation of root initiation (Nutaratat et al. 2016), cell division (Teale et al. 2006), cell elongation by altering cell wall plasticity (Sauer et al. 2013), cell differentiation (Tsavkelova et al. 2006; Sugawara et al. 2015), and response to directional stimuli (tropic response) and shoot branching (Halmann 1990; Grossmann 2010; Ali and Vora 2014).

The IAA response is concentration dependent and different plant tissues might respond in a distinct manner to varying amounts of endogenous IAA (Ludwig-Müller 2011). Regulation of IAA level (Homeostasis) is highly important for the plant to coordinate growth and tissue development. The plant tightly controls the optimum endogenous IAA level by various mechanisms namely: regulation of the rate of IAA biosynthesis; inactivation of the IAA by conjunction with amino acid, peptides or carbohydrates; irreversible degradation (oxidation), and by transportation to other parts of the plant (Olatunji et al. 2017).

2.3.1 IAA biosynthesis

IAA biosynthesis is extremely complex, and multiple pathways contribute to *de novo* IAA synthesis, and these pathway can be differentially or combinatory used in response to environmental signals (Ruiz Rosquete et al. 2012). In both plants and microorganisms, IAA is synthesized by two main routes namely Trp-dependant and Trp-independent pathways (Mohite 2013). The amino acid tryptophan (Trp) has been identified as main precursor for IAA biosynthesis for both plants and microorganisms (Costacurta and Vanderleyden 1995; Spaepen and Vanderleyden 2011; Herlina et al. 2017). The Trp-independent pathway is also believed to exist, it has been demonstrated in *Azospirillum brasilense* (Prinsen et al. 1993) as well in *Arabidopsis thaliana* (Normanly et al. 1993), *Lemna gibba* (Baldi et al. 1991), and maize mutant *orange pericarp* (Wright et al. 1991). However, little is known with regard to biochemical and genetic basis for Trp-independent pathway (Mashiguchi et al. 2011; Mano and Nemoto 2012), so far have been only cloned genes encoding Trp-dependent biosynthetic enzymes (Bartel 1997) and have been as well isolated and characterized only biosynthetic intermediates of Trp-dependent pathways (Ljung et al. 2002; Pollmann et al. 2009).

Several different Trp-dependent pathways for synthesis of IAA have been characterized, and most pathways described in microorganism show similarity to those described in plants, although some intermediates can differ (Spaepen et al. 2007; Spaepen and Vanderleyden 2011).

A single microorganism or plant may possess multiple parallel Trp-dependent pathways and some of them may intersect with one another (Patten and Glick 1996; Mohite 2013). These pathways are classified according to their intermediates

namely: indole-3-acetonitrile (IAN), indole-3-acetamide (IAM), indole-3-pyruvic acid (IPyA), tryptamine (TAM), and tryptophan side-chain oxidase (TSO) pathways (FIGURE 2) (Spaepen et al. 2007; Senthil et al. 2010; Li et al. 2018).

a) Indole-3-Acetonitrile (IAN) pathway

In the indole-3-acetonitrile (IAN) pathway, tryptophan is first converted into indole-3-acetaldoxime (IAOx) by cytochrome p450 oxidoreductase enzymes (CYP79B2 and CYP79B3 genes) (Zhao et al. 2002). The CYP79B2/B3 genes have so far only been identified in Brassicaceae species (family that includes *Arabidopsis* and other cruciferous plants) (Duca et al. 1996). In the second step, the indole-3-acetaldoxime intermediate is converted into indole-3-acetonitrile by an acetaldoxime dehydratase. The indole-3-acetonitrile intermediate is either converted directly to IAA by a nitrilase enzyme (Reilly and Turner 2003) or is converted into indole-3-acetamide (IAM) by nitrilase or nitrile hydratase, which is further converted to indol-3-acetic acid (IAA) by IAM hydrolase/amidase (Duca et al. 2014).

b) Indole-3-Acetamide (IAM) pathway

The indole-3-acetamide (IAM) pathway is considered the most common pathway for IAA biosynthesis in phytopathogenic bacteria (Cerboneschi et al. 2016), and the genes that encode enzymes involved in IAM pathway have been cloned and sequenced (Kochar et al. 2011; Spaepen and Vanderleyden 2011) in many phytopathogens such as in tumor-forming bacterium *Pseudomonas syringae pv. savastanoi* (Comai and Kosuge 1980; Yang et al. 2007), and in *Agrobacterium tumefaciens* (Yamada et al. 1985). This pathway consist in two consecutive steps, first occur oxidative decarboxylation of tryptophan to IAM catalyzed by tryptophan 2-monooxygenase (encoded by *aux1/iaaM/tms-1* genes), then IAM is hydrolyzed to form IAA and ammonia, by IAM hydrolase/amidase (encoded by *aux2/iaaH/tms-2* genes) (Yang et al. 2007; Mano and Nemoto 2012; Kunkel and Harper 2018).

For long time IAM pathway has been considered restricted to bacteria, as no evidence could be found in plant (Spaepen et al. 2007; Mano and Nemoto 2012). However, recent researches have detected enzyme in the genome of *Arabidopsis thaliana*, named *AMI1*, which code an amidase with significant sequence similarity to the IAM-hydrolyzing bacterial amidases, able to catalyze the conversion of IAM to IAA (Pollmann et al. 2003). Nemoto et al. (2009) also isolated the *NtAMI1* gene from BY-2 cells of Tobacco (*Nicotiana tabacum*), which is equivalent to the *AMI1* gene of *Arabidopsis thaliana*.

c) IPyA pathway

The IPyA pathway is thought to be the main IAA biosynthetic pathway in land plants (Machackova et al. 2008; Tsugafune et al. 2017). In this pathway, tryptophan is first converted to IPyA by transaminase reaction (Patten and Glick 2002b), followed by decarboxylation of IPyA into indole-3-acetaldehyde (IAAld), which is further converted to IAA either by dehydrogenation (NAD-dependent indoleacetaldehyde dehydrogenase) or by oxidation (indoleacetaldehyde oxidase) (Costacurta and Vanderleyden 1995; Machackova et al. 2008).

The gene *IPDC* which encode indole-3-pyruvate decarboxylase, an enzyme that catalyzes the conversion of IPyA into IAAld for IAA synthesis, has been isolated and characterized in *A. brasilense*, and has been demonstrated that most of IAA synthesis is strongly mediated by *IPDC* (Costacurta et al., 1994). The importance of IPyA pathway in IAA production has been also demonstrated in plant growth-promoting bacterium *P. putida* 12-2*,* where the inactivation of *IPDC* gene by insertional mutagenesis results in loss of IAA production (Patten and Glick 2002a). However, the accepted role of indole-3-acetaldehyde (IAAld) as an intermediate in this pathway has been questioned by recent data. Genetic and biochemical evidences demonstrated that IAA biosynthesis is predominantly from tryptophan via two sequential enzymatic reactions (Tsugafune et al. 2017), being IPyA the sole intermediate (Zhao 2014; Sugawara et al. 2015; Cook and Ross 2016). The tryptophan is first

converted to IPyA by *TAAs* (gene family of tryptophan aminotransferase), then IPyA go through decarboxylation by *YUC* (gene family of flavin monooxygenases) to produce IAA (Zhao 2012). *TAA* and *YUC* are two families of enzymes widely found in various plant species, including *Arabidopsis*, maize, rice, and several other species (Cook et al. 2016; Kasahara 2016), being tryptophan a preferred substrate for *TAA* gene family, while IPyA can be converted into IAA by catalyze of *YUC* gene family or IPyA can go through substantial non enzymatic breakdown to produce IAA (Cook et al. 2016).

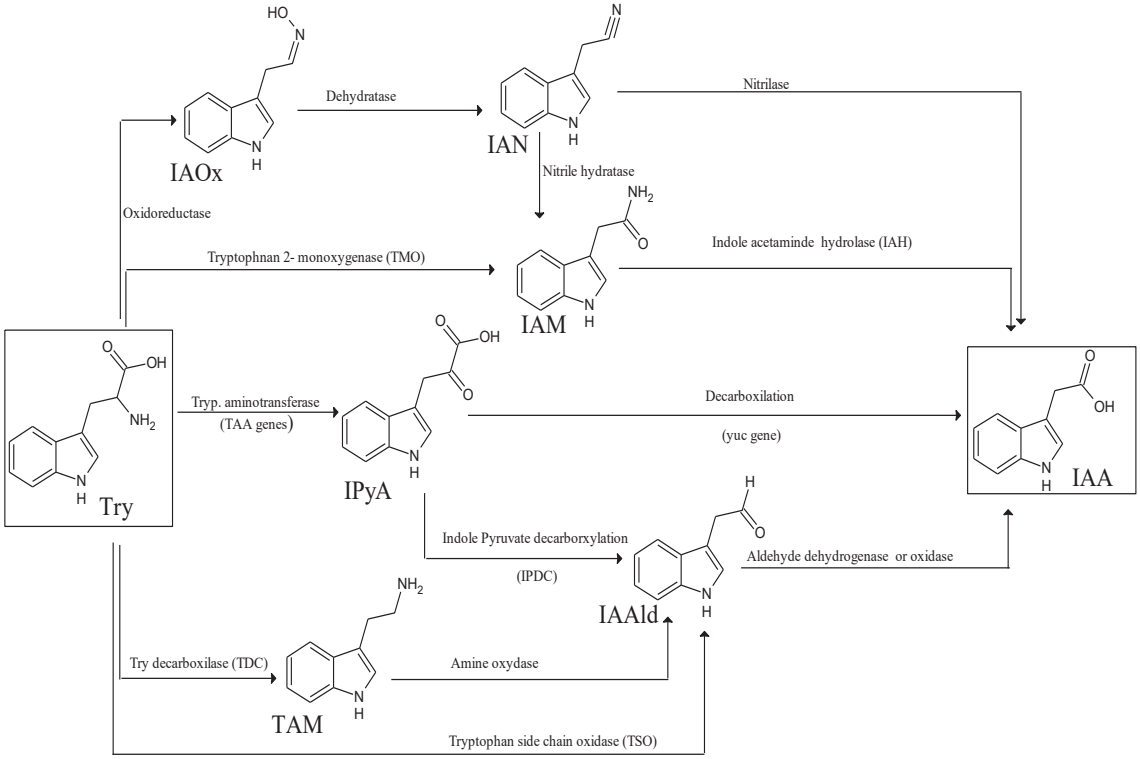
d) Tryptamine (TAM) pathway

The TAM pathway is similar to the IPyA pathway, the only difference between these two pathways is the order of the deamination and decarboxylation reactions. The IAA biosynthesis in this pathway initiate with decarboxylation of tryptopthan to TAM, which is then converted to IAAld by amine oxidase (Bunsangiam et al. 2019). The last step consists in conversion of IAAld into IAA by enzymatic action of IAAld dehydrogenase.

e) Tryptophan side chain oxidase (TSO) pathway

In this pathway, tryptophan is directly converted into indole-3-acetylaldehyde (IAAld) by a tryptophan side chain monooxygenase enzyme, which is then converted to IAA by action of IAAld dehydrogenase (Oberhansli et al. 1991).

FIGURE 2– IAA biosynthesis by Trp-dependent pathways



SOURCE: Adapted from Li et al. (2018)

2.3.2 Inactivation of IAA by conjugation

The free IAA can conjugate with amino acids, peptides, and proteins via amide bonds or with simple alcohols and sugars (e.g. glucose) by ester linkages (Normanly et al. 1993). In plants, the conjugated form of IAA represents approximately 95% of the IAA pool, and more than 80% of the IAA pool in *Arabidopsis* is present as amide derivatives (Bartel and Fink 1995). The most common amide conjugate in *Arabidopsis* are: IAA-Alanine (IAA-Ala), IAA-Leucine (IAA-Leu), IAA-Aspertate (IAA-Asp), and IAA-Glutamate (IAA-Glu) (Sauer et al. 2013).

Conjugation of endogenous IAA protect the free IAA from irreversibly oxidative degradation (Cohen and Bandurski 1982), inactivate excess IAA allowing rapid alteration of free IAA concentration, and act as temporary storage form of IAA in seeds and is a transport form in shoots (Sitbon et al. 1993; Rampey et al. 2004; Zazimalová et al. 2014).

The IAA-conjugates are not active auxins *per se*, the biological activities of IAA-conjugates are directly correlated to the ability of the plant tissue to enzymatically hydrolyze them to source free IAA (Bialek et al. 1983; LeClere et al. 2002), which has free carboxyl group that is essential for auxin activities.

2.3.3 Oxidative degradation of IAA

The over-production in plant or the exogenous application of IAA can trigger the oxidative degradation of IAA, contributing to the regulation of the level of IAA (Novák et al. 2012). The IAA degradation can occur either by oxidative decarboxylation of the side chain or by oxidation of indole ring without decarboxylation of the side chain. The decarboxylative oxidation of IAA is catalyzed by IAA-oxidase/peroxidase and leads to formation of degradation products such as oxindole-3-methanol, indole-3-aldehyde, methylene oxindole and indole-3-carboxilic acid. The IAA degradation without decarboxylation involves complex pathways, and results in formation of oxindole derivates such as oxindole-3-acetic acid (OxIAA) and dioxindole-3-acetic acid (diOxIAA) (Lalit 2002).

2.4 BIOSYNTHESIS OF MICROBIAL SECONDARY METABOLITES

The biosynthesis of secondary metabolites usually occurs at stationary phase (idiophase), when medium is depleted and as result the cells stop dividing (Weinberg 1969; Ruiz et al. 2010). When cells are growing actively, the secondary metabolites are either very less or are not formed, thus cells growth and secondary metabolites production is inversely related.

The synthesis of secondary metabolites is genetic in nature (Okada and Seyedsayamdost 2017), but is greatly influenced by culture conditions. Genes that trigger the biosynthesis of secondary metabolites can be over expressed by the change or manipulation of cultural conditions such as media composition (carbon, nitrogen, and phosphorus sources, trace elements), salinity, temperature, pH, and by addition of precursor (Bode et al. 2002; Netzker et al. 2015).

2.4.1 Carbon source

Carbon is the most important medium component, provides both energy for microorganisms and carbon units for biosynthesis of all metabolites (Singh et al. 2017; Pan et al. 2019). The alteration on the type and concentration of carbon sources has been recognized to have a significant influence on microbial secondary metabolite production (Luchese and Harrigan 1993; Ruiz et al. 2010). The carbon regulation of secondary metabolite production could be, for example, by catabolic repression which involves the inhibition of formation of certain enzymes by the catabolic products of carbon source (Bunch and Harris 1986; Demain 2006).

Many enzymes and secondary metabolites have been reported to be affected by carbon catabolic regulation (Spížek and Tichý 1995; Sanchez and Demain 2002). One distinctive characteristic of secondary metabolism is its association with low growth levels (Sánchez et al. 2010), this is, the carbon source rapidly assimilated by microorganism is often used first to produce cells (biomass), and the synthesis of secondary metabolite often occur after most of biomass has been produced. For instance, glucose has been reported to be excellent for growth but interferes with the biosynthesis of many secondary metabolite, whereas polysaccharides (e.g., starch), oligosaccharides (e.g., lactose), and oils (e.g., soybean oil) are often found to be better than glucose as carbon sources for secondary metabolite production (Martin and Demain 1980).

On the media where the initial growth rate is slower, production of secondary metabolites can become growth associated (Bunch and Harris 1986). However, depending on the type of microorganism, different undesirable carbon catabolic effects on secondary metabolite production can be observed (Romero-Rodríguez et al. 2017).

2.4.2 Nitrogen source

Nitrogen source is required for the synthesis of essential proteins, nucleic acids, and secondary metabolites (Pan et al. 2019). The microorganism can use both inorganic nitrogen source such as nitrate, nitrite and ammonium ions and/or organic nitrogen source like urea and amino acids for production of secondary metabolites (Singh et al. 2017), but preferences varies among microorganisms.

The production of secondary metabolites is negatively affected by nitrogen source favorable for growth such as ammonium (Demain 1986; Tudzynski 2014; Sharma and Jha 2015). As result, the slow assimilated nitrogen sources are often used for secondary metabolite production.

Nitrogen regulation of secondary metabolites production has been well demonstrated in Cyanobacteria and consists in repression of the pathways of assimilation of some nitrogen sources when other easily assimilated source of nitrogen is available to the cells. Rückert and Giani (2004) reported that nitrate, nitrite and ammonium are all used as inorganic nitrogen source by the Cyanobacteria, but when ammonium is available in culture medium in addition to one of these nitrogen source, the ammonium repressed the uptake of these other nitrogen source. Likewise, Herrero et al. (2001) reported that most of the genes encoding nitrogen assimilation enzymes are repressed by ammonium in Cyanobacteria.

In general, the nitrogen source rapidly assimilated such as ammonia, represses enzymes involved in the use of other nitrogen sources such as nitrite reductase, nitrate reductase, arginase, glutamate dehydrogenase, ornithine transaminase, extracellular protease, acetamidase, and threonine dehydratase (Martin and Demain 1980; Demain 1986).

In addition to above mentioned, the type and availability of nitrogen source also regulate the secondary metabolite production. Aharonowitz and Demain (1979) demonstrated that ammonium (NH^{4+}) when was added as the sole nitrogen source strongly inhibited and/or repressed the antibiotic production, whereas certain amino acid including asparagine stimulated the antibiotic (cephalosporin) production in *Streptoinyces clavuligerus*. They also observed that the production was highest under nitrogen limitation even when ammonium (NH^{4+}) was the sole nitrogen source. Likewise, Giordano et al. (1999) reported that bikaverin and gibberellins production by *Gibberella fujikuroi* began after nitrogen source depletion. Sánchez et al. (1981) reported that high NH_4Cl concentration in the medium led to 38% reduction in antibiotic (penicillin G) formation in *Penicillium chrysogenum* NRRR 1951.

2.4.3 Carbon-Nitrogen balance

The Carbon-Nitrogen (C-N) balance has been reported to regulate the secondary metabolite production (López et al. 2003). Brzonkalik et al. (2012) studied

the effect of different C-N rations on production of alternariol (AOH), alternariol monomethylether (AME) and tenuazonic acid (TA) by *Alternaria alternata* DSM 12633, and observed that carbon to nitrogen (C-N) ratio was one of factors that influenced their production. They demonstrated clearly that alternariol is produced in the stationary growth phase after nitrogen depletion, and that an excess of carbon has a positive impact on alternariol production. On the other hand, Dinarvand et al. (2013) obtained higher production of enzymes under excess of nitrogen and carbon limitation.

2.4.4 Metal ion

The biosynthesis of the secondary metabolites can be enhanced or repressed by addition of certain metal micronutrients to the fermentation medium. Some metal ions such as Fe, Cu, Mn, Zn, Co, Ni, Mo, Mg, are used as cofactors by certain microbial enzymes involved in biosynthesis of secondary metabolite, and many other metal micronutrients are also directly or indirectly involved in the secondary metabolism of microbes (Dubey et al. 2019). For example, Failla and Niehaus (1986) observed that Zn^{2+} regulates the synthesis of versicolorin A in a mutant strain of *Aspergillus parasiticus*. Likewise, Fe^{3+}, Cu^{2+} and Zn^{2+} ions have been shown to enhance canthaxanthin production of *D. natronolimnaea* HS-1 (Nasrabadi and Razavi 2010). Heavy metal ions La^{3+}, Ce^{3+} and Nd^{3+} had been reported to have stimulatory effect on carotenoid synthesis in *Phaffia rhodozyma* (Pettit 2011).

2.4.5 Phosphate

Phosphate ions are essential for all energy requiring enzymatic reactions for synthesis of protein and nucleic acids, carbon and nitrogen metabolism, biomass production, as well as in the regulation of secondary metabolite biosynthesis (Spížek and Tichý 1995).

The synthesis of secondary metabolite often reported in literature are negatively regulated by phosphate, their synthesis occur only under phosphate-limiting nutritional conditions (Luchese and Harrigan 1993; Martín 2004) including

spiramycin (Lounès et al. 1996), actinorhodin (Hobbs et al. 1990), nanaomycin (Masuma et al. 1986) and actinorhodin (Doull and Vining 1990).

2.4.6 Salinity

The secondary metabolites produced by a variety of microorganism may be regulated by environmental factors including salinity. Suitable salinity is needed for normal microbial growth, but high salt stress may trigger synthesis pathway to restore osmotic imbalance, thus activating silent genes encoding secondary metabolite (Huang et al. 2011; Wang et al. 2011; Pan et al. 2019).

2.4.7 Temperature

Temperature is an important factor that affects the bacterial growth and modulates secondary metabolite gene expression. Lind et al. (2016) reported that the regulation of secondary metabolism by the velvet protein complex was temperature-responsive in *Aspergillus fumigatus*.

The synthesis of secondary metabolite is directly influenced by optimal temperature of microbial enzyme activity (Pan et al. 2019). Cultivation of microorganism at higher temperature often weakened and reduce enzyme production (Thi Nguyen and Tran 2018).

2.4.8 External pH

The regulator effect of pH on the synthesis of microbial secondary metabolite had been reported in many studies. These studies presented genetic evidence that external pH exerts great influence over secondary metabolite gene expression. Gardiner et al. (2009) observed that low pH regulates the production of deoxynivalenol by *Fusarium graminearum.* Keller et al. (1997) reported that sterigmatocystin and aflatoxin biosynthesis in *Aspergillus* spp. was regulated by pH. Likewise, Espeso et al. (1993) reported that fungal penicillin biosynthetic gene expression was regulated by pH.

2.4.9 Precursor

The addition of a biosynthetic precursor (intermediate) to culture medium, that is rate limiting during secondary metabolite formation, is able to increase the yield or to directs the biosynthesis route toward desired secondary metabolite (Drew and Demain, 1977; Demain, 1998).

Several classes of secondary metabolites use aromatic amino acids or other metabolites derived from the shikimate pathways (e.g. flavonoids and alkaloids) as precursors (Pickens et al. 2011), for example, amino acid L-tryptophan has been reported to enhance the synthesis of IAA when exogenously supplied in the culture medium (Bharucha et al., 2013; Costacurta et al., 1994; Hasuty et al., 2018).

2.5 OPTIMIZATION OF SECONDARY METABOLITE PRODUCTION

2.5.1 Classical “One-Factor-At-Time” method

The one-factor-at-time (OFAT) method has been the most preferred choice for initial stage of optimization of medium components as well as for fermentation conditions. This method consists in changing one variable at a time while keeping other variables constant (at fixed level) (Frey et al. 2003; Soni et al. 2007; Chen et al. 2009). The OFAT is simply and convenience for screening short list of variables and the results of experiments can be analyzed by using simple graphs without the aid of sophisticate statistical programs (Panda et al. 2007). However, this method is extremely laborious, time consuming and expensive when involves the analysis of a large number of variables, and often does not bring about the effect of interaction among various parameters (Soni et al. 2007; Nor et al. 2017).

2.5.2 Statistical method - Two level full Factorial Designs

Two-level (2^k) full Factorial Designs are the most powerful screening designs and require experiments to be carried out at all possible combinations of the two levels of each of the k factors considered. The low and high levels of the factors in the design are expressed as (−1) and (+1) in coded units, respectively.

The two-level (2^k) full factorial designs allows the estimation of the effect of a factor at different levels of the other factors, as well as the magnitude of interactions effect (antagonistic or synergistic effects) between two or more factors on the response (Syed et al. 2013).

However, the two-level (2^k) full factorial designs become infeasible when involve a large number of factors. For example, a 2^k design with 8 factors (2^8) has 256 combinations, therefore would be required 256 experiments. Certainly, it would be difficult to maintain experimental conditions unchanged during a large number of experiments, as well as to interpret the large size of factorial experiment especially when there are interactions between factors (Durakovic 2017).

On the other hand, the Fractionate Factorial Designs enable the evaluation of a large number of input factors with a reduced number of experiments required (Bezerra et al. 2008), by fractionating a full factorial 2^k design into a 2^{k-p} design, where p is the number of generators chosen to fractionate the design. For example, a full factorial 2^k design with four (4) input factors can be half-fractionated (2^{4-1} = 8 experiments), and five (5) input factors can be quarter fractionated (2^{5-2} = 8 experiments) (Fukuda et al. 2018).

2.5.3 Plackett-Burman Design

Plackett-Burman Design (PBD) is a special type of two-level Fractionate Factorial Designs, is often used early in the experimentation phase to screen out non-contributing factors or to identify factors with significant effect to the response, which are then optimized by Response Surface Methodology in the subsequent studies. It allows the estimation of main effects for (*4n-1*) factors using a minimal number of experiments (*n*), where *n* represents multiples of 4, and starts from 8 and goes until 100 with the exception of *n* = 92 (*n* = 8, 12, 16, 20, 24, 28, 32, 36). For example, 4, 5, 6, or 7 factors would require 8 experimental runs, whereas 8, 9, 10, or 11 factors would require 12 experimental runs.

The Plackett-Burman Design should be used to study main effects when the factors have no interactions or are negligible, otherwise the effect of a certain factor would be enhanced or masked by other factors (Cheng 2006).

The construction of Plackett-Burman experimental design is based in Hadamard matrices, and the elements are either identified by (-) or (+) which

designate the lower and higher levels of the corresponding factor, respectively (Plackett and Burman 1946). However, caution must be taken when setting the level differential, as a small differential may not show any effect and a large differential for a sensitive factor can mask other factors (Rajput et al. 2016; Fukuda et al. 2018).

2.5.4 Response Surface Methodology

Response Surface Methodology (RSM) is a collection of mathematical and statistical techniques often used for optimization of multiple variables with minimum/reduced number of experiments (Kunamneni and Ellaiah 2002; Zhang et al. 2009; Yadavalli et al. 2013). It is useful for setting up a series of experiments (design) able to yield adequate and reliable data to build a empirical models, as well as to study the effects of interaction between factors and to determine the region of optimum conditions (input factors) that lead to the production of desirable responses (Chen et al. 2009; Barışçı and Turkay 2016). The RSM typically consist in the following steps:

- Screening of important factors using Plackett-Burman Design, for example;
- Choice of appropriate experimental design, and selection of experimental matrix. The Central Composite Design (CCD) and the Box Bohnken Design (BBD) are the two RSM designs commonly used for response optimization (Ahsan et al. 2017). However, CCD is one of the most preferred optimization designs, which is a five levels design (−α, −1, 0, +1, +α), created from a 2^k factorial (or 2^{k-p}) design and augmented with center points and axial points (α) (Fukuda et al. 2018). The axial points depend on the number of variables and can be calculated by $\alpha = 2^{(k-p)/4}$ (Bezerra et al. 2008);
- Running of experiments accordingly to experimental matrix chosen;
- The results of the experiments are then mathematically and statistically treated to fit a second-order model (polynomial function). The coefficient of the linear terms (e.g., X_1 , X_2, X_3), interaction terms (X_1X_2, X_1X_2, X_1X_3, and X_2X_3), and quadratic terms (e.g., X_1^2, X_2^2, and X_3^3) should be included in the mathematical model based on their p-values

through application of Analysis of Variance (ANOVA) (Fukuda et al. 2018);

- Evaluation of the model´s fitness;
- Verification of the necessity and possibility of performing a displacement in direction to the optimal region;
- Determination of optimum values for each input factor. The surface contour plots (3D surface and contour graphs) visually describe the relationship between variables and response. The surface contour plots are generated as a combination of two input factors with the others maintained at their respective zero levels.

REFERENCES

Afzal I, Khan Z, Sikandar S, Shahzad S (2019) Plant beneficial endophytic bacteria : Mechanisms , diversity , host range and genetic determinants. Microbiol Res J 221:36–49. https://doi.org/10.1016/j.micres.2019.02.001

Aharonowitz Y, Demain AL (1979) Nitrogen nutrition and regulation of cephalosporin production in *Streptomyces clavuligerus*. Can J Microbiol 25:61–67. https://doi.org/10.1139/m79-010

Ahsan T, Chen J, Wu Y, Irfan M (2017) Application of response surface methodology for optimization of medium components for the production of secondary metabolites by *Streptomyces diastatochromogenes* KX852460. AMB Express 7:1–10. https://doi.org/10.1186/s13568-017-0388-z

Aktuganov G, Melentjev A, Galimzianova N, et al (2008) Wide-range antifungal antagonism of *Paenibacillus ehimensis* IB-X-b and its dependence on chitinase and b-1, 3-glucanase production. Can J Microbiol 54:577–587. https://doi.org/10.1139/w08-043

Alam M, Khan MA, Imtiaz M, et al (2020) Indole-3-acetic acid rescues plant growth and yield of salinity stressed tomato (*Lycopersicon esculentum* L.). Gesunde Pflanz 72:87–95. https://doi.org/10.1007/s10343-019-00489-z

Ali B, Hayat S, Aiman Hasan S, Ahmad A (2008) A comparative effect of IAA and 4-Cl-IAA on growth, nodulation and nitrogen fixation in *Vigna radiata* (L.) Wilczek. Acta Physiol Plant 30:35–41. https://doi.org/10.1007/s11738-007-0088-4

Ali MM, Vora D (2014) Potential of Endophytic Bacteria Isolated from *Vitex negundo* L . to Produce Auxin. Res J Recent Sci 3:38–42

Alijani Z, Amini J, Ashengroph M, Bahramnejad B (2019) Antifungal activity of volatile compounds produced by *Staphylococcus sciuri* strain MarR44 and its potential for the biocontrol of *Colletotrichum nymphaeae* , causal agent strawberry anthracnose. Int J Food Microbiol 307:108276. https://doi.org/10.1016/j.ijfoodmicro.2019.108276

Alori ET, Glick BR, Babalola OO (2017) Microbial phosphorus solubilization and its potential for use in sustainable agriculture. Front Microbiol. https://doi.org/10.3389/fmicb.2017.00971

Amaresan N, Velusamy J, Thajuddin N (2014) Isolation and characterization of endophytic bacteria associated with chilli (*Capsicum annuum*) grown in coastal agricultural ecosystem. Indian J Biotechnol 13:247–255

Anjum N, Chandra R (2015) Endophytic bacteria : Optimizaton of isolation procedure from various medicinal plants and their preliminary characterization. Asian J Pharm Clin Res 8:233–238

Aswathy AJ, Jasim B, Jyothis M, Radhakrishnan EK (2013) Identification of two strains of *Paenibacillus* sp. as indole 3 acetic acid-producing rhizome-associated endophytic bacteria from Curcuma longa. 3 Biotech 3:219–224. https://doi.org/10.1007/s13205-012-0086-0

Aziz K, Nawaz M, Nazir J, et al (2015) Isolation, characterization and effect of auxin producing bacteria on growth of *Triticum aestivum*. J Anim Plant Sci 25:1003–

1007

Baldi BG, Maher BR, Slovin JP, Cohen JD (1991) Stable isotope labeling, *in vivo*, of D- and L-tryptophan pools in *Lemna gibba* and the low incorporation of label into indole-3-acetic acid. Plant Physiol 95:1203–1208. https://doi.org/10.1104/pp.95.4.1203

Bandara WMMS, Seneviratne G, Kulasooriya SA (2006) Interactions among endophytic bacteria and fungi: Effects and potentials. J Biosci 31:645–650. https://doi.org/10.1007/BF02708417

Barışçı S, Turkay O (2016) Optimization and modelling using the response surface methodology (RSM) for ciprofloxacin removal by electrocoagulation. Water Sci Technol 73:1673–1679. https://doi.org/10.2166/wst.2015.649

Bartel B (1997) Auxin Biosynthesis. Annu Rev Plant Physiol Plant Mol Biol 48:51–66. https://doi.org/10.1146/annurev.arplant.48.1.51

Bartel B, Fink GR (1995) ILR1, an amylohydrolase that releases active Indole-3-acetic acid from conjugates. Science (80-) 268:1745–1748. https://doi.org/10.1126/science.7792599

Beneduzi A, Ambrosini A, Passaglia LMP (2012) Plant growth-promoting rhizobacteria (PGPR): Their potential as antagonists and biocontrol agents. Genet Mol Biol 4:1044–1051

Bezerra MA, Santelli RE, Oliveira EP, et al (2008) Response surface methodology (RSM) as a tool for optimization in analytical chemistry. Talanta 76:965–977. https://doi.org/10.1016/j.talanta.2008.05.019

Bharucha U, Patel K, Trivedi UB (2013) Optimization of indole acetic acid production by *Pseudomonas putida* UB1 and its effect as plant growth-promoting rhizobacteria on Mustard (*Brassica nigra*). Agric Res 2:215–221. https://doi.org/10.1007/s40003-013-0065-7

Bialek K, Meudt WJ, Cohen JD (1983) Indole-3-acetic Acid (IAA) and IAA conjugates applied to bean stem sections: IAA content and the growth response. Plant Physiol 73:130–134. https://doi.org/10.1104/pp.73.1.130

Bloemberg G V., Lugtenberg BJJ (2001) Molecular basis of plant growth promotion and biocontrol by rhizobacteria. Curr Opin Plant Biol 4:343–350. https://doi.org/10.1016/S1369-5266(00)00183-7

Bode HB, Bethe B, Höfs R, Zeeck A (2002) Big effects from small changes: Possible ways to explore nature's chemical diversity. ChemBioChem 3:619–627. https://doi.org/10.1002/1439-7633(20020703)3:7<619::AID-CBIC619>3.0.CO;2-9

Bononi L, Chiaramonte JB, Pansa CC, et al (2020) Phosphorus-solubilizing *Trichoderma* spp . from Amazon soils improve soybean plant growth. Sci Rep 1–13. https://doi.org/10.1038/s41598-020-59793-8

Brzonkalik K, Hümmer D, Syldatk C, Neumann A (2012) Influence of pH and carbon to nitrogen ratio on mycotoxin production by *Alternaria alternata* in submerged cultivation. AMB Express. https://doi.org/10.1186/2191-0855-2-28

Bunch AW, Harris RE (1986) The manipulation of micro-organisms for the production of secondary metabolites. Biotechnol. Genet. Eng. Rev. 4:117–144

Bunsangiam S, Sakpuntoon V, Srisuk N, et al (2019) Biosynthetic pathway of indole-3-acetic acid in basidiomycetous yeast *Rhodosporidiobolus fluvialis*. Mycobiology 47:292–300. https://doi.org/10.1080/12298093.2019.1638672

Cao X, Yang H, Shang C, et al (2019) The roles of auxin biosynthesis *YUCCA* gene family in plants. Int J Mol Sci 20:8–10. https://doi.org/10.3390/ijms20246343

Capobianchi MR, Giombini E, Rozera G (2013) Next-generation sequencing technology in clinical virology. Clin Microbiol Infect 19:15–22. https://doi.org/10.1111/1469-0691.12056

Caulier S, Nannan C, Gillis A, Licciardi F (2019) Overview of the antimicrobial compounds produced by members of the *Bacillus subtilis* group. 10:1–19. https://doi.org/10.3389/fmicb.2019.00302

Cerboneschi M, Decorosi F, Biancalani C, et al (2016) Indole-3-acetic acid in plant–pathogen interactions: a key molecule for in planta bacterial virulence and fitness. Res Microbiol 167:774–787. https://doi.org/10.1016/j.resmic.2016.09.002

Chen XC, Bai JX, Cao JM, et al (2009) Medium optimization for the production of cyclic adenosine 3′,5′-monophosphate by *Microbacterium* sp. no. 205 using response surface methodology. Bioresour Technol 100:919–924. https://doi.org/10.1016/j.biortech.2008.07.062

Cheng CS (2006) Projection properties of factorial designs for factor screening. Screen Methods Exp Ind Drug Discov Genet 156–168. https://doi.org/10.1007/0-387-28014-6_7

Clarridge Jill E. (2004) Impact of 16S rRNA gene sequence analysis for identification of bacteria on Clinical Microbiology and Infectious Diseases. Clin Microbiol Rev 17:840–862. https://doi.org/10.1128/CMR.17.4.840

Cohen JD, Bandurski RS (1982) Chemistry and Physiology of the bound auxins. Ann Rev Plant Physlol 403–430. https://doi.org/10.1016/S1876-0813(08)60299-7

Comai L, Kosuge T (1980) Involvement of plasmid deoxyribonucleic acid in indoleacetic acid synthesis in *Pseudomonas savastanoi*. J Bacteriol 143:950–957. https://doi.org/10.1128/jb.143.2.950-957.1980

Cook SD, Nichols DS, Smith J, et al (2016) Auxin biosynthesis: Are the indole-3-acetic acid and phenylacetic acid biosynthesis pathways mirror images? Plant Physiol 171:1230–1241. https://doi.org/10.1104/pp.16.00454

Cook SD, Ross JJ (2016) The auxins, IAA and PAA, are synthesized by similar steps catalyzed by different enzymes. Plant Signal Behav 11:1–4. https://doi.org/10.1080/15592324.2016.1250993

Costacurta A, Keijers V, Vanderleyden J (1994) Molecular cloning and sequence analysis of an *Azospirillum brasilense* indole-3-pyruvate decarboxylase gene. Mol Gen Genet 243:463–472. https://doi.org/10.1007/BF00280477

Costacurta A, Vanderleyden J (1995) Synthesis of phytohormones by plant-associated bacteria. Crit Rev Microbiol 21:1–18. https://doi.org/10.3109/10408419509113531

Datta KS, Varma SK, Angrish R, et al (1997) Alleviation of salt stress by plant growth regulators in *Triticum aestivum* L. Biol. Plant. 40:269–275

Demain AL (2006) Overproduction of microbial metabolites and enzymes due to

alteration of regulation. Adv Biochem Eng Vol 1 I:113–142. https://doi.org/10.1007/bfb0044732

Demain AL (1986) Regulation of secondary metabolism in fungi. Pure Appl Chem 58:219–226. https://doi.org/10.1351/pac198658020219

Demain AL (1998) Induction of microbial secondary metabolism. Int Microbiol 1:259–264. https://doi.org/10.2436/im.v1i4.26

Deurenberg RH, Bathoorn E, Chlebowicz MA, et al (2017) Application of next generation sequencing in clinical microbiology and infection prevention. J Biotechnol 243:16–24. https://doi.org/10.1016/j.jbiotec.2016.12.022

Dinarvand M, Rezaee M, Masomian M, et al (2013) Effect of C/N ratio and media optimization through Response Surface Methodology on simultaneous productions of intra- and extracellular inulinase and invertase from *Aspergillus niger*. Biomed Res Int. https://doi.org/10.1155/2013/508968

Donoso R, Leiva-Novoa P, Zúñiga A, et al (2016) Biochemical and genetic basis of indole-3-acetic acid (auxin phytohormone) degradation by the plant growth promoting rhizobacterium *Paraburkholderia phytofirmans* PsJN. Appl Environ Microbiol. https://doi.org/10.1128/AEM.01991-16

Doull JL, Vining LC (1990) Nutritional control of actinorhodin production by *Streptomyces coelicolor* A3(2): suppressive effects of nitrogen and phosphate. Appl Microbiol Biotechnol 32:449–454. https://doi.org/10.1007/BF00903781

Drew SW, Demain AL (1977) Effect of primary metabolites on secondary metabolism. Annu Rev Microbiol 31:343–356. https://doi.org/10.1146/annurev.mi.31.100177.002015

Dubey MK, Meena M, Aamir M, et al (2019) Regulation and role of metal ions in secondary metabolite production by microorganisms. Elsevier B.V.

Duca D, Lorv J, Patten CL, et al (1996) Indole-3-acetic acid in plant – microbe interactions. Can J Microbiol. https://doi.org/10.1139/m96-032

Duca D, Rose DR, Glick BR (2014) Characterization of a nitrilase and a nitrile hydratase from *Pseudomonas* sp. strain UW4 that converts indole-3-acetonitrile to indole-3-acetic acid. Appl Environ Microbiol 80:4640–4649. https://doi.org/10.1128/AEM.00649-14

Durakovic B (2017) Design of experiments application, concepts, examples: State of the art. Period Eng Nat Sci 5:421–439. https://doi.org/10.21533/pen.v5i3.145

Dutta D, Puzari KC, Gogoi R, Dutta P (2014) Endophytes: Exploitation as a tool in plant protection. Brazilian Arch Biol Technol 57:621–629. https://doi.org/10.1590/S1516-8913201402043

Egamberdieva D, Ahmad P (2018) Plant Microbiome: Stress Response, Microorgan. Springer Nature Singapore Pte Ltd, Singapore

Erica de OA (2015) Rizobacteria in the control of pest insects in agriculture. African J Plant Sci 9:368–373. https://doi.org/10.5897/ajps2015.1318

Espeso EA, Tilburn J, Arst HN, Peñalva MA (1993) pH regulation is a major determinant in expression of a fungal penicillin biosynthetic gene. EMBO J 12:3947–3956. https://doi.org/10.1002/j.1460-2075.1993.tb06072.x

Failla LJ, Niehaus WG (1986) Regulation of Zn^{2+} uptake and versicolorin A synthesis in a mutant strain of *Aspergillus parasiticus*. Exp Mycol 10:35–41. https://doi.org/10.1016/0147-5975(86)90028-9

Figueiredo M do VB, Bonifacio A, Rodrigues AC, Araujo FF de (2016) Plant growth-promoting rhizobacteria: Key Mechanisms of Action. Microbial-Mediated Induc Syst Resist Plants 1–226. https://doi.org/10.1007/978-981-10-0388-2

Frank A, Saldierna Guzmán J, Shay J (2017) Transmission of bacterial endophytes. Microorganisms 5:70. https://doi.org/10.3390/microorganisms5040070

Frey DD, Engelhardt F, Greitzer EM (2003) A role for “one-factor-at-a-time” experimentation in parameter design. Res Eng Des 14:65–74. https://doi.org/10.1007/s00163-002-0026-9

Fu SF, Wei JY, Chen HW, et al (2015) Indole-3-acetic acid: A widespread physiological code in interactions of fungi with other organisms. Plant Signal Behav 10:. https://doi.org/10.1080/15592324.2015.1048052

Fukuda IM, Pinto CFF, Moreira CDS, et al (2018) Design of experiments (DoE) applied to pharmaceutical and analytical quality by design (QbD). Brazilian J Pharm Sci 54:1–16. https://doi.org/10.1590/s2175-97902018000001006

Gana, A S (2011) The role of synthetic growth hormones in crop multiplication and improvement. African J Biotechnol 10:10330–10334. https://doi.org/10.5897/AJB10.301

Ganeshan G, Kumar AM (2007) *Pseudomonas fluorescens*, a potential bacterial antagonist to control plant diseases. J Plant Interact 9145:123–134. https://doi.org/10.1080/17429140600907043

Gardiner DM, Osborne S, Kazan K, Manners JM (2009) Low pH regulates the production of deoxynivalenol by *Fusarium graminearum*. Microbiology 155:3149–3156. https://doi.org/10.1099/mic.0.029546-0

Garza DR, Dutilh BE (2015) From cultured to uncultured genome sequences : metagenomics and modeling microbial ecosystems. Cell Mol Life Sci 72:4287–4308. https://doi.org/10.1007/s00018-015-2004-1

Gaspar T, Kevers C, Faivre-Rampant O, et al (2003) Changing concepts in plant hormone action. Vitr Cell Dev Biol - Plant 39:85–106. https://doi.org/10.1079/IVP2002393

Gautam CK, Madhav M, Sinha A, Jabez Osborne W (2016) VIT-CMJ2: Endophyte of *Agaricus bisporus* in production of bioactive compounds. Iran J Biotechnol 14:19–24. https://doi.org/10.15171/ijb.1287

Giordano W, Avalos J, Cerda-Olmedo E, Domenech CE (1999) Nitrogen availability and production of bikaverin and gibberellins in *Gibberella fujikuroi*. FEMS Microbiol Lett 173:389–393. https://doi.org/10.1111/j.1574-6968.1999.tb13530.x

Glick BR (2012) Plant growth-promoting bacteria: Mechanisms and Applications. Scientifica (Cairo) 2012:. https://doi.org/http://dx.doi.org/10.6064/2012/963401

Gouda S, Das G, Sen SK, et al (2016) Endophytes: A treasure house of bioactive compounds of medicinal importance. Front Microbiol 7:1–8. https://doi.org/10.3389/fmicb.2016.01538

Gray WM (2004) Hormonal regulation of plant growth and development. PLoS Biol

2:. https://doi.org/10.1371/journal.pbio.0020311

Grossmann K (2010) Auxin herbicides: Current status of mechanism and mode of action. Pest Manag Sci 66:113–120. https://doi.org/10.1002/ps.1860

Gunatilaka AAL (2006) A.A.L. Gunatilaka. Natural products from plant-associated microorganisms: Distribution, structural diversity, bioactivity and implication of their occurrence. J.Net.Prod . 2006, 69: 509-526. J Nat Prod 69:509–526

Guo H, Glaeser SP, Alabid I, et al (2017) The abundance of endofungal bacterium *Rhizobium radiobacter* (syn. *Agrobacterium tumefaciens*) increases in its fungal host *Piriformospora indica* during the tripartite sebacinalean symbiosis with higher plants. Front Microbiol 8:1–13. https://doi.org/10.3389/fmicb.2017.00629

Halmann M (1990) Synthetic plant growth regulators. Adv Agron 43:47–105. https://doi.org/10.1016/S0065-2113(08)60476-9

Hasuty A, Choliq A, Hidayat I (2018) Production of indole acetic acid (IAA) by *Serratia marcescens* subsp. marcescens and rhodococcus aff. qingshengii. Int J Agric Technol 14:299–312

Herlina L, Pukan KK, Mustikaningtyas D (2017) The endophytic bacteria producing IAA (Indole Acetic Acid) in *Arachis hypogaea*. 1:31–35. https://doi.org/10.13057/cellbioldev/t010106

Herrero A, Muro-pastor AM, Flores E (2001) MINIREVIEW Nitrogen control in Cyanobacteria. 183:411–425. https://doi.org/10.1128/JB.183.2.411

Hobbs G, Frazer CM, Gardner DCJ, et al (1990) Pigmented antibiotic production by *Streptomyces coelicolor* A3(2): Kinetics and the influence of nutrients. J Gen Microbiol 136:2291–2296. https://doi.org/10.1099/00221287-136-11-2291

Huang J, Lu C, Qian X, et al (2011) Effect of salinity on the growth, biological activity and secondary metabolites of some marine fungi. Acta Oceanol Sin 30:118–123. https://doi.org/10.1007/s13131-011-0126-3

Hurst CJ, Crawford RL, Garland JL, et al (2016) Manual of environmental microbiology, Third edit. American Society for Microbiology Press, Washington, DC. 20036–2904, USA

Kalayu G (2019) Phosphate solubilizing microorganisms: Promising approach as biofertilizers. Int J Agron 2019:. https://doi.org/10.1155/2019/4917256

Kandel S, Joubert P, Doty S (2017) Bacterial endophyte colonization and distribution within plants. Microorganisms 5:77. https://doi.org/10.3390/microorganisms5040077

Kasahara H (2016) Current aspects of auxin biosynthesis in plants. Biosci Biotechnol Biochem 80:34–42. https://doi.org/10.1080/09168451.2015.1086259

Keller NP, Nesbitt C, Sarr B, et al (1997) pH regulation of sterigmatocystin and aflatoxin biosynthesis in *Aspergillus* spp. Phytopathology 87:643–648. https://doi.org/10.1094/PHYTO.1997.87.6.643

Kochar M, Upadhyay A, Srivastava S (2011) Indole-3-acetic acid biosynthesis in the biocontrol strain *Pseudomonas fluorescens* Psd and plant growth regulation by hormone overexpression. Res Microbiol 162:426–435. https://doi.org/10.1016/j.resmic.2011.03.006

Kumar S, Khapre A (2017) Phytocapping technology for sustainable management of landfill sites, Environmen. Springer

Kunamneni A, Ellaiah P (2002) Response surface optimization of the critical medium components for the production of alkaline protease by a newly isolated *Bacillus* sp. J Pharm Pharm Sci 3:272–278

Kunkel BN, Harper CP (2018) The roles of auxin during interactions between bacterial plant pathogens and their hosts. J Exp Bot 69:245–254. https://doi.org/10.1093/jxb/erx447

Lacava PT, Li W, Araujo WL, et al (2007) The endophyte *Curtobacterium flaccumfaciens* reduces symptoms caused by xylella fastidiosa in *Catharanthus roseus*. Jounal Microbiol 45:388–393

Lalit MS (2002) Plant Growth and Development. Hormones and Environment. 155–168

LeClere S, Tellez R, Rampey RA, et al (2002) Characterization of a family of IAA-amino acid conjugate hydrolases from *Arabidopsis*. J Biol Chem 277:20446–20452. https://doi.org/10.1074/jbc.M111955200

Li M, Guo R, Yu F, et al (2018) Indole-3-acetic acid biosynthesis pathways in the plant-beneficial bacterium *Arthrobacter pascens* zz21. Int J Mol Sci 19:. https://doi.org/10.3390/ijms19020443

Lind AL, Smith TD, Saterlee T, et al (2016) Regulation of secondary metabolism by the velvet complex is temperature-responsive in *Aspergillus*. G3 Genes, Genomes, Genet 6:4023–4033. https://doi.org/10.1534/g3.116.033084

Ljung K, Hull AK, Kowalczyk M, et al (2002) Biosynthesis, conjugation, catabolism and homeostasis of indole-3-acetic acid in *Arabidopsis thaliana*. Plant Mol Biol 50:309–332. https://doi.org/10.1023/A:1016024017872

Lodewyckx C, Vangronsveld J, Porteous F, et al (2015) Critical reviews in plant sciences endophytic bacteria and their potential applications. 2689:37–41. https://doi.org/10.1080/0735-260291044377

López JLC, Pérez JAS, Sevilla JMF, et al (2003) Production of lovastatin by *Aspergillus terreus*: effects of the C:N ratio and the principal nutrients on growth and metabolite production. Enzyme Microb Technol 33:270–277. https://doi.org/10.1016/S0141-0229(03)00130-3

Lounès A, Lebrihi A, Benslimane C, et al (1996) Regulation of spiramycin synthesis in *Streptomyces ambofaciens*: Effects of glucose and inorganic phosphate. Appl Microbiol Biotechnol 45:204–211. https://doi.org/10.1007/s002530050671

Luchese RH, Harrigan WF (1993) Biosynthesis of aflatoxin-the role of nutritional factors. J Appied Bacteriol 5–14

Ludwig-Müller J (2011) Auxin conjugates: Their role for plant development and in the evolution of land plants. J Exp Bot 62:1757–1773. https://doi.org/10.1093/jxb/erq412

Machackova I, Zazimalova E, George EF (2008) Plant Growth Regulators: Auxins, their Analogues and Inhibitors, Plant Prop. Springer Netherlands, Netherlands

Maela PM, Serepa-dlamini MH (2019) Current understanding of bacterial endophytes , their diversity, colonization and their roles in promoting plant growth. Appl

Microbiol Open Access 5:1–12. https://doi.org/10.4172/2471-9315.1000157

Malik DK, Sindhu SS (2011) Production of indole acetic acid by *Pseudomonas* sp.: Effect of coinoculation with *Mesorhizobium* sp. Cicer on nodulation and plant growth of chickpea (*Cicer arietinum*). Physiol Mol Biol Plants 17:25–32. https://doi.org/10.1007/s12298-010-0041-7

Mano Y, Nemoto K (2012) The pathway of auxin biosynthesis in plants. J Exp Bot 63:2853–2872. https://doi.org/10.1093/jxb/ers091

Martín JF (2004) Phosphate control of the biosynthesis of antibiotics and other secondary metabolites is mediated by the PhoR-PhoP system: An unfinished story. 186:5197–5201. https://doi.org/10.1128/JB.186.16.5197

Martin JF, Demain AL (1980) Control of antibiotic biosynthesis. Microbiol Rev 44:230–251

Masclaux-Daubresse C, Daniel-Vedele F, Dechorgnat J, et al (2010) Nitrogen uptake, assimilation and remobilization in plants: Challenges for sustainable and productive agriculture. Ann Bot 105:1141–1157. https://doi.org/10.1093/aob/mcq028

Mashiguchi K, Tanaka K, Sakai T, et al (2011) The main auxin biosynthesis pathway in *Arabidopsis*. Pnas 108:18512–18517. https://doi.org/10.1073/pnas.1108434108/-/DCSupplemental.www.pnas.org/cgi/doi/10.1073/pnas.1108434108

Masuma R, Tanaka Y, Ōmura S, Tanaka H (1986) Production of nanaomycin and other antibiotics by phosphate-depressed fermentation using phosphate-trapping agents. J Antibiot (Tokyo) 39:1557–1564. https://doi.org/10.7164/antibiotics.39.1557

Miche L, Balandreau J (2001) Effects of rice seed surface sterilization with hypchlorite on iniculated *Burkholderia vietnamiesis*. Appl Environ Microbiol 67:3046–3052. https://doi.org/10.1128/AEM.67.7.3046-3052.2001

Mohite B (2013) Isolation and characterization of indole acetic acid (IAA) producing bacteria from rhizospheric soil and its effect on plant growth. J Soil Sci Plant Nutr 13:638–649. https://doi.org/10.4067/S0718-95162013005000051

Muzzamal H, Sarwar R, Sajid I, Hasnain S (2012) Isolation, identification and screening of endophytic bacteria antagonistic to biofilm formers. Pak J Zool 44:249–257

Nannipieri P, Giagnoni L, Landi L, Renella G (2011) Phosphorus in action, Phosphorus. Springer, New York

Nasrabadi MRN, Razavi SH (2010) Enhancement of canthaxanthin production from *Dietzia natronolimnaea* HS-1 in a fed-batch process using trace elements and statistical methods. Brazilian J Chem Eng 27:517–529. https://doi.org/10.1590/s0104-66322010000400003

Nemoto K, Hara M, Suzuki M, et al (2009) The *NtAMI1* gene functions in cell division of tobacco BY-2 cells in the presence of indole-3-acetamide. FEBS Lett 583:487–492. https://doi.org/10.1016/j.febslet.2008.12.049

Netzker T, Fischer J, Weber J, et al (2015) Microbial communication leading to the activation of silent fungal secondary metabolite gene clusters. Front Microbiol

6:1–13. https://doi.org/10.3389/fmicb.2015.00299

Nor NM, Mohamed MS, Loh TC, et al (2017) Comparative analyses on medium optimization using one-factor-at-a-time, response surface methodology, and artificial neural network for lysine – methionine biosynthesis by *Pediococcus pentosaceus* RF-1. Biotechnol Biotechnol Equip 5:935–947. https://doi.org/10.1080/13102818.2017.1335177

Normanly J, Cohen JD, Fink GR (1993) *Arabidopsis thaliana* auxotrophs reveal a tryptophan-independent biosynthetic pathway for indole-3-acetic acid. Proc Natl Acad Sci U S A 90:10355–10359. https://doi.org/10.1073/pnas.90.21.10355

Novák O, Hényková E, Sairanen I, et al (2012) Tissue-specific profiling of the *Arabidopsis thaliana* auxin metabolome. Plant J 72:523–536. https://doi.org/10.1111/j.1365-313X.2012.05085.x

Nutaratat P, Srisuk N, Arunrattiyakorn P, Limtong S (2016) Fed-batch fermentation of indole-3-acetic acid production in stirred tank fermenter by red yeast *Rhodosporidium paludigenum*. Biotechnol Bioprocess Eng 21:414–421. https://doi.org/10.1007/s12257-015-0819-0

Oberhansli T, Defago G, Haas D (1991) Indole-3-acetic acid (IAA) synthesis in the biocontrol strain CHA0 of *Pseudomonas fluorescens*: Role of tryptophan side chain oxidase. J Gen Microbiol 137:2273–2279. https://doi.org/10.1099/00221287-137-10-2273

Ohyama T (2010) Nitrogen as a major essential element of plants. Nitrogen Assim plants 1–17

Okada BK, Seyedsayamdost MR (2017) Antibiotic dialogues: Induction of silent biosynthetic gene clusters by exogenous small molecules. FEMS Microbiol Rev 41:19–33. https://doi.org/10.1093/femsre/fuw035

Olatunji D, Geelen D, Verstraeten I (2017) Control of endogenous auxin levels in plant root development. Int J Mol Sci 18:. https://doi.org/10.3390/ijms18122587

Palaniyandi SA, Yang SH, Zhang L, Suh JW (2013) Effects of Actinobacteria on plant disease suppression and growth promotion. Appl Microbiol Biotechnol 97:9621–9636. https://doi.org/10.1007/s00253-013-5206-1

Pan R, Bai X, Chen J, et al (2019) Exploring structural diversity of microbe secondary metabolites using OSMAC strategy: A literature review. Front Microbiol 10:1–20. https://doi.org/10.3389/fmicb.2019.00294

Panda BP, Ali M, Javed S (2007) Fermentation process optimization. Res. J. Microbiol. 2:201–208

Pařízková B, Pernisová M, Novák O (2017) What has been seen cannot be unseen-detecting auxin *in vivo*. Int J Mol Sci 18:1–26. https://doi.org/10.3390/ijms18122736

Park JY, Fox LK, Seo KS, et al (2011) Comparison of phenotypic and genotypic methods for the species identification of coagulase-negative staphylococcal isolates from bovine intramammary infections. Vet Microbiol 147:142–148. https://doi.org/10.1016/j.vetmic.2010.06.020

Patten CL, Glick BR (1996) Bacterial biosynthesis of indole-3-acetic acid. Can J Microbiol 42:207–220. https://doi.org/10.1139/m96-032

Patten CL, Glick BR (2002a) Role of *Pseudomonas putida* indoleacetic acid in development of the host plant root system. Appl Environ Microbiol 68:3795–801. https://doi.org/10.1128/AEM.68.8.3795

Patten CL, Glick BR (2002b) Regulation of indoleacetic acid production in *Pseudomonas putida* GR12-2 by tryptophan and the stationary-phase sigma factor RpoS. Can J Microbiol 48:635–642. https://doi.org/10.1139/w02-053

Peker N, Garcia-Croes S, Dijkhuizen B, et al (2019) A comparison of three different bioinformatics analyses of the 16S-23S rRNA encoding region for bacterial identification. Front Microbiol 10:1–13. https://doi.org/10.3389/fmicb.2019.00620

Petti CA (2007) Detection and identification of microorganisms by gene amplification and sequencing. Clin Infect Dis 44:1108–1114. https://doi.org/10.1086/512818

Petti CA, Polage CR, Schreckenberger P (2005) The role of 16S rRNA gene sequencing in identification. J Clin Microbiol 43:6123–6125. https://doi.org/10.1128/JCM.43.12.6123

Pettit RK (2011) Minireview Small-molecule elicitation of microbial secondary metabolites. 4:471–478. https://doi.org/10.1111/j.1751-7915.2010.00196.x

Pickens LB, Tang Y, Chooi Y-H (2011) Metabolic engineering for the production of natural products. Annu Rev Chem Biomol Eng 23:211–236. https://doi.org/10.1038/jid.2014.371

Plackett RL, Burman JP (1946) The design of optimum multifactorial experiments. Oxford Journals 33:305–325

Pollmann S, Düchting P, Weiler EW (2009) Tryptophan-dependent indole-3-acetic acid biosynthesis by “IAA-synthase” proceeds via indole-3-acetamide. Phytochemistry 70:523–531. https://doi.org/10.1016/j.phytochem.2009.01.021

Pollmann S, Neu D, Weiler EW (2003) Molecular cloning and characterization of an amidase from *Arabidopsis thaliana* capable of converting indole-3-acetamide into the plant growth hormone, indole-3- acetic acid. Phytochemistry 62:293–300

Poza-Carrión C, Jiménez-Vicente E, Navarro-Rodríguez M, et al (2014) Kinetics of *nif* gene expression in a nitrogen-fixing bacterium. J Bacteriol 196:595–603. https://doi.org/10.1128/JB.00942-13

Prinsen E, Costacurta A, Michiels K, et al (1993) *Azospirillum brasilence* indole-3-acetic acid biosynthesis: Evidence for a non-tryptophan dependent pathway. 609–615

Puri A, Padda KP, Chanway CP (2017) Beneficial effects of bacterial endophytes on forest tree species, Sustainabl. Springer International Publishing

Rajput KN, Patel KC, Trivedi UB (2016) Screening and selection of medium components for cyclodextrin glucanotransferase production by new alkaliphile *Microbacterium terrae* KNR 9 using Plackett-Burman Design. 2016:. https://doi.org/10.1155/2016/3584807

Rampey RA, LeClere2 S, Kowalczyk M, et al (2004) A family of auxin-conjugate hydrolases that contributes to free indole-3-acetic acid levels during *Arabidopsis* germination. Plant Physiol 135:978–988. https://doi.org/10.1104/pp.104.039677

Reilly CO, Turner PD (2003) The nitrilase family of CN hydrolysing enzymes – a comparative study. 1161–1174. https://doi.org/10.1046/j.1365-

2672.2003.02123.x

Rincón A, Priha O, Sotta B, et al (2003) Comparative effects of auxin transport inhibitors on rhizogenesis and mycorrhizal establishment of spruce seedlings inoculated with *Laccaria bicolor*. Tree Physiol 23:785–791. https://doi.org/10.1093/treephys/23.11.785

Romero-Rodríguez A, Rocha D, Ruiz-Villafán B, et al (2017) Carbon catabolite regulation in *Streptomyces*: new insights and lessons learned. World J Microbiol Biotechnol 33:0. https://doi.org/10.1007/s11274-017-2328-0

Rosenblueth M, Martínez-Romero E (2006) Bacterial endophytes and their interactions with hosts. Mol Plant-Microbe Interact 19:827–837. https://doi.org/10.1094/MPMI-19-0827

Rückert GVON, Giani A (2004) Effect of nitrate and ammonium on the growth and protein concentration of *Microcystis viridis* Lemmermann (Cyanobacteria). Rev Bras Bot 27:325–331

Ruiz B, Chávez A, Forero A, et al (2010) Production of microbial secondary metabolites: Regulation by the carbon source. Crit Rev Microbiol 36:146–167. https://doi.org/10.3109/10408410903489576

Ruiz Rosquete M, Barbez E, Kleine-Vehn J (2012) Cellular auxin homeostasis: Gatekeeping is housekeeping. Mol Plant 5:772–786. https://doi.org/10.1093/mp/ssr109

Ryan RP, Germaine K, Franks A, et al (2008) Bacterial endophytes: recent developments and applications. FEMS Microbiol Lett 278:1–9. https://doi.org/10.1111/j.1574-6968.2007.00918.x

Sánchez S, Chávez A, Forero A, et al (2010) Carbon source regulation of antibiotic production. J Antibiot (Tokyo) 63:442–459. https://doi.org/10.1038/ja.2010.78

Sanchez S, Demain AL (2002) Metabolic regulation of fermentation processes. Enzyme Microb Technol 31:895–906. https://doi.org/10.1016/S0141-0229(02)00172-2

Sánchez S, Paniagua L, Mateos RC, et al (1981) Nitrogen regulation of Penicillin G biosynthesis in *Penicillium Chrysogenum*. Ferment Prod 147–154. https://doi.org/10.1016/b978-0-08-025385-5.50031-6

Santi C, Bogusz D, Franche C (2015) Biological nitrogen fixation in non-legume plants. Ann Bot. https://doi.org/10.1093/aob/mct048

Santoyo G, Moreno-Hagelsieb G, del Carmen Orozco-Mosqueda M, Glick BR (2016) Plant growth-promoting bacterial endophytes. Microbiol Res 183:92–99. https://doi.org/10.1016/j.micres.2015.11.008

Sauer M, Robert S, Kleine-Vehn J (2013) Auxin: Simply complicated. J Exp Bot 64:2565–2577. https://doi.org/10.1093/jxb/ert139

Sentausa E, Fournier P (2013) Advantages and limitations of genomics in prokaryotic taxonomy. Clin Microbiol Infect 19:790–795. https://doi.org/10.1111/1469-0691.12181

Senthil N, Elangovan K, Rajkumar S, Bavya M (2010) Studies on indole acetic acid production and phosphate solubilization from forest soil bacteria. Biotechnology 4:3–6

Senthilraj R, Prasad GS, Janakiraman K (2016) Sequence-based identification of microbial contaminants in non-parenteral products. Brazilian J Pharm Sci 52:329–336. https://doi.org/10.1590/S1984-82502016000200011

Sharma B, Jha DK (2015) Role of nitrogen sources in regulation of fungal secondary metabolism. Fungal Biomol Sources, Appl Recent Dev 213–224. https://doi.org/10.1002/9781118958308.ch16

Sharon JA, Hathwaik LT, Glenn GM, et al (2016) Isolation of efficient phosphate solubilizing bacteria capable of enhancing tomato plant growth. J Soil Sci Plant Nutr 16:525–536

Shi T-Q, Peng H, Zeng S-Y, et al (2017) Microbial production of plant hormones: Opportunities and challenges. Bioengineered 8:124–128. https://doi.org/10.1080/21655979.2016.1212138

Singh DP, Singh BH, Prabha R (2016) Microbial Inoculants in Sustainable Agricultural Productivity. Springer.

Singh V, Haque S, Niwas R, et al (2017) Strategies for fermentation medium optimization: An in-depth review. Front Microbiol 7:. https://doi.org/10.3389/fmicb.2016.02087

Sitbon F, Östin A, Sundberg B, et al (1993) Conjugation of indole-3-acetic acid (IAA) in wild-type and IAA-overproducing transgenic tobacco plants, and identification of the main conjugates by frit-fast atom bombardment liquid chromatography-mass spectrometry. Plant Physiol 101:313–320. https://doi.org/10.1104/pp.101.1.313

Soni P, Singh M, Kamble AL, Banerjee UC (2007) Response surface optimization of the critical medium components for carbonyl reductase production by *Candida viswanathii* MTCC 5158. Bioresour Technol 98:829–833. https://doi.org/10.1016/j.biortech.2006.03.008

Spaepen S, Vanderleyden J (2011) Auxin and plant-microbe interactions. Cold Spring Harb Perspect Biol 3:1–13. https://doi.org/10.1101/cshperspect.a001438

Spaepen S, Vanderleyden J, Remans R (2007) Indole-3-acetic acid in microbial and microorganism-plant signaling. FEMS Microbiol Rev 31:425–448. https://doi.org/10.1111/j.1574-6976.2007.00072.x

Spížek J, Tichý P (1995) Some aspects of overproduction of secondary metabolites. Folia Microbiol (Praha) 40:43–50. https://doi.org/10.1007/BF02816527

Sugawara S, Mashiguchi K, Tanaka K, et al (2015) Distinct characteristics of indole-3-acetic acid and phenylacetic acid, two common auxins in plants. Plant Cell Physiol 56:1641–1654. https://doi.org/10.1093/pcp/pcv088

Suhandono S, Kusumawardhani MK, Aditiawati P (2016) Isolation and molecular identification of endophytic bacteria from rambutan fruits (Nephelium lappaceum L.) Cultivar Binjai. HAYATI J Biosci 23:39–44. https://doi.org/10.1016/j.hjb.2016.01.005

Syed MB, Seraman S, Rajendran A, Thangavelu V (2013) Optimization of compactin production by Plackett-Burman Method using *Penicillium citrinum*. Int J ChemTech Res 5:1393–1400

Teale WD, Paponov IA, Palme K (2006) Auxin in action: Signalling, transport and the

control of plant growth and development. Nat Rev Mol Cell Biol 7:847–859. https://doi.org/10.1038/nrm2020

Tenorio-salgado S, Tinoco R, Vazquez-duhalt R, et al (2013) Identification of volatile compounds produced by the bacterium *Burkholderia tropica* that inhibit the growth of fungal pathogens. Bioengineered 4:236–243. https://doi.org/http://dx.doi.org/10.4161/bioe.23808

Thi Nguyen HY, Tran GB (2018) Optimization of fermentation conditions and media for production of glucose isomerase from *Bacillus megaterium* using Response Surface Methodology. Scientifica (Cairo) 2018:. https://doi.org/10.1155/2018/6842843

Toldrá F (2008) Meat Biotechnology, Meat Biote. Springer Science + Business Media, LLC, New York

Tsavkelova EA, Klimova SY, Cherdyntseva TA, Netrusov AI (2006) Microbial producers of plant growth stimulators and their practical use: A review. Appl Biochem Microbiol 42:117–126. https://doi.org/10.1134/S0003683806020013

Tsugafune S, Mashiguchi K, Fukui K, et al (2017) Yucasin DF , a potent and persistent inhibitor of auxin biosynthesis in plants. Sci Rep 1–13. https://doi.org/10.1038/s41598-017-14332-w

Tudzynski B (2014) Nitrogen regulation of fungal secondary metabolism in fungi. Front Microbiol 5:1–15. https://doi.org/10.3389/fmicb.2014.00656

Varma A, C. Kharkwal A (2009) Symbiotic Fungi, Principles and practice. Springer-Verlag Berlin Heidelberg

Vetrovsky T, Baldrian P (2013) The variability of the 16S rRNA gene in bacterial genomes and its consequences for bacterial community analyses. 8:1–10. https://doi.org/10.1371/journal.pone.0057923

Wade W (2002) Unculturable bacteria - the uncharacterized organisms that cause oral infections. J R Soc Med 95:81–83

Wanderley CDS, Faria RT de, Ventura MU, Vendrame W (2014) The effect of plant growth regulators on height control in potted *Arundina graminifolia* orchids (Growth regulators in *Arundina graminifolia*). Acta Sci Agron 36:489. https://doi.org/10.4025/actasciagron.v36i4.18085

Wang Y, Lu Z, Sun K, Zhu W (2011) Effects of high salt stress on secondary metabolite production in the marine-derived fungus *Spicaria elegans*. Mar Drugs 9:535–542. https://doi.org/10.3390/md9040535

Weinberg ED (1969) Biosynthesis of secondary metabolites: Roles of trace metals. Adv Microb Physiol 4:1–44. https://doi.org/10.1016/S0065-2911(08)60438-5

Weyens N, van der Lelie D, Taghavi S, Vangronsveld J (2009) Phytoremediation: plant-endophyte partnerships take the challenge. Curr Opin Biotechnol 20:248–254. https://doi.org/10.1016/j.copbio.2009.02.012

Wilson D (2020) Endophyte: the evolution of a term, and clarification of its use and definition. Nord Soc Oikos 73:274–276

Woo PCY, Lau SKP, Teng JLL, et al (2008) Then and now: Use of 16S rDNA gene sequencing for bacterial identification and discovery of novel bacteria in clinical microbiology laboratories. Clin Microbiol Infect 14:908–934.

https://doi.org/10.1111/j.1469-0691.2008.02070.x

Wright AD, Sampson MB, Neuffer MG, et al (1991) Indole-3-acetic acid biosynthesis in the mutant maize orange pericarp, a tryptophan auxotroph. Science (80-) 254:998–1000. https://doi.org/10.1126/science.254.5034.998

Yadavalli R, Rao R, Rao CS (2013) Response Surface Methodological Approach to optimize process parameters for the biomass production of *Chlorella pyrenoidosa*. Int J Bio-Technology 3:37–48

Yamada T, Palm CJ, Brooks B, Kosuge T (1985) Nucleotide sequences of the *Pseudomonas savastanoi* indoleacetic acid genes show homology with *Agrobacterium tumefaciens* T-DNA. Proc Natl Acad Sci U S A 82:6522–6526. https://doi.org/10.1073/pnas.82.19.6522

Yamakawa T, Kurahashi O, Ishida K, et al (1979) Stability of indole-3-acetic acid to autoclaving, aeration and light illumination. Agric Biol Chem 43:879–880. https://doi.org/10.1080/00021369.1979.10863551

Yang S, Zhang Q, Guo J, et al (2007) Global effect of indole-3-acetic acid biosynthesis on multiple virulence factors of *Erwinia chrysanthemi* 3937. Appl Environ Microbiol 73:1079–1088. https://doi.org/10.1128/AEM.01770-06

Zazimalová E, Petrasek J, Benková E (2014) Auxin and its role in plant development. Auxin Its Role Plant Dev 1–444. https://doi.org/10.1007/978-3-7091-1526-8

Zhang CH, Ma YJ, Yang FX, et al (2009) Optimization of medium composition for butyric acid production by *Clostridium thermobutyricum* using Response Surface Methodology. Bioresour Technol 100:4284–4288. https://doi.org/10.1016/j.biortech.2009.03.022

Zhao Y (2010) Auxin biosynthesis and its role in plant development. Annu Rev Plant Biol 61:49–64. https://doi.org/10.1146/annurev-arplant-042809-112308.Auxin

Zhao Y (2014) Auxin Biosynthesis. Arab B 12:e0173. https://doi.org/10.1199/tab.0173

Zhao Y (2012) Auxin biosynthesis: A simple two-step pathway converts tryptophan to indole-3-acetic acid in plants. Mol Plant 5:334–338. https://doi.org/10.1093/mp/ssr104

Zhao Y, Hull AK, Gupta NR, et al (2002) Trp-dependent auxin biosynthesis in *Arabidopsis* : involvement of cytochrome P450s CYP79B2 and CYP79B3. 3100–3112. https://doi.org/10.1101/gad.1035402.5

Zhu F, Qu L, Hong X, Sun X (2011) Isolation and characterization of a phosphate-solubilizing halophilic bacterium *Kushneria* sp . YCWA18 from Daqiao Saltern on the Coast of Yellow Sea of China. Evidence-based Complement Altern Med 2011:. https://doi.org/10.1155/2011/615032

3 ISOLATION OF ENDOPHYTIC BACTERIA AND SELECTION OF BETTER IAA PRODUCERS

3.1 MATERIAL AND METHODS

3.1.1 Mushroom collection

The wild mushrooms were collected at the Polytechnic Center (25°26'57.52"S, 49°13'53.12"W and 25°26'52.51"S, 49°13'1.99"W) and at Botanical Garden (25°26'49,19"S, 49°14'11,64"W and 25°26'49,09"S, 49°14'16,16"W) of the Federal University of Paraná Campus, municipality of Curitiba-PR, during period of September to November of 2017 and April to May of 2018. The collected mushrooms were taken to the Bioprocess Engineering Laboratory – CENBAPAR (25°26'56,08"S, 49°14'04,52"W) for isolation of endophytic bacteria.

3.1.2 Mushroom surface sterilization

Mushrooms were surface sterilized following the method described by Gautam et al. (2016) with slight modifications. Mushrooms were first washed with tap water to remove dust and adhering soil residues. Then, mushrooms were immediately surface sterilized by rinsining it in 70% EtOH for 1min, 2.5% NaClO for 2min, 70% EtOH for 30s, and washing three times in autoclaved dH_2O. Following, the mushrooms were surface dried with sterile paper towels.

3.1.3 Assessment of surface sterilization

In order to check the effectiveness of the disinfection process, two drops of the last rinse in autoclaved dH_2O was inoculated in Petri dishes containing nutrient agar (NA) medium. The Petri dish was sealed with parafilm paper and taked to incubation in darkness, at 30^{o}C for 48h. The absence of bacterial growth on the Petri dish means that the surface sterilization was effective.

3.1.4 Isolation of endophytic bacteria

In a laminar air flow cabinet, inner portions of surface sterilized mushrooms were cut in 1x1x1cm pieces with a sterile scalpel. Then, three pieces were immediately inoculated separately in the same Petri dish containing NA medium and sealed using parafilm tape; they were also inoculated in a tube containing nutrient broth (NB) medium, then taken to incubation in darkness, at 30ºC in order to recover the maximum possible bacterial endophytes. After 48h of incubation, the endophytic bacteria cultivated in NB medium were subcultured in NA medium. After all, morphologically different bacterial colonies were selected and repeatedly streaked on NA medium until pure cultures were obtained. The pure bacterial colonies were then characterized based on morphology (e.g. color), Gram´s staining, oxygen requirements, and biochemical testing following protocols described by Johnson and Case (2014). All isolated endophytic bacteria were stored in 20% glycerol at -20ºC, for other subsequent experiments.

3.1.5 Screening of isolates for IAA production

Bacterial colonies were aseptically inoculated in 10mL NB medium, then incubated in darkness, at 30ºC for 48h. After incubation, 120µL of cell suspension was transferred to sterile conical tube (15mL) containing 5mL of medium for IAA production (TABLE 1), pH adjusted to 7.0, then taken to incubation on shaker at 120rpm, 30ºC for 16h. Ended the incubation period, an aliquot of 250µL was aseptically inoculated in 150mL Erlenmeyer flask containing 25mL of medium for IAA production supplemented with 0.5mg/mL L-tryptophan. The Erlenmeyer flask was then incubated on shaker at 120rpm, 30ºC for 5 days. The experiments were performed in triplicate.

The production of IAA was qualitatively and quantitatively determined by colorimetric method using Salkowski's reagent (Gordon and Weber 1950). After fermentation, 2mL aliquot of each sample was taken to centrifugation at 3500rpm for 10min. In a clean tube was mixed 1mL of culture supernatant with 2mL of Salkowski's reagent (50mL Perchloric acid 35% and 0.5mL of $FeCl_3$ 0.5M). The mixture was then kept on darkness for 30min at room temperature. After 30min, the development of pink color was considered as positive for IAA production. The

intensity of color development is proportional to IAA production, and hence the resulting colors were qualitatively rated as following: (-) No IAA production, (+) Low IAA production, (++) Medium IAA production, and (+++) High IAA production. The samples that exhibited color intensity qualitatively classified as medium and high IAA production were further quantified by measuring absorbance at 530nm in spectrophotometer. The IAA concentration was determined from authentic IAA standard curve.

TABLE 1 – Medium for IAA production

Medium components	Concentration
K_2HPO_4	1.0 g/L
$MgSO_4.7H_2O$	0.2 g/L
NaCl	1.0 g/L
$CaCl_2.6H_2O$	0.06 g/L
Glycerol	48.34 g/L
Corn steep liquor	9.09 g/L

3.1.6 Phosphate solubilization

All isolates were qualitatively assessed for phosphate solubilizing activity according to the method described by Nautiyal (1999). Brifly, bacterial culture (10µL), previously grown in NB medium, was inoculated in the middle of Petri dishes containing National Botanical Research Institute's Phosphate (NBRIP) solid medium, and then incubated in darkness, at 30^{o}C for 7 days. The ability to solubilize phosphate was detected by the formation of clear halo around bacterial colony and qualitatively rated as (+), whereas the lack of formation of halo around bacterial colony was classified as (-).

3.1.7 Nitrogen fixation activity

All isolates were tested for nitrogen fixation activity through the methodology described by Goswami et al. (2015). Bacterial colony was aseptically inoculated in a tube with 8mL of nitrogen-free bromothymol blue (NfB) medium, then incubated at 30^{o}C for 4 days. Color change, from green to blue, was a positive indicator for nitrogen fixation activity and qualitatively classified as (+) whereas the lack of change of color was classified as (-) which means no nitrogen fixation activity.

3.2 RESULTS AND DISCUSSION

In total, seventy-one (71) endophytic bacteria with different morphologies, oxygen requirements, and biochemical characteristics were isolated from surface sterilized mushrooms (TABLE 2). In agreement with our result, Ramalashmi et al. (2018) isolated a high number of endophytic bacteria with the use of NA medium. Likewise, Anjum and Chandra (2015) reported successful isolation of about 35 endophytic bacteria from four medicinal plants using NA medium.

This study demonstrated the occurrence and diversity of culturable endophyte bacteria from wild mushrooms (FIGURE 3), however the recovering of these microorganisms depended mainly on the isolation media and surface sterilization. In the present study, NA and NB media have been used as an initial strategy for recovering endophytic bacteria from mushrooms. NB has the same formulation as NA, the only difference is that the NB medium is liquid (does not contain agar for solidification). The strategy of using both liquid and solid/semi-solid media for initial growth is important because certain bacterial strains might have their growth inhibited by oxygen. Pure cultures of endophytic bacteria were obtained by streaking on NA medium, since NA medium allows separation of bacterial colonies (visible to the naked eye). This is not the first study to report the isolation of endophytic bacteria from mushrooms. Gautam et al. (2016) reported the isolation endophytic bacteria *Enterobacter* sp. from edible mushroom *Agaricus bisporus*, with ability to produce bioactive compounds.

The protocol used for sterilization of mushrooms was found effective, as confirmed by the absence of any colony growth on the NA plates where aliquots of dH_2O used for final rinse of mushroom surface were inoculated. Therefore, bacterial isolates obtained from sterilized mushrooms can be considered endophytic. Moreover, the huge number of endophytic bacteria isolated from mushrooms means that the surface sterilization protocol used was effective at eliminating the epiphytic microorganisms, without affecting or affecting in minimal magnitude the endophytic microorganisms.

The IAA production capacity was used as the main characteristic for the selection of endophytic bacteria capable of promoting plant growth. Based on qualitative analysis, eight (8) endophytic bacteria were classified as medium (++) and high (+++) producers of IAA (TABLE 2). Quantitative analysis of these 8 endophytic bacteria

revealed that only two (2) isolates, namely *Cog placa 12ST1* and *Cog tubo umido 2*, were the efficient producers of IAA (124–140 µg/mL), and this production of IAA is equal to or higher than many other bacteria previously reported by many authors (TABLE 3).

Endophytic bacteria may exhibit multiple traits for promotion of plant growth. The results of this study revealed that some isolated endophytic bacteria, in addition to IAA production capability, also have other mechanisms for plant growth promotion such as phosphate solubilization and nitrogen fixation activity (FIGURE 4). Approximately 35% were able to solubiblize phosphate, and 27% exhibited ability to fix nitrogen (TABLE 2).

FIGURE 3 – Some wild mushrooms collected. Names are coded.

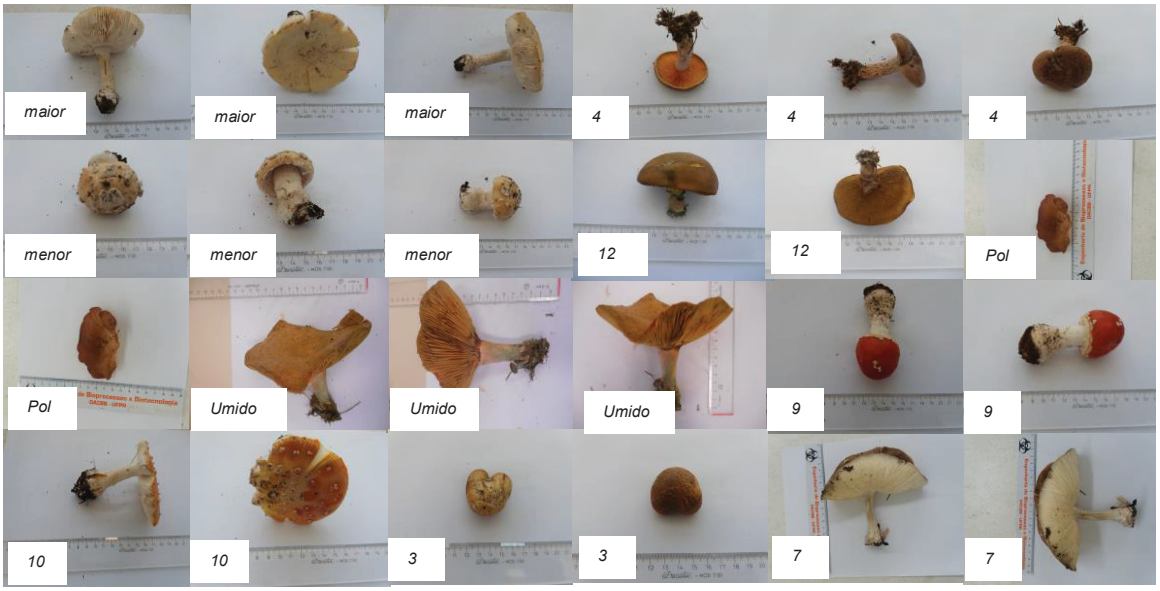


FIGURE 4 – a) Phosphate solubilization activity shown by formation of clear zone around bacterial growth. b) N_2 fixation activity detected by color changing of culture.

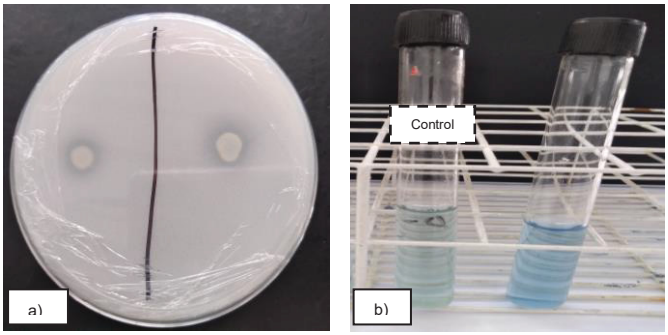


TABLE 2 – Morphology, oxygen and biochemical characteristics of isolates, along with IAA production and other PGP traits

				Morphological Characteristics			Biochemical Characteristics				Oxygen requirements				IAA production		Other PGP traits	
Mushroom Code	Isolation tissue	Initial growth media	Isolate code	Colony color	Gram staining	Cell shape	Fermentation of sugars	H_2S production	Indol	Motility	Obligate aerobic	Obrigate Anaerobic	Facultative anaerobic	Microaerophile	Qualitative	Quantitative (µg/mL)	Phosphate solubilization	N_2 Fixation
1	Stem	NA	*Calo placa 1-1*	white	+ve	Bacillus	-	-	-	-	X				-		-	-
			Calo placa 1-2	white	-ve	Coccus	-	-	-	-			X		-		-	-
			Calo placa1-3	white	-ve	Coccus	-	-	-	-			X		-		+	-
		NB	*Calo tubo 1-1*	yellow	-ve	Coccus	-	-	-	-			X		-		-	+
			Calo tubo 1-2	white	-ve	Diplococcus	-	-	-	-			X		-		+	-
3	Cap	NA	*Cog placa 3-1*	yellow	- ve	Streptococcus	-	-	-	-		X			+++	89.9± 8	-	-
			Cog placa 3-2	yellow	+ve	Coccus	Glu/Lac/Suc	-	-	+			X		-		-	-
		NB	*Cog tubo 3-1*	yellow	+ve	Coccus	-	-	-	-		X			-		+	-
4	Cap	NA	*Calo placa 4-1*	cream	-ve	Coccus	-	-	-	-		X			-		-	+
			Calo placa 4-2	yellow	-ve	Bacillus	-	-	-	-			X		+		-	-
			Calo placa 4-2A	cream	+ve	Coccus	-	-	-	-		X			-		-	+
			Calo placa 4-3	white	+ve	Cocccus	Glu	-	-	-		X			+		-	-
6	Stem	NA	*Calo placa 6-1*	yellow	-ve	Coccus	-	-	-	-		X			-		-	-
		NB	*Calo tubo 6-1*	white	+ve	Coccus	Glu	-	-	-	X				-		-	-
	Cap	NA	*Cog placa 6-1*	yellow	+ve	Bacillus	Glu	-	-	-		X			-		-	+
			Cog placa 6-2	white	+ve	Bacillus	-	-	-	-			X		-		-	-
7	Stem	NB	*Calo tubo 7-1*	cream	+ve	Coccus	Glu	-	-	+			X		++	29±11	-	-
		NA	*Calo placa7-1*	cream	+ve	Coccus	Glu	-	-	+			X		-		+	-
9	Stem	NA	*Calo placa 9-1*	yellow	+ve	Diplococcus	-	-	-	-			X		-		-	+
			Calo placa 9-2	yellow	+ve	Coccus	-	-	-	-				X	-		+	-
		NB	*Calo tubo 9-1*	yellow	-ve	Coccus	Glu/Lac/Suc	-	-	+			X		-		+	-
10	Stem	NB	*Calo tubo 10-1A*	yellow	+ve	Diplococcus	Glu/Lac/Suc	-	+	+			X		++	21±1	+	-
			Calo tubo 10-1	cream	+ve	Coccus	Glu	-	-	+				X	+++	96±25	+	-
		NA	*Calo placa 10-2*	cream	+ve	Bacillus	Glu/Lac/Suc	-	-	+	X				-		+	+
			Calo placa 10-3	cream	+ve	Coccus	-	-	-	-			X		-		-	+
			Calo placa 10-3A	transparent	-ve	Bacillus	-	-	-	-			X		-		-	-
11	Cap	NA	*Cog placa 11-1*	white	+ve	Bacillus	-	-	-	+	X				+++	68± 31	-	+
		NB	*Cog tubo 11-1*	white	+ve	Bacillus	Gluc	-	-	+			X		+		-	+

			Cog tubo 11-1A	transparent	-ve	Coccus	-	-	-	-		X			-		-	-
12	Cap	NA	*Cog placa 12ST1*	white	-ve	Diplococus	Glu	-	-	+			X		+++	139±66	+	+
			Cog placa 12ST2	cream	+ve	Bacillus	Glu	-	-	+			X		++	8±2	-	-
Maior	Cap	NB	*Cog tubo maior-1*	yellow	-ve	Bacillus	-	-	+	-			X		-		+	-
			Cog tubo maior-1A	transparent	+ve	Diplococcus	-	-	+	-			X		-		+	-
			Cog tubo maior-2	cream	+ve	Bacillus	Glu/Lac/Suc	-	-	-			X		-		-	-
			Cog tubo maior-3	yellow	-ve	Coccus	-	-	+	+			X		-		+	-
	Stem	NB	*Calo tubo maior-3A*	yellow	-ve	Bacillus	-	-	+	-			X		-		+	-
Menor	Cap	NB	*Cog tubo menor-1*	white	+ve	Bacillus	-	-	-	-	X				-		-	-
			Cog tubo menor-1A	yellow	+ve	Bacillus	-	-	-	-				X	-		-	-
		NA	*Cog placa menor -1*	cream	+ve	Coccus	-	-	-	-	X				-		+	-
Grande	Cap	NA	*Cog placa grande -1*	yellow	-ve	Bacillus	-	-	-	-			X				-	-
		NB	*Cog tubo grande -1*	transparent	+ve	Coccus	Glu/Lac/Suc	-	+	+			X		-		+	-
			Cog tubo grande -1A	transparent	+ve	Coccus	Glu/Lac/Suc	-	+	+			X		-		-	-
			Cog tubo grande -2	white	+ve	Bacillus	Glu/Lac/Suc	-	-	+				X	+		-	-
			Cog tubo grande -2A	white	-ve	Coccus	Glu	-	+	+			X				-	-
			Cog tubo grande -3	white	+ve	Bacillus	-	-	-	-			X		-		-	-
			Cog tubo grande -3A	yellow	+ve	Bacillus	-	-	-	-			X		-		-	-
Pol	Cap	NA	*Cog placa pol-1*	yellow	+ve	Coccus	Glu	-	+	+			X		+		+	-
	Cap	NB	*Cog tubo pol-1*	cream	- ve	Bacillus	-	-	-	-			X		-		-	-
Umido	Cap	NA	*Cog placa umido -1*	yellow	+ve	Coccus	Glu/Lac/Suc	-	+	+			X		-		-	+
	Cap	NB	*Cog tubo umido 1*	cream	-ve	Bacillus	Glu/Lac/Suc	-	-	-			X		-		-	-
			Cog tubo umido 2	cream	-ve	Bacillus	Glu/Lac/Suc	-	+	+			X		+++	124±47	+	+
			Cog tubo umido 3	yellow	+ve	Coccus		-	-	+			X		+		-	-
			Cog tubo umido 3A	yellow	-ve	Coccus	Glu/Lac/Suc	-	+	+			X		-		+	-
			Cog tubo umido 4	white	+ve	Coccus	Glu	-	-	+			X		-		+	-
			Cog tubo umido 4A	white	-ve	Coccus	Glu/Lac/Suc	-	+	+			X		+		+	+
A	Cap	NA	*Cog placa A-1*	yellow	+ve	Coccus	Glu	-	-	+			X		+		+	-
			Cog placa A-2	yellow	+ve	Coccus	-	-	-	-			X		+		+	-
			Cog placa A-3	cream	+ve	Bacillus	Glu	-	-	-			X		+		+	-
			Cog placa A-4	white	+ve	Bacillus	Glu	-	-	-		X			+		-	-
B	Cap	NA	*Cog placa B-1*	cream	+ve	Coccus	-	-	-	-			X		-		-	+
			Cog placa B-2	cream	+ve	Coccus	-	-	-	-			X		+		-	-
			Cog placa B-2A	transparent	+ve	Coccus	-	-	-	-			X		+		-	-
			Cog placa B-3	cream	+ve	Coccus	-	-	-	-			X				+	-
		NB	*Cog placa B-1A*	yellow	+ve	Bacillus	-	-	-	-				X	+		-	+
			Cog placa B-2A	white	+ve	Coccus	-	-	-	-				X	-		-	+
			Cog placa B-2	yellow	-ve	Coccus	-	-	-	-				X	+		-	+
			Cog placa B-3A	cream	-ve	Coccus	-	-	-	-			X		+		-	+
			Cog placa B-3	white	+ve	Bacillus	-	-	-	-			X		-		-	+

C	Cap	NA	*Cog placa C-1*	yellow	+ve	Bacillus	-	-	-	-	X				+		-	-
			Cog placa C-2	yellow	+ve	Coccus	-	-	-	-			X		+		-	-
			Cog placa C-3	cream	+ve	Bacillus	-	-	-	-			X		+		-	-

-, +, ++, +++, see Materials and Methods section; +ve means positive; –ve means negative; Glu – Glucose; Lac – Lactose; Suc – Sucrose

TABLE 3 – Bacterial strains producing IAA reported in the literature

Microoganisms	IAA (µg/mL)	Reference
Enterobacter cloacea H3	12.28	Widowati et al. (2019)
Enterobacter ludwigii	30	Shoebitz et al. (2009)
Enterobacter asburiae strain PS2	32	Ahemad and Khan (2010)
Bacterial isolate DPY-05	67.18 (optimized)	Dasri et al. (2014)
Enterobacter cloacae MSR1	112	Khalifa et al. (2016)
Pseudomonas aeruginosa	132	Sasirekha et al. (2012)

3.3 CONCLUSION

The study demonstrated that wild mushrooms harbor a variety of cultivable endophytic bacteria, with diverse plant growth promotion traits. Among seventy-one (71) endophytic bacterial strains isolated from wild mushrooms, 35% were able to solubilize phosphate, 27% were able to fix atmospheric nitrogen and 38% were able to produce IAA. Eight (8) endophytic bacteria were qualititatively classified as good IAA producers. Based on the quantitative analysis of the production of IAA, two (2) bacterial strains called *Cog placa 12ST1* and *Cog tubo umido 2* were considered efficient producers of IAA, therefore they were selected for the study of medium and conditions optimization for IAA production.

In the later study, the strains *Cog placa 12ST1* and *Cog tubo umido 2* were identified by molecular technique as *Enterobacter* sp. and *Providencia* sp., respectively.

REFERENCES

Ahemad M, Khan MS (2010) Plant growth promoting activities of phosphate-solubilizing *Enterobacter asburiae* as influenced by fungicides. EurAsian J Biosci 88–95. https://doi.org/10.5053/ejobios.2010.4.0.11

Anjum N, Chandra R (2015) Endophytic bacteria: Optimizaton of isolation procedure from various medicinal plants and their preliminary characterization. Asian J Pharm Clin Res 8: 233–238

Dasri K, Kaewharn J, Kanso S, Sangchanjiradet S (2014) Optimization of indole-3-acetic acid (IAA) production by Rhizobacteria isolated from epiphytic orchids. KKU Res J 19:268–275

Gautam CK, Madhav M, Sinha A, Jabez Osborne W (2016) VIT-CMJ2: Endophyte of *Agaricus bisporus* in production of bioactive compounds. Iran J Biotechnol 14:19–24. https://doi.org/10.15171/ijb.1287

Gordon SA, Weber RP (1950) Colorimetric Estimation of Indoleacetic Acid. 192–195

Goswami D, Parmar S, Vaghela H, et al (2015) Describing *Paenibacillus mucilaginosus* strain N3 as an efficient plant growth promoting rhizobacteria (PGPR). Cogent Food Agric 163:1–13. https://doi.org/10.1080/23311932.2014.1000714

Johnson TR, Case CL (2014) Laboratory Experiments in Microbiology, Tenth Edit. Pearson, Harlow

Khalifa AYZ, Saleh FA (2016) Characterization of the plant growth promoting bacterium , *Enterobacter cloacae* MSR1, isolated from roots of non-nodulating *Medicago sativa*. Saudi J Biol Sci 23:79–86.

https://doi.org/10.1016/j.sjbs.2015.06.008

Ramalashmi K, Prasanna VK, Magesh K, et al (2018) A potential surface sterilization technique and culture media for the isolation of endophytic bacteria from *Acalypha indica* and its antibacterial activity. J Med Plants Stud 6:181–184

Sasirekha B, Shivakumar S, Sullia SB (2012) Statistical optimization for improved indole-3-acetic acid (iaa) production by *Pseudomonas aeruginosa* and demonstration of enhanced plant growth promotion. J Soil Sci Plant Nutr 12:863–873. https://doi.org/10.4067/s0718-95162012005000038

Shoebitz M, Ribaudo CM, Pardo MA, et al (2009) Plant growth promoting properties of a strain of *Enterobacter ludwigii* isolated from Lolium perenne rhizosphere. Soil Biol Biochem 41:1768–1774. https://doi.org/10.1016/j.soilbio.2007.12.031

Nautiyal CS (1999) An efficient microbiological growth medium for screening phosphate solubilizing microorganisms. FEMS Microbiol Lett 170:265–270. https://doi.org/10.1016/S0378-1097(98)00555-2

Widowati T, Nuriyanah, Sukiman H (2019) Production of indole acetic acid by *Enterobacter cloacea* H3 isolated from Mungbean (Vigna radiata) and its potential supporting the growth of soybean seedling. IOP Conf Ser Earth Environ Sci 308. https://doi.org/10.1088/1755-1315/308/1/012040

4 ARTICLE 1: OPTIMIZED PRODUCTION OF INDOL-3-ACETIC ACID FOR AGRICULTURE APPLICATION BY A NOVEL *Enterobacter* sp. ISOLATED FROM WILD MUSHROOM *Boletus edulis*

Marcelino Lourenço Xavier[a], Susan Grace Karp[a], Carlos Ricardo Soccol[a] *

a. Federal University of Paraná, Department of Bioprocess Engineering and Biotechnology, Centro Politécnico, 81531-990, Curitiba, Paraná, Brazil

Manuscript formatted according to the guidelines of Planta (An International Journal of Plant Biology)

4.1 ABSTRACT

Strain *Enterobacter* sp. was isolated from fruiting body of a common edible mushroom *Boletus edulis* and identified using 16S rRNA. The presence of Enterobacteriaceae family within the fruiting body of *Boletus edulis* was confirmed by metagenomic analysis.
The ability to produce Indol-3-Acetic Acid (IAA) was assessed by colorimetric method and confirmed by HPLC analysis. The IAA production was optimized using classical "One-Factor-At-Time" method and statistical methods namely: Two Level Full Factorial Design, Plackett-Burman Design and Response Surface Methodology. Starch and yeast extract were found as the best carbon and nitrogen sources, respectively. The starch/yeast extract ratio for higher IAA production was 60/4.146 (g/L) or simply 14.47. Starch and L-tryptophan were the input factors found significant to the IAA production and their optimal concentration were 52.556 g/L and 15mg/mL, respectively. Under optimized concentrations of starch and L-tryptophan, *Enterobacter* sp. strain produced 1838.8±67.8 µg/mL of IAA, representing about 12-fold increase when compared to IAA produced under un-optimized fermentation medium. The effect of incubation time on IAA production revealed 168h as suitable, with horary IAA production of 13.8±0.9 µg/mL.h (2311.0±155.7 µg/mL).
According to *in vitro* studies, the strain *Enterobacter* sp. showed ability to solubilize phosphate, fix atmospheric N_2, produce NH_3, had antagonistic activity against *Gibberella* sp. and *Fusarium moniliforme*, and delayed sporulation of *Aspergillus niger*.
In vivo studies, bacterial suspension of the strain *Enterobacter* sp. containing IAA promoted higher germination rate and dry weight of soybean plant than bacterial suspension without IAA (control).

Keywords: endophytic bacteria; Indol-3-Acetic Acid; plant growth promotion traits; mushroom; metagenomic analysis

4.2 INTRODUCTION

According to United Nations (2015, 2019) reports, the current world population of 7.6 billion is projected to grow to around 8.5 billion in 2030, 9.7 billion in 2050, and 10.9 billion in 2100, and the demand for food and other agricultural products is anticipated to increase by 50% between 2012 and 2050.

During the last century, world agriculture experienced higher increase in crop yield, due to the increased use of chemical fertilizers, pesticides, and intensive irrigated farming system (Tilman et al. 2002; Mateo-Sagasta et al. 2017). This, in turn, led to substantial environmental problems such as water scarcities, depletion of soil quality, water and air pollution, and emergence of pathogens.

The negative effect of the intensive use of agrochemicals is now clearly visible, for example, the pesticide poisoning account for 1 million/year worldwide

death and chronic diseases (Aktar et al. 2009), lower agriculture yield due to degradation of soils (Kopittke et al. 2019), and huge losses of harvestable produce due to the emergence of resistant pathogens (Forget 1994). Therefore, the world agriculture is more likely to have lower growth rate in yields compared to the past. This calls to urgent actions such as adoption of strategy of sustainable agriculture, able to increase the crop production while preserving the environment.

The environmentally friendly approach suggested in this paper is the use of Plant Growth Promoting Bacteria (PGPB). PGPB are bacteria with ability to stimulate plant growth through production of phytohormone (auxin, cytokinin, and gibberellins), nutrient acquisition (nitrogen fixation, phosphate solubilization, and iron sequestration by siderophores), production of HCN which confer resistance against plant pathogens, and by production of enzyme 1-aminocyclopropane-1-carboxylate (ACC) which control the level of ethylene biosynthesis in response to biotic or abiotic stress (Khan et al. 2016a; Kandel et al. 2017). However, the most reported mechanism predominantly used to explain the positive effects of PGPB for plant growth promotion is their ability to produce auxin (Olanrewaju et al. 2017), being indole-3-acetic acid (IAA) the most relevant and common natural auxin, synthesized mainly through tryptophan dependent pathway (Spaepen and Vanderleyden 2011).

The IAA controls various plant physiological processes including cell division and elongation, tissue differentiation, root initiation and phototropic response (Kumla et al. 2020). Thus, IAA produced by PGPB, particularly endophyte bacteria has attracted a great deal of attention in scientific community. Many studies have reported growth enhancement by increasing plant height and/or biomass when plants were inoculated with endophyte bacteria capable of producing IAA (Shi et al. 2009; Xin et al. 2009; Khan et al. 2016b; Bhutani et al. 2018; Ribeiro et al. 2018).

Several endophyte bacteria, such as *Pseudomonas* (Chen et al. 2017; Tamošiūnė et al. 2018) *Stenotrophomonas*, *Bacillus*, *Pantoea*, *Serratia* (Etminani and Harighi 2018), *Enterobacter* (Macedo-Raygoza et al. 2019; Panigrahi et al. 2019; Silva et al. 2019) and *Arthrobacter* (Hernández-Soberano et al. 2020) have proved to have plant growth and biocontrol activities. However, member of genus *Bacillus* are the most used and preferred in agriculture due to their ability to produce endospores allowing them to survive in stress conditions such as hot climate and salinity (Torres et al. 2020).

Therefore, the present study was focused in bacterial Indole-3-Acetic Acid, rather than bacterial cell. The study aimed to optimize the Indole-3-Acetic Acid produced by strain *Enterobacter* sp*.* isolated from the fruiting body of wild mushroom *Boletus edulis.*

4.3 MATERIAL AND METHODS

4.3.1 Isolation

The wild mushroom morphologically identified as *Boletus edulis* (Porcino) was collected in Pinus Forest located in Federal University of Paraná Campus - Polytechnic Center, Brazil, in April of 2018. The endophyte bacteria was isolated from mushroom through surface sterilization method (Gautam et al. 2016). Mushroom was first washed with tap water to remove dust and adhering soil particles. Then, surface sterilized by a chemical disinfection treatment, which consisted on consecutive rinse in 70% EtOH for 1min, 2.5% NACIO for 2min, 70% EtOH for 30s, and three times in autoclaved dH_2O. Following, the inner part of mushroom was cut in blocks of $1cm^3$ with scalpel sterile blade then inoculated in Nutrient Agar (NA), and incubated for 24h, in darkness, at 30ºC. The bacteria colony was purified by repeated streaking on NA. The last dH_2O rinse was used as negative control to check the efficacy of disinfection treatment. The pure endophyte bacteria was then stored in 20% glycerol at -20ºC.

4.3.2 Bacterial DNA extraction

The extraction of gDNA was carried out following Cheng and Jiang (2006) methodology with slight modifications. Briefly, bacterial strain was grown in 10mL nutrient broth medium at 30ºC, in darkness, for 48h. The bacterial culture was then centrifuged at 5000rpm for 15min. The supernatant was discharged, and the pellet was washed two times with 400µL STE Buffer (100mM NaCl, 10mM Tris/HCl, 1mM EDTA, pH 8.0). The resulting pellet was suspended in 400µL TE Buffer (10mM Tris/HCl, 1mM EDTA, pH 8.0), then mixed by vortex with 200µL of saturated phenol. The mixture was centrifuged at 1300g for 5min at 4ºC, and then collected the upper aqueous phase (320µL) to a clean tube. In tube was added 80µL of TE Buffer and

200µL of chloroform, then centrifuged at 13000g for 5min at 4ºC.The aqueous phase (320µL) was collected and precipitated with 3 volumes of isopropanol, then centrifuged at 12000g for 20min at 4ºC. The upper aqueous phase (150µL) was collected to a clean tube and stored at -20ºC for the subsequent experiments.

4.3.3 Bacterial DNA amplification and sequencing

The bacterial 16S ribosomal DNA (rDNA) was amplified following Cheng and Jiang (2006) methodology with minor modifications. The primers used for DNA amplification were 27F (5'-AGAGTTTGATCCTGGCTCAG-3') and 1512R (5′-TACCTTGTTACGACTT-3′)(Lefebvre et al. 2010). The PCR reaction mixture consisted of 5µL of 1x PCR buffer, 1µL of $MgCl_2$ (50mM), 0.55µL of dNTPs mix (0.22mM), 0.5µL of 27F (0.2mM), 0.5µL of 1512R (0.2mM), 0.2µL of Taq DNA polymerase(1U), 1µL of template DNA (50ng/µL), and 16.25mL of Milli-Q water to a final volume of 25µL. The amplification reaction was performed in Veriti Thermal Cycler (AB Applied Biosystems) under the following thermal cycling conditions: 5min at 95°C, 30 cycles (1min at 93°C, 1min at 50°C and 1min 30s at 72°C), followed by a final elongation cycle at 72°C for 5min. The PCR product was assessed on 0.7% agarose gel. The PCR product was purified and sequenced by ACTGene Análises Moleculares Ltda. (Alvorada, RS-Brazil), using own protocols.

4.3.4 Bioinformatic analysis

Sequences were analyzed and edited using sequence alignment editor software (BioEdit 7.2) and aligned through CLUSTALW using MEGA version X (Kumar et al. 2018). The consensus sequence was used to identifying the highly similar sequences at nucleotide database collection of NCBI (National Center of Biotechnology Information) using BLAST search online program. The closely related sequences obtained were aligned through MAFFT version 7 online program (Katoh et al. 2018). The phylogenetic tree was constructed using MEGA version X (Kumar et al. 2018). The evolutionary history was inferred by Maximum Likelihood method and Hasegawa-Kishino-Yano model with Gamma distribution rate (Hasegawa et al. 1985), and bootstrap consensus tree inferred from 1000 replicates.

4.3.5 Metagenomic analysis

The metagenomic analysis of *Boletus edulis* fruiting body was done by GoGenetic (Federal University of Paraná Campus - Polytechnic Center, Curitiba-Brazil) using the following protocol. Twenty nanograms of metagenomic DNA were used as a template for 18 cycles of amplification of the V4 region of the 16S rDNA of bacteria and archaea, using primers 515F/806R (Caporaso et al. 2012) and GoTaq Master Mix (Promega). The PCR product was analyzed by electrophoresis in 1.5% agarose and quantified using Qubit dsDNA HS kit (Invitrogen). The 10 pM mixture of the amplicons were sequenced in Illumina MiSeq apparatus using MiSeq Reagent 500V2 (Illumina), generating 250 base sequences. DNA sequences were analyzed through QIIME (Quantitative Insights Into Microbial Ecology) version 1.9.2 (Caporaso et al. 2010). The sequences were grouped into OTUs (Operational Taxonomic Units) considering 97% identity with database of 16S/18S SILVA 132 (Quast et al. 2013).

4.3.6 Fermentation

A colony of the isolated bacterial strain was aseptically inoculated in 10mL Nutrient Broth (NB) medium, then incubated in darkness, at 30°C for 48h. After incubation, 120µL of cell suspension was transferred to sterile conical tube (15mL) containing 5mL of un-optimized medium for IAA production (1.0 g/L K_2HPO_4, 0.2 g/L $MgSO_4.7H_2O$, 1.0 g/L NaCl, 0.06 g/L $CaCl_2.6H_2O$, 48.34 g/L glycerol, 9.09g/L corn steep liquor, pH 5.0), then taken to incubation on shaker at 120rpm, 30°C for 16h. Ended the incubation period, an aliquot of 250µL was aseptically inoculated in 150mL Erlenmeyer flask containing 25mL of un-optimized medium for IAA production supplemented with 0.5mg/mL L-tryptophan. The Erlenmeyer flask was then incubated on shaker at 120 rpm, 30°C for 5 days.

4.3.6.1 Quantification of IAA production by colorimetric method

The concentration of IAA was assessed by Salkowski´s method (Gordon and Weber 1950). After fermentation, 2mL aliquot was taken to centrifugation at 3500rpm for 10min. In a clean tube was mixed 1mL of culture supernatant with 2mL of Salkowski's reagent (50mL Perchloric acid 35% and 0.5mL of $FeCl_3$ 0.5M). The

mixture was then kept on darkness for 30min at room temperature. The absorbance was measured at 530nm in a spectrophotometer, and the IAA concentration was determined by comparison with the standard curve of authentic IAA.

4.3.6.2 High Performance Liquid Chromatography (HPLC)

The HPLC analysis was performed according to Sheikhian and Bina (2016) using Agilent technologies 1260 Infinity HPLC system (Varian Inc., Palo Alto, CA, USA). HPLC column Microsorb 60-8, C18, 21.4x250mm (Varian) was used as stationary phase and 1% water-Acetic Acid/methanol (50:50 v/v) as mobile phase. The elution rate was 0.5mL/min, and the UV wavelength detector was set at 283nm.

4.3.7 IAA optimization production

4.3.7.1 Screening of the C-sources by One-Factor-At-Time (OFAT)

Three C-sources (24.17g/L glucose, 24.17g/L mannitol, and 24.17g/L starch) were separately added in the un-optimized medium for IAA production replacing 48.34gL glycerol, while other medium components were kept constant. After fermentation, the IAA production was assessed by Salkowski´s method (Gordon and Weber 1950).

4.3.7.2 Screening of the N-sources by One-Factor-At-Time (OFAT)

The screening of C-sources by OFAT found starch as the best C-source, and hence starch replaced glycerol in the un-optimized medium for the screening of N-sources. Three (3) N-sources namely peptone (2.6906g/L), yeast extract (3.3633g/L) and urea (0.7214g/L), were separately added in the un-optimzed medium replacing corn steep liquor (9.09g/L), while the other medium components were kept constant. As positive control, the un-optimized medium was used, which had glycerol (48.34g/L) and corn steep liquor (9.09g/L) as C and N sources, respectively. The IAA production was quantified through Salkowski´s method (Gordon and Weber 1950).

4.3.7.3 Two-level full Factorial Design

The OFAT found starch and yeast extract as the best C and N sources, respectively. The effect of each variable and their interaction on the IAA production was assessed by 2^2 Factorial Design. The 2^2 Factorial Designs consisted on two factors and two levels (-1 and +1). For starch, the coded value for high level (+1) was 60g/L and for low level (-1) was 24.17g/L. The coded value for yeast extract was 4.146g/L for the high level (+1) and 1.68g/L for the low level (-1). Four (4) experiments were run in duplicate, as shown in TABLE 4.

TABLE 4 – 2^2 Factorial Design matrix, along with IAA production

Run order	Starch	Yeast extract	IAA production (µg/mL)
1	1	1	613
2	1	-1	522
3	-1	-1	399
4	-1	-1	396
5	-1	1	229
6	1	-1	486
7	1	1	610
8	-1	1	214

4.3.7.4 Plackett-Burman Design

The Plackett-Burman experimental design was developed using Minitab Statistical Software, to identify factors that make significant contribution to IAA production. The Plackett-Burman Design assumes that there is no interaction among factors, and is based on the first order polynomial model given by the following equation:

$$y=\beta o+\sum \beta i Xi \quad (1)$$

Where, y is the response, βo is the model intercept, βi is the variable estimates, and Xi is the independent factor level (Zeinab et al. 2015).

The Minitab Statistical Software set fourteen (14) runs to study three (3) variables at three different concentration levels namely: low (-1) and high (+1), and central level (0), and the corresponding coded values are shown in TABLE 5, and the experimental design matrix is shown in TABLE 6.

TABLE 5 – Coded values of the Plackett-Burman Design

Variables	Units	Levels		
		Low (-1)	0	High (+1)
L-tryptophan	mg/mL	0.4	1.2	2.4
Starch*	g/L	10.0	25.0	40.0
Initial pH	-	5.0	7.0	9.0

(*) the ratio of starch/yeast extract was 14.47

TABLE 6 – Plackett-Burman Design matrix, along with IAA production

Run order	L-tryptophan	Initial pH	Starch	IAA (µg/mL)
1	0	0	0	238
2	-1	1	-1	179
3	-1	1	1	46
4	1	-1	-1	212
5	-1	-1	1	143
6	1	1	1	247
7	-1	-1	-1	45
8	1	1	-1	377
9	1	1	-1	255
10	1	-1	1	755
11	1	-1	1	710
12	-1	-1	-1	49
13	0	0	0	244
14	-1	1	1	358

4.3.7.5 Response Surface Methodology

The central composite design (CCD) of Response Surface Methodology (RSM) was used to determine the optimal concentration of the two variables selected by the Placket Burman Design, namely L-tryptophan and starch. Each variable was studied at five levels (-1.4142; -1; 0; +1; +1.4142). The experimental design was formulated with the aid of Minitab Statistical Software, and the coded values are shown in TABLE 7. The experimental design consisted on fourteen (14) runs, with four (4) cube points, three (3) centre points in the cube, four (4) axial points, and three (3) centre points in axial, as presented in TABLE 8.

The response (IAA production) was used to estimate variable coefficients to generate the empirical mathematical model, which follows a quadratic model given by the equation 2 (Pan and Fan 2008):

$$y=\beta o+\sum \beta iXi+\sum \beta iiXi^2+\sum \beta ijXiXj \quad (2)$$

Where y is the predicted response (IAA production), βo is the intercept, βi is the linear coefficient, βii is the squared coefficient, and βij is the interaction coefficient, Xi and Xj are the coded values of the variables.

The effect of variables on the IAA production was accessed by F-test of ANOVA and their significance was evaluated through p-value. The 3D response surface and 2D contour plot were plotted using Minitab Statistical Software. The fitness or accuracy of the RSM model was accessed by R^2 and adjusted R^2. The resulted model was validated by running experiments at optimal conditions.

TABLE 7 – Coded values of CCD

Variables	Units	Levels				
		-1.4142	-1	0	+1	+1.4142
L-tryptophan	mg/mL	2.9289	5	10	15	17.0711
Starch*	g/L	21.4436	26	37	48	52.5564

(*) the ratio of starch/yeast extract was 14.47

TABLE 8 – CCD matrix, along with IAA production

			IAA production (µg/mL)	
Run order	L-tryptophan	Starch	Experimental	Predicted
1	1	-1	959.39	950.85
2	0	0	1307.46	1417.50
3	-1	1	1324.03	1403.71
4	-1	-1	1072.65	1049.81
5	0	0	1368.23	1417.50
6	1	1	1575.41	1669.39
7	0	0	1445.58	1417.50
8	0	0	1373.76	1417.50
9	1.4142	0	1362.71	1317.02
10	-1.4142	0	1224.59	1199.14
11	0	0	1533.98	1417.50
12	0	0	1475.97	1417.50
13	0	1.4142	1766.02	1657.96
14	0	-1.4142	862.71	899.64

4.3.7.6 Effect of incubation time on IAA production

The effect of incubation time was assessed under optimized concentrations of starch and L-tryptophan. Aliquot for IAA quantification was taken at every 3h interval in the first 24hours of fermentation, then at intervals of 24h in the subsequent fermentation periods.

4.3.8 Plant growth promotion traits

4.3.8.1 Phosphate solubilization

The National Botanical Research Institute's Phosphate (NBRIP) solid medium (Nautiyal 1999), was used to assess the ability of bacterial strain to solubilise phosphate. Bacterial culture (10µL) previously growth in nutrient broth medium, was inoculated in the middle of Petri plate containing NBRIP solid medium, then incubated in darkness, at 30ºC for 7 days. The ability to solubilise phosphate was detected by the formation of clear halo around bacterium colony, and the width of the clear zone was determined through equation 3 (Yulianti and Rakhmawati 2018):

$$\text{Width of the clear zone} = \text{Halo zone diameter} - \text{Colony diameter} \quad (3)$$

4.3.8.2 Nitrogen fixing activity

The ability to fix nitrogen was qualitatively assessed through methodology described by Goswami et al. (2015). Bacterial colony was aseptically inoculated in tube with 8mL of nitrogen-free bromothymol blue (NfB) medium, then incubated in darkness, at 30ºC, for 4 days. The ability to fix nitrogen was evaluated by colour change from green to blue.

4.3.8.3 Ammonia production

Bacterial colony was inoculated in tube with 8mL peptone water, then incubated at 30ºC for 4 days. After incubation, 0.5mL of Nessler's reagent (K_2HgI_4, Potassium Tetraiodomercurate II) was added in tube, and the change of color from brown to yellow confirms the production of ammonia (Goswami et al. 2015).

4.3.8.4 Hydrogen Cyanide (HCN) production

The ability of the bacterial isolate to produce HCN was qualitatively accessed following methodology described by Durve (2017). Briefly, bacterial colony was streaked on Petri plate containing NA medium amended with 4.4 g/L of glycine. A

sterile whatman filter paper was soaked in 0.5% of picric acid solution, then put in the top of the Petri plate, then incubated at 30ºC for 7 days. The development of red color after incubation was considered positive for HCN production.

4.3.8.5 Antifungal activity

The antagonistic activity against *Aspergillus niger*, *Fusarium moniliforme* and *Gibberella* sp. was assessed by method described by Das et al. (2015). Briefly, 1cm^2 mycelial block of phytopathogenic fungi was inoculated in the middle of the Petri plate containing PDA medium, and in two opposite sides (at distance of 2.5cm from fungal block) was streaked the bacterial strain, then incubated at 30ºC for 7 days in darkness. Plate without bacterial isolate was used as negative control. The antagonism activity was assessed by growth inhibition and sporulation. The relative growth inhibition was expressed by equation 4 (Lahlali et al. 2007):

$$Relative\ Growth\ Inhibition = \frac{Ri - Rc}{Rc} \times 100\% \quad (4)$$

Where, Ri is length of hyphal growth toward bacterial isolate, and Rc is length of hyphal growth on control.

4.3.9 Effect of bacterial suspension containing IAA on germination and growth of soybean plant

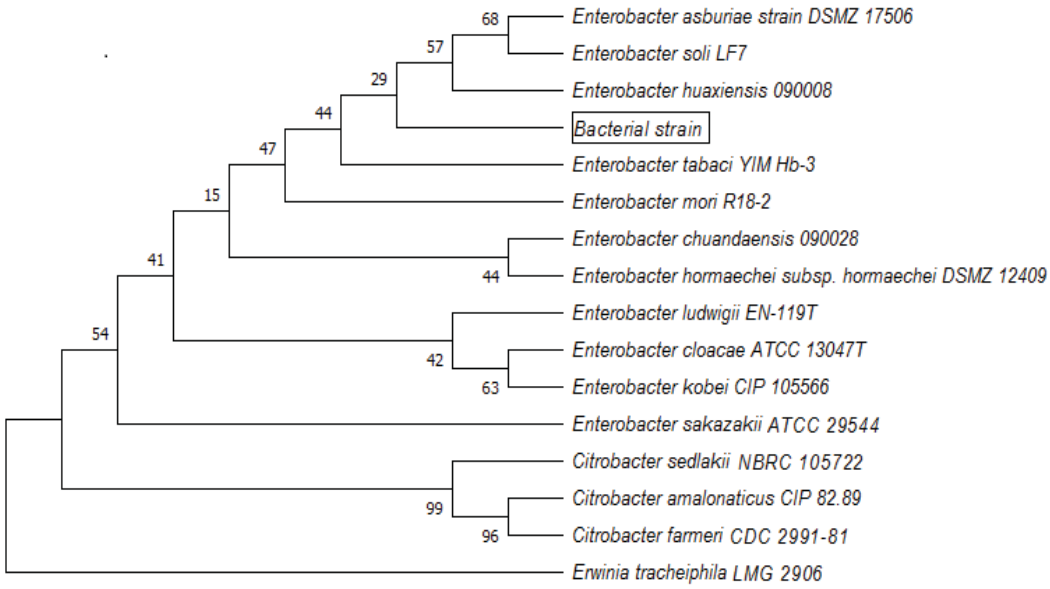
The evaluation of the effect of bacterial suspension containing IAA on soybean culture was performed in greenhouse. The soybean seeds were treated with water (0µg/mL of bacterial IAA), bacterial suspension containing 5µg/mL of IAA and bacterial suspension containing 15µg/mL of IAA, corresponding to control, treatment 1, and treatment 2, respectively. For each treatment were used 10 seeds. The seeds were sown in 1L pots containing moist sand. The germination analysis was performed daily for 14 days. The first foliar application of treatments (control, treatment 1 and treatment 2) was carried out fifteen (15) days after seedling emergence, and the second at 30 day. The harvest was performed at 45 days after seed emergence and assessed the height and dry weight of root and aerial part of the plant.

4.4 RESULTS

4.4.1 Molecular Identification

Comparing 16S rDNA sequence of the bacterial isolate with the NCBI database, the highest similarity (99.08%) was obtained with members of the *Enterobacter* genera, as shown in the phylogenetic tree (FIGURE 5). The nucleotide sequence was submitted to GenBank with accession number MT422205.

FIGURE 5 – Phylogenetic tree



4.4.2 Metagenomic analysis

The bacteria diversity within *Boletus edulis* fruiting body is totally described or covered, and can be used to characterize the endophytic microbiota as demonstrated by the rarefaction curve (FIGURE 6). The most detected family was Ruminococcaceae (43.73%), followed by Lachnospiraceae (22.88%), Bifidobacteriaceae (15.35%), Erysipelotrichaceae (5.52%), Bacteroidaceae (4.18%), Lactobacillaceae (2.44%), Enterobacteriaceae (2.13%), and Rikenellaceae (1.89%). Other families with relative abundance less than 1% represented 1.88% (FIGURE 7).

FIGURE 6 – Rarefaction curve of the observed OTUs (97% similarity)

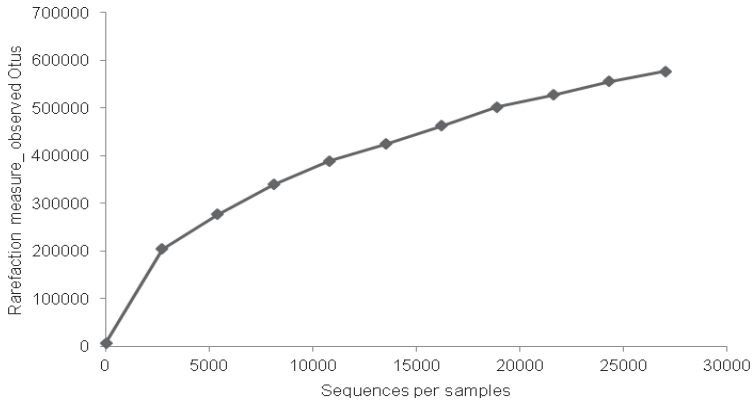
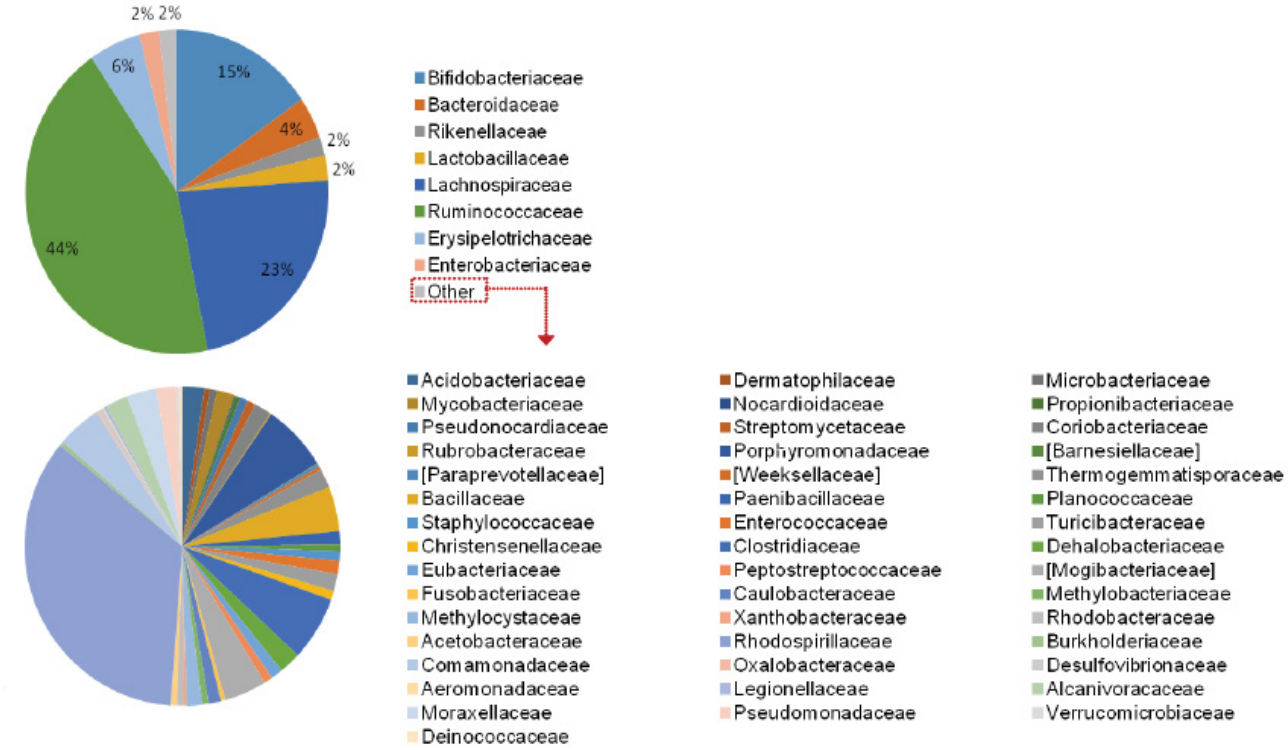


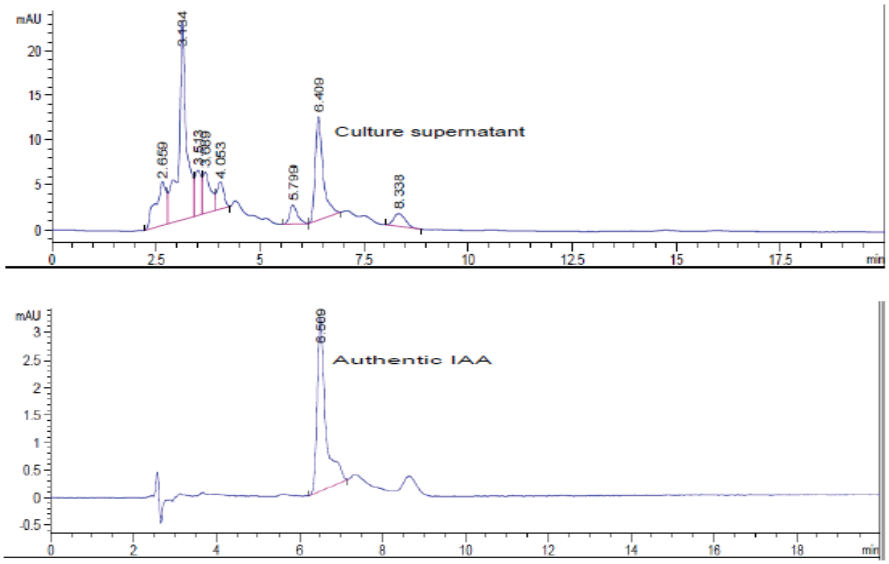
FIGURE 7 – Bacterial diversity in *Boletus edulis* fruiting body, at family level



4.4.3 HPLC analysis of IAA production

The IAA production was confirmed by HPLC analysis. Strong peak was observed at 6.409min in the chromatogram corresponding to culture supernatant, and the authentic IAA is recorded at the retention time 6.509min (FIGURE 8).

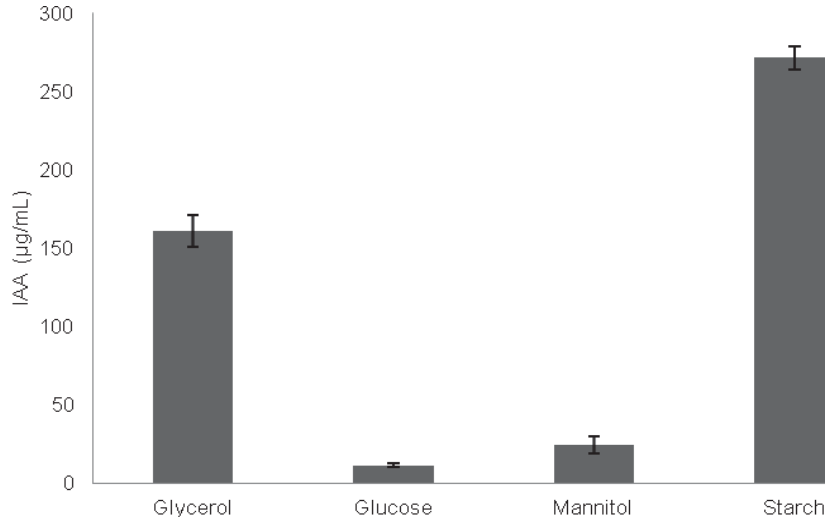
FIGURE 8 – HPLC analysis



4.4.4 Screening of the C-sources by One-Factor-At-Time (OFAT)

The effect of different C-sources on the IAA production is shown in FIGURE 9. Among C-sources tested, starch was the best C-source producing 271.5 ±7.1 µg/mL of IAA.

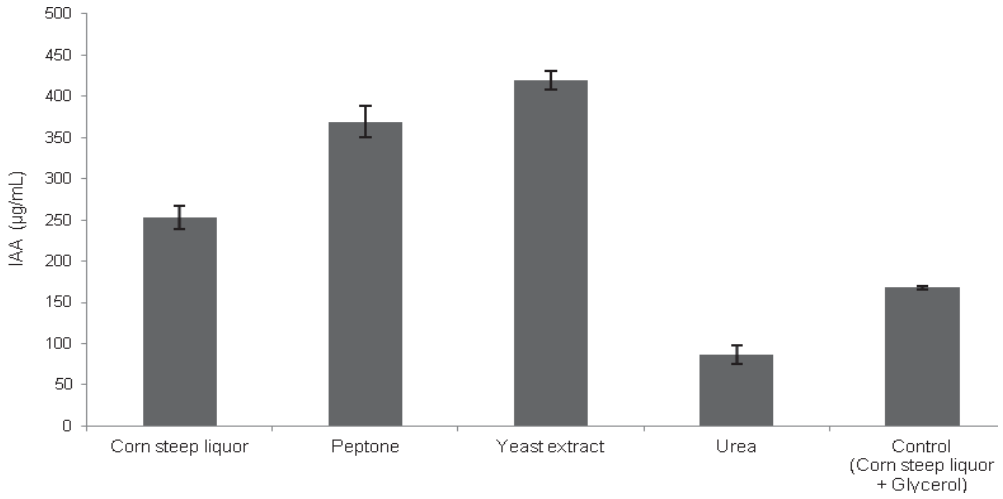
FIGURE 9 – Screening of C-sources for IAA production. Each value is mean of 3 replications. Vertical bars represent standard error.



4.4.5 Screening of the N-sources by One-Factor-At-Time (OFAT)

Among all N-sources tested, yeast extract was the best N-source producing 419.5µg/mL of IAA, followed by peptone 368.9µg/mL IAA, and corn steep liquor 253.20µg/mL IAA (FIGURE 10).

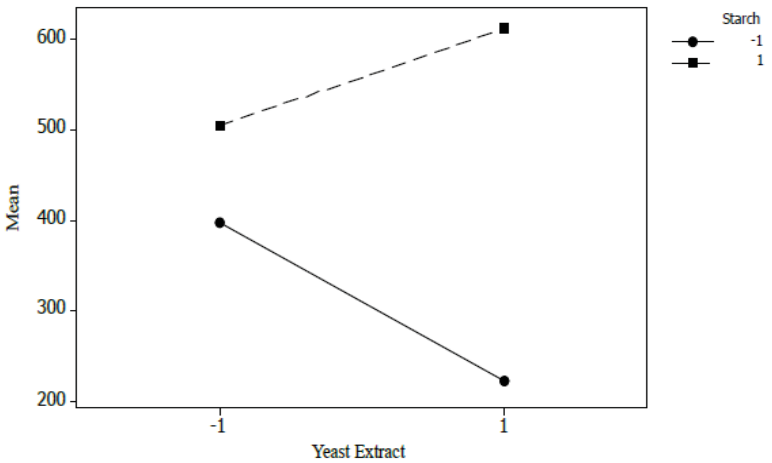
FIGURE 10 – Screening of N-sources for IAA production. Each value is mean of 3 replications. Vertical bars represent standard error.



4.4.6 Two-level Full Factorial Design

The result of 2^2 Factorial Designs is shown in FIGURE 11. The mean response is maximum when starch and yeast extract were both at high levels (+1).

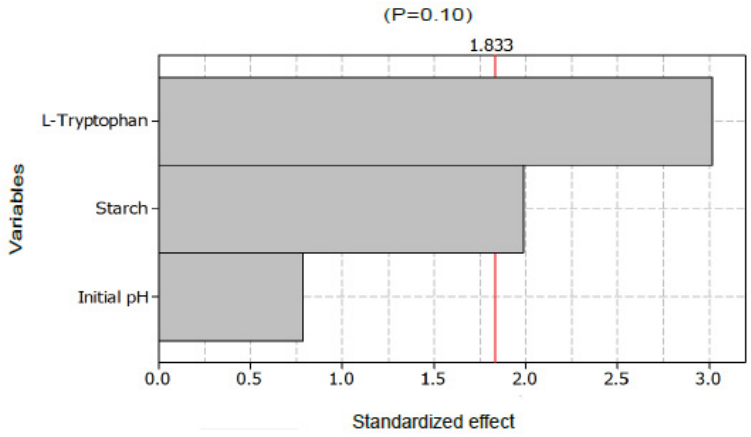
FIGURE 11 – Effect of starch and yeast extract on IAA production



4.4.7 Plackett-Burman Design

The Pareto chart of standardized effects (FIGURE 12) was used to show the result of the PBD. The factors with confidence level above 90% were considered significant on IAA production.

FIGURE 12 – Pareto chart of standardized effects



4.4.8 Response Surface Methodology

L-tryptophan and starch were found significant at 90% confidence level by Plackett-Burman Design, hence were further optimized using Central Composite Design of RSM. The experimental results of Central Composite Design were fitted in the following quadratic polynomial model:

$$y = 1417.50 + 41.68Xi + 268.11Xj - 79.71Xi^2 - 69.35Xj^2 + 91.16XiXj \quad (5)$$

Where, y is the predicted response (IAA production), Xi and Xj is L-tryptophan and starch concentrations, respectively.

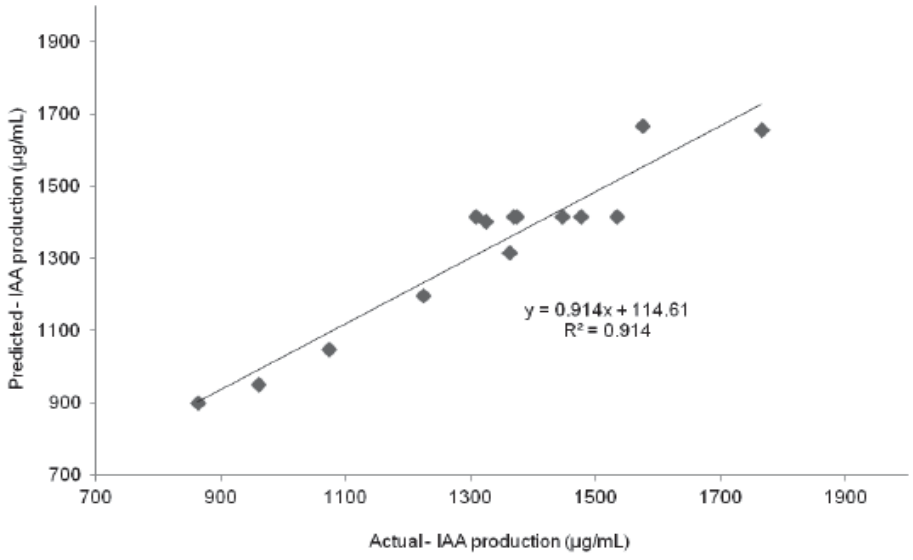
The ANOVA results were validated by testing the assumptions of normal distribution, homogeneity of variance, and independence (figures not shown). All assumptions were satisfied, therefore, the coefficient and p-values were not biased.

The coefficient of determination R^2 of the model was 94.19%, which means good fit. The R^2 of the model suggests that 5.81% of all variations in the IAA production are caused by uncertainties and cannot be explained by the model.

The R^2 always lies between 0% and 100%, and higher R^2 values ($R^2 \cong 100\%$) are preferable as indicates that the model perfectly fits the data. However, according to Hegde et al. (2013), higher R^2 does not mean that the model is accurate, it may be deceiving because doesn't take into account the degree of freedom in the model determination, this is, the R^2 increases with the addition of new variable, doesn't matter whether the new variable is statically significant or not.

The adjusted R^2 attempt to overcome that issue, particularly when there is more than one variable in the model, as it measures the proportion of the variation explained by only independent variables that really help in explaining the response. In the present study, the adjusted R^2 was 89.21%, and according to Saedon et al. (2012) adjusted R^2 > 80%, are within the acceptable level. These findings were strengthened by the plot of actual versus predicted IAA production (FIGURE 13), where can easily be observed that the data points falls close to the straight line, with degree of correlation (R^2) of 0.914. Thus, the quadratic model is very accurate.

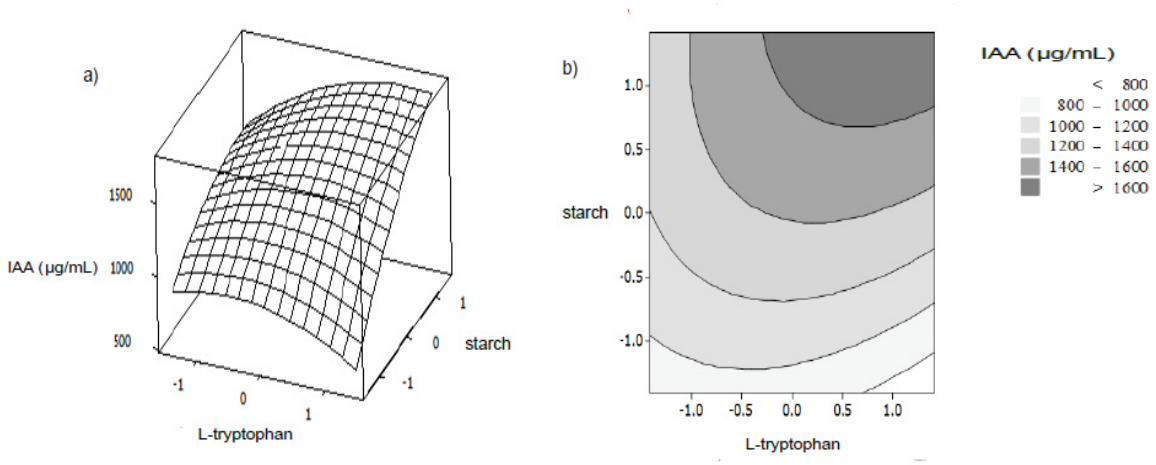
FIGURE 13 – Actual values versus Predicted values for IAA production



The optimal concentration range of the two variables is shown in 3D response surface and contour plot (FIGURE 14). The theoretical optimal

concentrations suggested by Minitab Statistical Software were: starch (+1.4142) and L-tryptophan (+1). The RSM model validation resulted in 1838.8±67.8 µg/mL of IAA production.

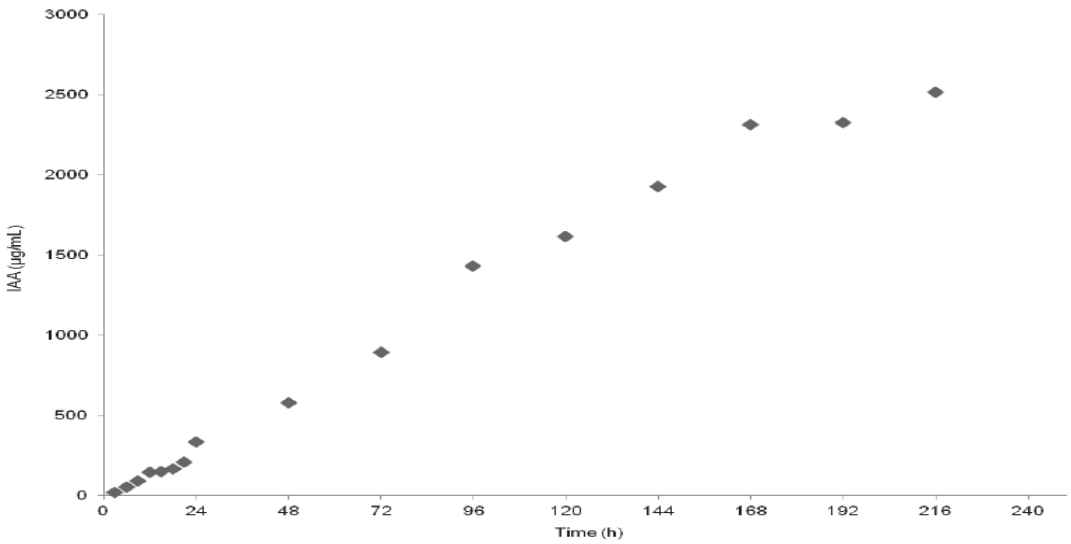
FIGURE 14 – Interaction effect of L-tryptophan versus starch on IAA production. a) 3D response surface. b) 2D contour plot



4.4.9 Effect of incubation time on IAA production

The effect of different incubation periods was investigated in the optimized concentration of starch and L-tryptophan and the results are shown in FIGURE 15.

FIGURE 15 – Effect of incubation time on IAA production



4.4.10 Plant growth promotion traits

The plant growth promotion traits of the isolated bacterial strain are shown in TABLE 9.

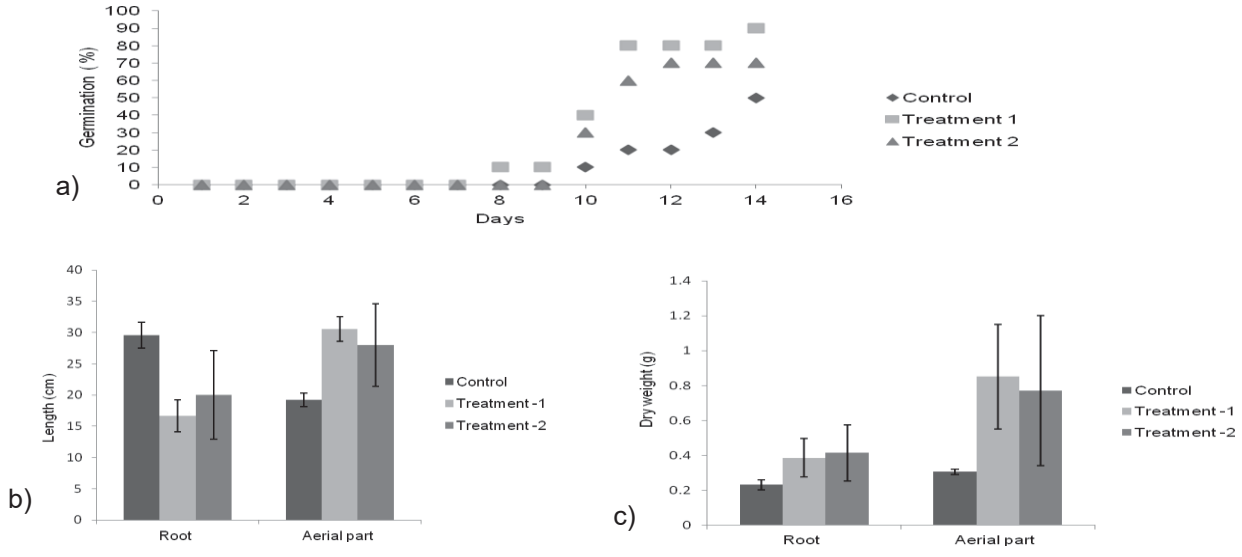
TABLE 9 – Summary of bacterial strain´s plant growth promoting traits.

Phosphate Solubilization width (mm)		5
N_2 Fixation Activity		+
NH_3 Production		+
HCN Production		-
Antagonistic Test	Sporulation	Relative growth inhibition (%)
Aspergillus niger	Delayed	0
Gibberella sp.	NA	7.6
Fusarium moniliforme	NA	17.14

4.4.11 Effect of bacterial suspension conatining IAA on germination and growth of soybean plant

The result of evaluation of the effect of bacterial suspension containing IAA on soybean culture under greenhouse conditions is shown in FIGURE 16. Both treatments 1 and 2 showed higher germination rate and dry weight than control (0µg/mL of IAA). Although, both treatments 1 and 2 showed root length inferior to control, the dry weight was superior.

FIGURE 16 – Effect of bacterial suspension containing IAA on soybean plant. a) Seed germination. b) Length. c) Dry weight



4.5 DISCUSSION

Comparing 16S rRNA sequence of the isolated bacterial strain with the NCBI database, maximum similarity (99.08%) was observed with various species of genus *Enterobacter*. In the phylogenetic tree, the bacterial isolate grouped with *Enterobacter* species (FIGURE 5). Hence, the isolated bacterial strain was identified as *Enterobacter* sp.

The high throughput 16S rRNA gene sequencing analysis of mushroom *Boletus edulis* fruiting body revealed in-depth the presence of several bacterial communities, confirming the presence of Enterobacteriaceae family with relative abundance of 2.13%, but was not classify further than family level.

Some authors have reported that 16S rRNA gene has low phylogenetic power at the species level (Janda and Abbott 2007; Morales-lópez et al. 2019). Therefore, multilocus sequence analysis (MLSA) and several housekeeping genes such as *rpoB*, *gyrB*, *dnaJ* and *recA* have been used to establish higher phylogenetic resolution (Hata et al. 2016). For instance, the identification of *Enterobacter* sp. *DMKU-RP206* was based on 16S rRNA and multilocus gene sequences *gyrB*, *rpoB*, *atpD*, and *infB* (Nutaratat et al. 2017), and *Enterobacter arachidis* was identified by combination of 16S rRNA with *rpoB* gene sequence analysis (Madhaiyan et al. 2010).

The IAA production by bacterial strain *Enterobacter* sp. was assessed through Salvolwski´s reagent (Gordon and Weber 1950) and quantified using UV-Vis spectrophotometer. The IAA production by *Enterobacter* sp. was confirmed by HPLC.

Bacterial strain *Enterobacter* sp. produced 154µg/mL of IAA under un-optimized fermentation medium. This production is higher than IAA produced by many other *Enterobacter* species reported earlier, for example, *Enterobacter cloacae* MSR1 produced 112µg/mL (Khalifa et al. 2016), *Enterobacter ludwigii* produced approximately 30µg/mL (Shoebitz et al. 2009), *Enterobacter asburiae* strain PS2 produced a maximum 32µg/mL (Ahemad and Khan 2010), and *Enterobacter arachidis* produced 5.57µg/mL.

The OFAT found starch as the best carbon source for IAA production (271.5 ±7.1µg/mL) among C-sources tested. The replacement of glycerol by starch showed positive effect, whereas negative effect was observed when glycerol was replaced by glucose and mannitol. Similarly, Myo et al. (2019) found starch as the most suitable

C-source for IAA production by *Streptomyces fradiae* NKZ-259. Nalini and Rao (2014) as well found starch as being one of the three best C-source out of twelve C-sources tested for IAA production by *Rhizobium* species. However, Balaji et al. (2012) observed total inhibitor effect when starch, glucose, lactose and cellulose were added in medium for IAA production, being only glycerol the C-source that stimulated the IAA production by *Pseudomonas* species.

Among N-sources tested for IAA production, yeast extract was distinguished as the best. Likewise, Nutaratat et al. (2017) observed that the highest amount of IAA was obtained when yeast extract was used for IAA production by *Enterobacter* sp. DMKU-RP206. Balaji et al. (2012) also found yeast extract as the best N-source followed by beef extract in IAA production by *Pseudomonas* species. Therefore, these finding corroborates with the statement of Bhutani et al. (2018) that the utilization of C and N-source are exclusive for each bacterial strain.

The 2^2 Factorial Design revealed that yeast extract was sensitive to mean response at both levels of starch, but maximum yield was observed when starch was kept at high level. The mean response was maximum when starch and yeast extract were both kept at high levels (+1), which correspond to starch/yeast extract ratio of 60/4.146 g/L (14.47). The Carbon/Nitrogen (C/N) balance has been reported to regulate the secondary metabolite production (López et al. 2003). Brzonkalik et al. (2012) studied the effect of different C/N ratios on production of alternariol (AOH), alternariol monomethylether (AME) and tenuazonic acid (TA) by *Alternaria alternata* DSM 12633, and observed that carbon to nitrogen (C/N) ratio was one of factors that influenced their production. They demonstrated clearly that alternariol is produced in the stationary growth phase after nitrogen depletion, and that an excess of carbon has a positive impact on alternariol production. On the other hand, Dinarvand et al. (2013) obtained higher production of enzymes under conditions of carbon limitation and an excess of nitrogen.

The Plackett-Burman Design found starch and L-tryptophan as variables that significantly influence the IAA production, and were further optimized by Response Surface Methodology. The optimal concentration of starch and L-tryptophan was 52.556g/L and 15mg/mL, respectively. The RSM model was validated using the optimal concentration of starch and L-tryptophan above mentioned and resulted in production of 1838.8±67.8 µg/mL of IAA. This represent about 12-fold increases when compared with the IAA produced in un-optimized fermentation medium. The

error between the actual and predicted value (1749µg/mL) was 4.9%. According to Rahimi and Mokrtari (2018) errors between the actual and predicted values lower than 12% are acceptable. Therefore, RSM model of the present study is able to accurately predict the IAA production.

The effect of incubation time on IAA production by bacterial isolate (*Enterobacter* sp.) revealed gradual increase in the IAA production from the start of the analysis at 3h up until 216h. The maximum IAA production was observed in 216h (2563.4±150.2 µg/mL). However, the IAA production at incubation periods of 168h (2311.0±155.7 µg/mL), 192h (2323.9.0±122.7 µg/mL) and 216h (2563.4±150.2 µg/mL), were not statistical different ($p<0.05$). From 168h, the increase in incubation period did not resulted in significant increase in IAA production, thus the incubation time for higher IAA production was considered 168h (7 days). Similarly, Nutaratat et al. (2017) observed maximum IAA production (448.5 µg/mL) *by Enterobacter* sp. DMKU-RP206 after 10 days, but decided to reduce the fermentation time to 5 days (414.2 µg/mL of IAA) as to reduce the costs of production.

In vitro studies also demonstrated that the isolated bacterial strain is able to solubilize phosphate, therefore is able to improve phosphorous uptake by the plant. Member of the genus *Enterobacter* have been reported to show phosphate solubization trait, for example, *Enterobacter cloacae* MSR1 (Khalifa et al. 2016), and *Enterobacter* sp. ENB1 (Delgado et al. 2014). Moreover, the isolated bacterial strain showed ability to fix atmospheric nitrogen and to produce ammonia. With regard to fungal mycelium growth inhibition, the isolated bacteria showed antifungal activity against *Gibberella* sp. and *Fusarium moniliforme*, with relative growth inhibition of 7.6% and 17.14%, respectively. Additionally, was able to delay *Aspergillus niger* sporulation. However, was negative for HCN production and was unable to inhibit the growth of *Aspergillus niger.*

The result of evaluation of the effect of bacterial suspension containing IAA on soybean plant under controlled conditions revealed higher germination rate, length of the aerial part and dry weight of both treatments 1 and 2 (bacterial suspension containing IAA) than control (0µg/mL of IAA).

4.6 CONCLUSION

To our knowledge, this is the first report addressing optimization of IAA produced by *Enterobacter* sp. strain isolated from mushroom fruiting body, particularly *Boletus edulis*. The culture conditions were successfully optimized using both classical and statistical approaches and resulted in about 12-fold increase in IAA production. This result demonstrated that the bacterial strain *Enterobacter* sp. has great potential for Indole-3-Acetic Acid production. Moreover, this study demonstrated that mushrooms harbor various bacterial groups, which can be easily isolated and be exploited for agricultural applications in way of biofertilizer or bioenhancer for plant growth promotion.

ACKNOWLEDGEMENTS

The author is thankful to CAPES (Coordenação de Aperfeiçoamento de Pessoal de Nível Superior) for the research scholarship.

CONFLICT OF INTEREST

The author declares no conflict of interest.

REFERENCES

Ahemad M, Khan MS (2010) Plant growth promoting activities of phosphate-solubilizing *Enterobacter asburiae* as influenced by fungicides. EurAsian J Biosci 88–95. https://doi.org/10.5053/ejobios.2010.4.0.11

Aktar W, Sengupta D, Chowdhury A (2009) Impact of pesticides use in agriculture: Their benefits and hazards. Interdiscip Toxicol 2:1–12. https://doi.org/10.2478/v10102-009-0001-7

Anushi J, Jain A, Mudaliyar V, Durve-Gupta A (2017) Isolation, characterization and application of endophytic bacteria isolated from medicinal plants. Int Res J Nat Appl Scieneces 4:3–20

Balaji N, Lavanya SS, Muthamizhselvi S, Tamilarasan K (2012) Optimization of fermentation condition for indole acetic acid production by *Pseudomonas* species . Journal, Int Biotechnol 3:797–803

Bhutani N, Maheshwari R, Negi M, Suneja P (2018) Optimization of IAA production by endophytic *Bacillus* spp. from *Vigna radiata* for their potential use as plant growth promoters. Isr J Plant Sci 65:83–96. https://doi.org/10.1163/22238980-00001025

Brzonkalik K, Hümmer D, Syldatk C, Neumann A (2012) Influence of pH and carbon to nitrogen ratio on mycotoxin production by *Alternaria alternata* in submerged cultivation. AMB Express. https://doi.org/10.1186/2191-0855-2-28

Caporaso JG, Kuczynsk J, Stombaugh J, et al (2010) QIIME allows analysis of high-throughput community sequencing data. Nat Methods 7:335–336. https://doi.org/10.1038/nmeth.f.303

Caporaso JG, Lauber CL, Walters WA, et al (2012) Ultra-high-throughput microbial community analysis on the Illumina HiSeq and MiSeq platforms. ISME J 6:1621–1624. https://doi.org/10.1038/ismej.2012.8

Chen B, Luo S, Wu Y, et al (2017) The effects of the endophytic bacterium *Pseudomonas fluorescens* Sasm05 and IAA on the plant growth and cadmium uptake of *Sedum alfredii* hance. Front Microbiol 8:1–13. https://doi.org/10.3389/fmicb.2017.02538

Cheng H, Jiang N (2006) Extremely rapid extraction of DNA from bacteria and yeasts. Biotechnol Lett 55–59. https://doi.org/10.1007/s10529-005-4688-z

Das MP, Devi PV, Yasmine Y (2015) A study on antagonistic potential of bacteria against phytopathogenic fungi. Int J Pharm Sci Rev Res 34:191–193

Delgado M, Mendez J, Rodríguez-Herrera R, et al (2014) Characterization of phosphate-solubilizing bacteria isolated from the arid soils of a semi-desert region of north-east Mexico. Biol Agric Hortic 30:211–217. https://doi.org/10.1080/01448765.2014.909742

Dinarvand M, Rezaee M, Masomian M, et al (2013) Effect of C/N ratio and media optimization through Response Surface Methodology on simultaneous productions of intra- and extracellular inulinase and invertase from *Aspergillus niger*. Biomed Res Int. https://doi.org/10.1155/2013/508968

Etminani F, Harighi B (2018) Isolation and identification of endophytic bacteria with plant growth promoting activity and biocontrol potential from wild pistachio trees. Plant Pathol J 34:208–217. https://doi.org/10.5423/PPJ.OA.07.2017.0158

Forget G (1994) Balancing the need for pesticides with the risk to human health. Impact Pestic Use Heal Dev Ctries 2–16

Gautam CK, Madhav M, Sinha A, Jabez Osborne W (2016) VIT-CMJ2: Endophyte of *Agaricus bisporus* in production of bioactive compounds. Iran J Biotechnol 14:19–24. https://doi.org/10.15171/ijb.1287

Gordon SA, Weber RP (1950) Colorimetric estimation of indoleacetic acid. 192–195

Goswami D, Parmar S, Vaghela H, et al (2015) Describing *Paenibacillus mucilaginosus* strain N3 as an efficient plant growth promoting rhizobacteria (PGPR). Cogent Food Agric 163:1–13. https://doi.org/10.1080/23311932.2014.1000714

Hasegawa M, Kishino H, Yano T aki (1985) Dating of the human-ape splitting by a molecular clock of mitochondrial DNA. J Mol Evol 22:160–174. https://doi.org/10.1007/BF02101694

Hata H, Natori T, Mizuno T, et al (2016) Phylogenetics of family Enterobacteriaceae and proposal to reclassify *Escherichia hermannii* and *Salmonella subterranea* as *Atlantibacter hermannii* and *Atlantibacter subterranea* gen. nov., comb. nov. Microbiol Immunol 303–311. https://doi.org/10.1111/1348-0421.12374

Hegde S, Bhadri G, Narsapur K, et al (2013) Statistical optimization of medium components by Response Surface Methodology for enhanced production of bacterial

cellulose by *Gluconacetobacter persimmonis*. Bioprocess Biotech 4:1–5. https://doi.org/10.4172/2155-9821.1000142

Hernández-Soberano C, Ruíz-Herrera LF, Valencia-Cantero E (2020) Endophytic bacteria *Arthrobacter agilis* UMCV2 and *Bacillus methylotrophicus* M4-96 stimulate achene germination, *in vitro* growth, and greenhouse yield of strawberry (Fragaria × ananassa). Sci Hortic (Amsterdam) 261:109005. https://doi.org/10.1016/j.scienta.2019.109005

Janda JM, Abbott SL (2007) 16S rRNA gene sequencing for bacterial identification in the diagnostic laboratory: Pluses , Perils , and Pitfalls. J Clin Microbiol 45:2761–2764. https://doi.org/10.1128/JCM.01228-07

Kandel S, Joubert P, Doty S (2017) Bacterial endophyte colonization and distribution within plants. Microorganisms 5:77. https://doi.org/10.3390/microorganisms5040077

Katoh K, Rozewicki J, Yamada KD (2018) MAFFT online service: Multiple sequence alignment, interactive sequence choice and visualization. Brief Bioinform 20:1160–1166. https://doi.org/10.1093/bib/bbx108

Khalifa AYZ, Alsyeeh AM, Almalki MA, Saleh FA (2016) Characterization of the plant growth promoting bacterium, *Enterobacter cloacae* MSR1, isolated from roots of non-nodulating *Medicago sativa*. Saudi J Biol Sci 23:79–86. https://doi.org/10.1016/j.sjbs.2015.06.008

Khan AL, Halo BA, Elyassi A, et al (2016a) Indole acetic acid and ACC deaminase from endophytic bacteria improves the growth of *Solanum lycopersicum*. Electron J Biotechnol 21:58–64. https://doi.org/10.1016/j.ejbt.2016.02.001

Khan Z, Rho H, Firrincieli A, et al (2016b) Growth enhancement and drought tolerance of hybrid poplar upon inoculation with endophyte consortia. Curr Plant Biol 6:38–47. https://doi.org/10.1016/j.cpb.2016.08.001

Kopittke PM, Menzies NW, Wang P, et al (2019) Soil and the intensification of agriculture for global food security. Environ Int 132:105078. https://doi.org/10.1016/j.envint.2019.105078

Kumar S, Stecher G, Li M, et al (2018) MEGA X: Molecular evolutionary genetics analysis across computing platforms. Mol Biol Evol 35:1547–1549. https://doi.org/10.1093/molbev/msy096

Kumla J, Suwannarach N, Matsui K, Lumyong S (2020) Biosynthetic pathway of indole-3-acetic acid in ectomycorrhizal fungi collected from northern Thailand. PLoS One 15:1–15. https://doi.org/10.1371/journal.pone.0227478

Lahlali R, Bajii M, Jijakli MH (2007) Isolation and evaluation of bacteria and fungi as biological control agents. Commun Agric Appl Biol Sci 973–982

Lefebvre O, Ha Nguyen TT, Al-Mamun A, et al (2010) T-RFLP reveals high - Proteobacteria diversity in microbial fuel cells enriched with domestic wastewater. J Appl Microbiol 109:839–850. https://doi.org/10.1111/j.1365-2672.2010.04735.x

López JLC, Pérez JAS, Sevilla JMF, et al (2003) Production of lovastatin by *Aspergillus terreus* : effects of the C:N ratio and the principal nutrients on growth and metabolite production. Enzyme Microb Technol 33:270–277. https://doi.org/10.1016/S0141-0229(03)00130-3

Macedo-Raygoza GM, Valdez-Salas B, Prado FM, et al (2019) *Enterobacter cloacae*, an endophyte that establishes a nutrient-transfer symbiosis with banana plants and protects against the black sigatoka pathogen. Front Microbiol 10:1–15. https://doi.org/10.3389/fmicb.2019.00804

Madhaiyan M, Poonguzhali S, Lee JS, et al (2010) *Enterobacter arachidis* sp. nov., a plant-growth-promoting diazotrophic bacterium isolated from rhizosphere soil of groundnut. Int J Syst Evol Microbiol 60:1559–1564. https://doi.org/10.1099/ijs.0.013664-0

Mateo-Sagasta J, Marjani S, Turral H, Burke J (2017) Water pollution from agriculture: a global review. FAO y IWMI 35. https://doi.org/http://www.fao.org/3/a-i7754e.pdf

Morales-lópez S, Yepes JA, Prada-herrera JC, Torres-jiménez A (2019) *Enterobacteria* in the 21 st century : a review focused on taxonomic changes. J Infect Dev Ctries. https://doi.org/10.3855/jidc.11216

Myo EM, Ge B, Ma J, et al (2019) Indole-3-acetic acid production by *Streptomyces fradiae* NKZ-259 and its formulation to enhance plant growth. BMC Microbiol 1–14. https://doi.org/https://doi.org/10.1186/s12866-019-1528-1

Nalini G, Rao YRKVT (2014) Effect of different carbon and nitrogen sources on growth and indole acetic acid production by *Rhizobium* species Isolated from cluster bean [*Cyamopsis tetragonoloba* (L .)]. Br Microbiol Researh J 4:1189–1197

Nautiyal CS (1999) An efficient microbiological growth medium for screening phosphate solubilizing microorganisms. FEMS Microbiol Lett 170:265–270. https://doi.org/10.1016/S0378-1097(98)00555-2

Nutaratat P, Monprasit A, Srisuk N (2017) High-yield production of indole-3-acetic acid by *Enterobacter* sp . DMKU-RP206 , a rice phyllosphere bacterium that possesses plant growth-promoting traits. 3 Biotech 7:1–15. https://doi.org/10.1007/s13205-017-0937-9

Olanrewaju OS, Glick BR, Babalola OO (2017) Mechanisms of action of plant growth promoting bacteria. World J Microbiol Biotechnol 33:0. https://doi.org/10.1007/s11274-017-2364-9

Pan CM, Fan YT (2008) Statistical optimization of process parameters on biohydrogen production from glucose by *Clostridium* sp . Fanp2. 99:3146–3154. https://doi.org/10.1016/j.biortech.2007.05.055

Panigrahi S, Mohanty S, Rath CC (2019) Characterization of endophytic bacteria *Enterobacter cloacae* MG00145 isolated from *Ocimum sanctum* with Indole Acetic Acid (IAA) production and plant growth promoting capabilities against selected crops. South African J Bot 000:1–10. https://doi.org/10.1016/j.sajb.2019.09.017

Quast C, Pruesse E, Yilmaz P, et al (2013) The SILVA ribosomal RNA gene database project: Improved data processing and web-based tools. Nucleic Acids Res 41:590–596. https://doi.org/10.1093/nar/gks1219

Rahimi M, Mokhtari J (2018) Modeling and Optimization of Waterproof-Breathable Thermo- Regulating Core-Shell Nanofiber / Net Structured Membrane for Protective Clothing Applications. https://doi.org/10.1002/pen.24776

Ribeiro VP, Marriel IE, Sousa SM de, et al (2018) Endophytic *Bacillus* strains enhance pearl millet growth and nutrient uptake under low-P. Brazilian J Microbiol 49:40–46. https://doi.org/10.1016/j.bjm.2018.06.005

Saedon JB, Soo SL, Aspinwall DK, et al (2012) Prediction and Optimization of Tool Life in Micromilling AISI D2 (~ 62 HRC) Hardened Steel. Eng Procedia 41:1674–1683. https://doi.org/10.1016/j.proeng.2012.07.367

Sheikhian L, Bina S (2016) Simultaneous extraction and HPLC determination of 3-indole butyric acid and 3-indole acetic acid in pea plant by using ionic liquid-modified silica as sorbent. J Chromatogr B Anal Technol Biomed Life Sci 1010:34–43. https://doi.org/10.1016/j.jchromb.2015.11.047

Shi Y, Lou K, Li C (2009) Promotion of plant growth by phytohormone-producing endophytic microbes of sugar beet. Biol Fertil Soils 45:645–653. https://doi.org/10.1007/s00374-009-0376-9

Shoebitz M, Ribaudo CM, Pardo MA, et al (2009) Plant growth promoting properties of a strain of *Enterobacter ludwigii* isolated from *Lolium perenne* rhizosphere. Soil Biol Biochem 41:1768–1774. https://doi.org/10.1016/j.soilbio.2007.12.031

Silva CF, Vitorino LC, Mendonça MAC, et al (2019) Screening of plant growth-promoting endophytic bacteria from the roots of the medicinal plant *Aloe vera*. South African J Bot 000:1–14. https://doi.org/10.1016/j.sajb.2019.09.019

Spaepen S, Vanderleyden J (2011) Auxin and plant-microbe interactions. Cold Spring Harb Perspect Biol 3:1–13. https://doi.org/10.1101/cshperspect.a001438

Tamošiūnė I, Stanienė G, Haimi P, et al (2018) Endophytic *Bacillus* and *Pseudomonas* spp. modulate apple shoot growth, cellular redox balance, and protein expression under *in vitro* conditions. Front Plant Sci 9:1–14. https://doi.org/10.3389/fpls.2018.00889

Tilman D, Cassman KG, Matson PA, et al (2002) Agricultural sustainability and intensive production practices. Nature 418:671–677. https://doi.org/10.1038/nature01014

Torres M, Llamas I, Torres B, et al (2020) Growth promotion on horticultural crops and antifungal activity of *Bacillus velezensis* XT1. Appl Soil Ecol 150:103453. https://doi.org/10.1016/j.apsoil.2019.103453

United Nations (2019) World population prospects 2019

United Nations (2015) The future of food and agriculture: Trends and challenges

Xin G, Zhang G, Kang JW, et al (2009) A diazotrophic, indole-3-acetic acid-producing endophyte from wild cottonwood. Biol Fertil Soils 45:669–674. https://doi.org/10.1007/s00374-009-0377-8

Yulianti E, Rakhmawati A (2018) Screening and characterization of phosphate solubilizing bacteria from isolate of thermophilic bacteria. Biophys Rev 090015: https://doi.org/10.1063/1.4995207

Zeinab AK, Faiza A, Fatma EM (2015) The Plackett-Burman Design to evaluate significant media components for antimicrobial production of *Lactobacillus rhamnosus*. IntJCurrMicrobiolAppSci 4:1082–1096

5 ARTICLE 2: *Providencia* sp. ISOLATED FROM *Lactarius deliciosus* FRUITING BODY WITH HIGH POTENTIAL TO PRODUCE INDOL-3-ACETIC ACID, SOLUBILZE PHOSPHATE AND FIX NITROGEN

Marcelino Lourenço Xavier[a], Susan Grace Karp[a], Vanete Thomaz-Soccol[a], Dão Pedro de Carvalho Neto[a], Manuel Hospinal-Santiani[a], Carlos Ricardo Soccol[a]*

[a.] Federal University of Paraná, Department of Bioprocess Engineering and Biotechnology, Centro Politécnico, 81531-990, Curitiba, Paraná, Brazil.

* Corresponding author. E-mail: soccol@ufpr.br

Manuscript formatted according to the guidelines of Applied Microbiology and Biotechnology

5.1 ABSTRACT

Bacterial strain *Providencia* sp. isolated from edible mushroom *Lactarius deliciosus* was found to produce high amount of Indol-3-Acetic Acid (IAA), 2880±11 µg/mL in 15h of incubation (horary production of 192±0.7 µg/mL.h) under following medium and fermentation conditions: K_2HPO4 (1g/L), $MgSO_4.7H_2O$ (0.2g/L), NaCl (1g/L), starch (24.17g/L), yeast extract (1.68g/L), L-tryptophan (9mg/mL), initial pH 5, shaking speed of 120 rpm, 30°C. The IAA concentration achieved by the optimized cultivation of *Providencia* sp. is comparable to the best results of the literature, however its high productivity of 192±0.7 µg/mL.h was never reported in the literature. In the optimization process, starch and yeast extract were found as the best C and N sources, respectively. Result of Plackett-Burman experimental design indicated L-tryptophan as the only factor that significantly affected the IAA production. The relationship between L-tryptophan and IAA production was described by simple linear regression model.
The strain *Providencia* sp. was also found positive for almost all plant growth promotion traits tested, namely: phosphate solubilization, nitrogen fixation, ammonia production, HCN production, and antifungal activity against *Fusarium moniliforme* and *Gibberella* sp.
The bacterial suspension of the strain *Providencia* sp. containing IAA was effective in germination and growth promotion (length and dry-weight) of *Glycine max* (soybean).

Keywords: Endophytic bacteria; indol-3-acetic acid; plant growth promotion traits

5.2 INTRODUCTION

The increased cost of chemical fertilizer and growing concern over their negative impact to the human health and environment is regarded as the key driver to the continuous search for microorganisms with great potential to increase the soil fertility and to stimulate the plant growth. Many of these microorganisms are isolated from plant surfaces (phylosphere and rhizosphere) and in inside plant tissues known as endophyte (Bhutani et al. 2018). Endophytic microorganisms, particularly bacteria, are being largely recognized as plant growth promoter (Frank et al. 2017; Macedo-Raygoza et al. 2019; Panigrahi et al. 2019; Hagaggi and Mohamed 2020). They promote plant growth through several mechanisms, which include phytohormones synthesis (auxin, cytokinin and gibberellins), suppression of ethylene production by 1-aminocyclopropane-1-carboxylate (ACC) deaminase, and by facilitating the availability and acquisition of essential nutrients which typically includes nitrogen, phosphorus and iron, as well as by production of antagonistic substances against phytopathogens (Santoyo et al. 2016).

Among mechanisms applied by endophyte bacteria to improve plant growth and development, the production Indol-3-Acetic Acid (IAA) is considered the most important (de Souza et al. 2015), synthesized mainly via tryptophan dependent pathway (Li et al. 2018). Indol-3-Acetic Acid is the main natural phytohormone of the auxin class, and is able to regulate many physiological processes in plants, such as cell division and elongation, seed germination, root initiation and development, phototropism, and fruit formation (Sauer et al. 2013).

Several endophytic as well as rhizospheric bacteria such as *Bacillus*, *Azotobacter*, *Pseudomonas*, *Azospirillum* have been reported as IAA producers (Bhutani et al. 2018). Da Costa et al. (2014) observed high IAA production by most of the genera belonging to the Enterobactereace family namely *Enterobacter, Escherichia, Grimontella, Klebsiella, Pantoea,* and *Rahnella*. Various studies have also reported that *Providencia* species are good IAA producer in addition to other plant growth promoting characteristics (Rana et al. 2011; Manjunath et al. 2013; Hussain et al. 2015; Islam et al. 2016), commonly found inhabiting soil and aquatic environment as well as endophytic of mangroves, chili and pepper plants (Amaresan et al. 2014; Aish et al. 2019).

Although the *Providencia* species are known to produce IAA, to the best of our knowledge there is no report about optimization of IAA produced by *Providencia* spp. as well as their isolation from the mushroom. Therefore, this is the first report of optimization of IAA produced by *Providencia* sp. isolated from mushroom *L. deliciosus*.

5.3 MATERIAL AND METHODS

5.3.1 Mushrooms collection

The edible mushrooms morphologically identified as *Lactarius deliciosus* were collected at Pinus Forest of Federal University of Paraná Campus - Polytechnic Center, Brazil, in April of 2018.

5.3.2 Isolation of endophytic bacteria

To isolate endophytic bacteria, mushrooms were surface sterilized following method described by Gautam et al. (2016) with slight medications. Mushrooms were consecutively washed with tap water, 70% EtOH for 1min, 2.5% NACIO for 2min, 70% EtOH for 30s, and three times in autoclaved dH_2O. Following, the inner part of mushrooms fruiting body was aseptically cut in blocks of $1cm^3$ then placed on Nutrient Agar (NA) plates, and incubated at 30ºC for 24h, in darkness. The endophyte bacterium was selected based on colony morphology and purified by repeated streaking on NA.

5.3.3 Molecular identification

The gDNA of the isolated bacteria was extracted by phenol/chloroform method described by Cheng and Jiang (2006). The gDNA was used as template for PCR. The 16S rRNA gene was amplified using universal primers 27F (5'-AGAGTTTGATCCTGGCTCAG-3') and 1512R (5′-TACCTTGTTACGACTT-3′). The amplification reaction was carried out in Veriti Thermal Cycler (AB Applied Biosystems), and the reaction mixture consisted on 5µL of 1x PCR buffer, 1µL of $MgCl_2$ (50mM), 0.55µL of dNTPs mix (0.22mM), 0.5µL of 27F (0.2mM), 0.5µL of 1512R (0.2mM), 0.2µL of Taq DNA polymerase(1U), 1µL of template DNA (50ng/µL), and 16.25mL of Milli-Q water. The thermal cycling conditions used were: 5min at 95°C, 30 cycles (1min at 93°C, 1min at 50°C and 1min 30s at 72°C), followed by a final elongation cycle at 72°C for 5min. The PCR product was analyzed by electrophoresis in 0.7% agarose gel and quantified in NanoVue spectrophotometer (GE Healthcare UK Limited). The PCR product was sequenced using the same primers at the ACTGene Análises Moleculares Ltda. (Alvorada, RS-Brazil). The nucleotide sequences obtained were visualized in BioEdit version 7.2 and aligned by CLUSTALW using MEGA version X (Kumar et al. 2018). The gene sequences were compared to nucleotide collection deposited in NCBI (National Center of Biotechnology Information) using BLAST server. The neighbor-joining (NJ) tree was constructed using MEGA version X (Kumar et al. 2018). The evolutionary history was computed using Maximum Likelihood method and Hasegawa-Kishino-Yano model

with Gamma distribution rate (Hasegawa et al. 1985). The Bootstrap confidence was inferred with 1000 replicates.

5.3.4 Metagenomic analysis

The 16S Illumina amplicon protocol was used to identify endophytic bacteria present in mushroom *L. deliciosus* fruiting body. The metagenomic DNA was amplified using primers 515F/806R targeting V4 region of 16S (Caporaso et al. 2012) and GoTaq Master Mix (Promega). The amplicons generated were sequenced in Illumina MiSeq apparatus using MiSeq Reagent 500V2 (Illumina). The DNA sequences were analyzed through QIIME (Quantitative Insights Into Microbial Ecology) version 1.9.2 (Caporaso et al. 2010), and grouped into OTUs (Operational Taxonomic Units) considering 97% identity of Silva 132 database (Quast et al. 2013).

5.3.5 IAA production

The bacterial strain was inoculated in 8mL Nutrient Broth (NB) medium, then incubated at 30°C, for 48h in darkness. Following, 120µL of bacterial suspension was inoculated in sterile 15mL conical tube containing 5mL of basic medium for IAA production (1.0 g/L K_2HPO_4, 0.2 g/L $MgSO_4.7H_2O$, 1.0 g/L NaCl, 48.34 g/L glycerol, 9.09g/L corn steep liquor, pH 5.0), then incubated on shaker at 120rpm, 30°C, for 16h. Subsequently, aliquot of 250µL was inoculated in 150mL Erlenmeyer flask containing 25mL of basic medium for IAA production supplemented with 0.5mg/mL of L-tryptophan previously filter sterilized. The Erlenmeyer flasks were then incubated on shaker at 120rpm, 30°C, for 96h. The concentration of IAA per mL of culture was quantified by colorimetric method described by Gordon and Weber (1950). Aliquot of 2mL of fermented culture was centrifuged at 3500rpm, room temperature, for 10min. One (1) mL of culture supernatant was mixed with 2mL of Salkowski's reagent (50mL Perchloric acid 35% and 0.5mL of $FeCl_3$ 0.5M), then kept in darkness, at room temperature for 30min. The development of pink colour indicates positive test for IAA production, and the colour intensity was quantitatively assessed in UV-Vis spectrophotometer set at 530nm. The absorbance obtained was then converted to IAA concentration by standard curve of IAA. The IAA production was then confirmed

by High Performance Liquid Chromatography (HPLC), performed according to Sheikhian and Bina (Sheikhian and Bina 2016), using Agilent Technologies 1260 Infinity HPLC System (Varian Inc., Palo Alto, CA, USA), equipped with HPLC column Microsorb 60-8, C18, 21.4x250mm (Varian). The mobile phase was 1% water - acetic acid/methanol (50:50 v/v). Elution was carried out at flow rate of 0.5 mL/min, and the absorbance was recorded at 283nm.

5.3.6 Optimization of IAA production

5.3.6.1 One-Factor-At-Time (OFAT)

Three C-sources vis. glucose (24.17g/L), mannitol (24.17g/L), and starch (24.17g/L) were separately added in the basic medium for IAA production replacing glycerol (48.34g/L), while other medium components were kept constant. Following, the C-source found as the best for IAA production, was subsequently used for the screening of N-sources (2.6906g/L peptone, 3.3633g/L yeast extract, 0.7214g/L urea and 9.09g/L corn steep liquor) by the same method OFAT. The basic medium which had glycerol (48.34g/L) and corn steep liquor (9.09g/L) as C and N sources, respectively, was used as positive control for N-sources screening. The IAA production was quantified by Salkowski´s reagent (Gordon and Weber 1950).

5.3.6.2 Two-level Factorial Design

Starch and yeast extract were found as the best C and N sources, respectively. The single effect and interaction effect of these factors on the IAA production was studied by 2^2 Factorial Design. Each factor was studied at two levels (low and high). For starch, the coded values corresponding to low level (-1) and high level (+1) were 24.17g/L and 60g/L, respectively. For yeast extract, 1.68g/L corresponded the low level (-1) whereas 4.146g/L was the high level (+1). The 2^2 factorial design consisted on four (4) experiments run in duplicate (TABLE 10).

TABLE 10 – 2^2 Factorial Design matrix, along with IAA production

Run order	Starch	Yeast extract	IAA production (µg/mL)
1	1	1	221
2	1	1	213
3	-1	1	170
4	-1	-1	258
5	-1	-1	264
6	-1	1	164
7	1	-1	210
8	1	-1	215

5.3.6.3 Plackett-Burman Design (PBD)

PBD is one of the most important statistical methods, classified as two-level Fractional Factorial Design. This method helps to find the most important factors among many factors, and hence enabling to discharge those that do not influence the response. Therefore, in this study was used PBD to select the most significant medium components affecting IAA production. The selected variables for study were L-tryptophan, starch and initial pH. Each variable was studied at three levels vis. low (-1) and high (+1), and central level (0), as shown in TABLE 11. Fourteen (14) trials design was generated with the aid of Minitab Statistical Software (TABLE 12).

TABLE 11 – Coded values of PBD

Variables	Units	Levels		
		-1	0	+1
L-tryptophan	mg/mL	1.00	3.00	6.00
Starch*	g/L	4.17	14.17	24.17
Initial pH	-	5.00	7.00	9.00

(*) the ratio of starch/yeast extract was 14.39

TABLE 12 – PBD matrix, along with IAA production

Run order	L-tryptophan	Starch	Initial pH	IAA (µg/mL)
1	-1	1	1	144
2	1	1	1	564
3	1	1	-1	668
4	1	1	-1	657
5	-1	1	-1	154
6	-1	-1	1	201
7	-1	-1	-1	150
8	1	-1	-1	615
9	0	0	0	337
10	-1	1	1	127
11	1	-1	1	641
12	0	0	0	326
13	1	-1	1	598
14	-1	-1	-1	144

5.3.6.4 Effect of L-tryptophan on IAA production

The PBD found L-tryptophan as the only factor that significantly affects the IAA production at 95% confidence level. Therefore, was studied the effect of L-tryptophan on IAA production at different concentrations (0.8; 1.6; 2.4; 3.2; 4.0; 4.8; 5.6 and 6.4 mg/mL). The experiment was performed by varying the concentration of L-tryptophan supplemented to the medium for IAA production (1g/L K_2HPO_4 , 0.2 g/L $MgSO_2.H_2O$, 1g/L NaCl, 24.17g/L starch, 1.68g/L yeast extract, initial pH 5.0). Each assay was done in triplicate. The incubation was done on shaker at 120rpm, 30ºC, for 96h.

5.3.6.5 Model validation

The model was validated by running experiment at three different concentrations of L-tryptophan (3; 6 and 9 mg/mL) supplemented to the fermentation medium for IAA production.

5.3.6.6 Effect of incubation time on IAA production

The effect of incubation time was assessed on the medium for IAA production (1g/L K_2HPO_4, 0.2 g/L $MgSO_2.H_2O$, 1g/L NaCl, 24.17g/L starch, 1.68g/L yeast extract, initial pH 5.0) supplemented with 9mg/mL of L-tryptophan. Aliquots for IAA quantification and bacterial growth analysis were taken at every 3h interval in 1st day of fermentation, then at intervals of 24h up to 9th day of fermentation.

5.3.6.7 Other plant growth promotion traits

The ability to solubilize phosphate was detected by formation of a clear zone around bacterial growth on National Botanical Research Institute's Phosphate (NBRIP) solid medium (Nautiyal 1999), and quantitatively determined by equation 6 (Yulianti and Rakhmawati 2018):

Width of the clear zone = Halo zone diameter - Colony diameter (6)

The ability to fix nitrogen was qualitatively assessed through methodology described by Goswami et al. (2015). Bacterial colony was aseptically inoculated in tube with 8mL of nitrogen-free bromothymol blue (NfB) medium, then incubated at 30°C for 4 days. Colour change from green to blue, was a positive indicator for nitrogen fixation activity.

The ammonia production was determined as well according to Goswami et al. (2015). The isolated bacterial strain was inoculated in 8mL peptone water and incubated for 4 days at 30°C. After incubation, 0.5mL of Nessler's reagent (K_2HgI_4, Potassium Tetraiodomercurate II) was added in tube. The change of color from brown to yellow was considered positive for production of ammonia.

The HCN production was qualitatively assessed by Saharan (2015) method. Bacterial colony was inoculated on nutrient agar (NA) medium plate amended with 4.4 g/L of glycine. A sterile whatman filter paper was soaked in 0.5% of picric acid solution, then placed to the top of the Petri plate. The plate was then incubated at 30°C for 7days. The development of red color after incubation was recorded as positive for HCN production.

The antifungal activity of the bacterial strain was tested against three (3) phytophatogens: *Aspergillus niger, Fusarium moniliforme* and *Gibberella* sp., using dual culture method on potato dextrose agar (PDA) medium (Das et al. 2015). Plates were incubated at 30°C for 7 days in darkness. Plate without bacterium was used as negative control. The antagonistic activity was assessed by growth inhibition, expressed by equation 7 (Lahlali et al. 2007):

$$Relative\ Growth\ Inhibition = \frac{Ri - Rc}{Rc} \times 100\% \quad (7)$$

Where, Ri is length of hyphal growth toward bacterial strain, and RC is length of hyphal growth on control.

5.3.7 Effect of bacterial suspension containing IAA on germination and growth of *Glycine max* (soybean)

The study was carried out in a greenhouse, and consisted on three treatments vis. water (0µg/mL of bacterial IAA), bacterial suspension containing 5µg/mL and bacterial suspension containing 15µg/mL, corresponding to the control,

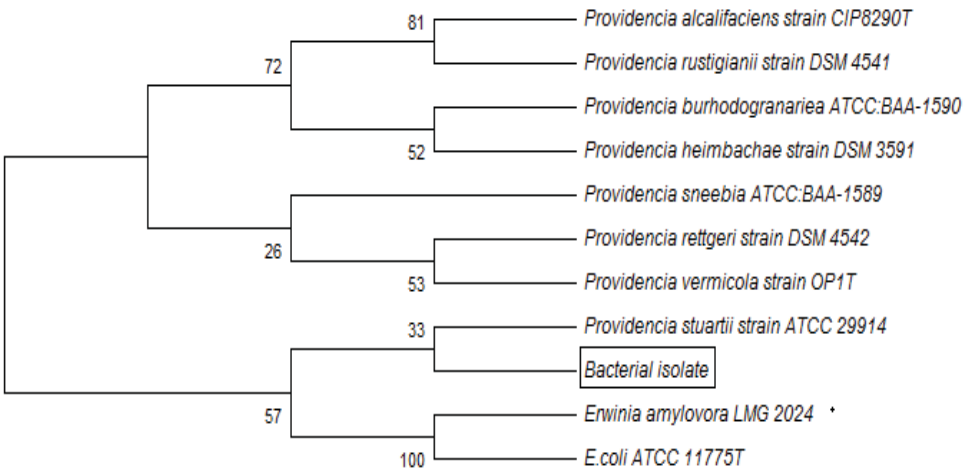
treatment 1, and treatment 2, respectively. The seed density per treatment was in number of ten (10). The treatments were applied directly to seed and via foliar. The seeds treated were sown in 1L pots with moist sand. The first foliar application of treatments was carried out fifteen (15) days after seedling emergence, and the second at 30 day. The irrigation was done by conventional irrigation system. The harvest was performed at 45 days after seed emergence. The agronomic characteristics of the soybean evaluated were germination, length and dry weight of root and aerial part of the plant. To determine the dry masses, plants were dried in oven set at 70°C, until constant mass.

5.4 RESULTS

5.4.1 Molecular identification

The 16S rDNA sequence of bacterial strain was compared to nucleotide sequences of NCBI database using BLAST server. Higher similarity (98.37%) was obtained with members of genus *Providencia*. The phylogenic position of the isolated bacterial strain in relation to *Providencia* species is shown in FIGURE 17. The nucleotide sequence was deposited in GenBank with accession number MT422206.

FIGURE 17 – Phylogenetic tree



5.4.2 Metagenomic analysis

The rarefaction curve of the operational taxonomic units (OTUs) (FIGURE 18) showed that the sequencing depth covered nearly all bacterial communities, and hence can be used to characterize the endophytic microbiota of the *L. deliciosus* fruiting body. The composition of endophytic bacteria at family level is dominated by Pseudomonadaceae with relative abundance of 99% (FIGURE 19).

FIGURE 18 – Rarefaction curve of the observed OTUs (97% similarity)

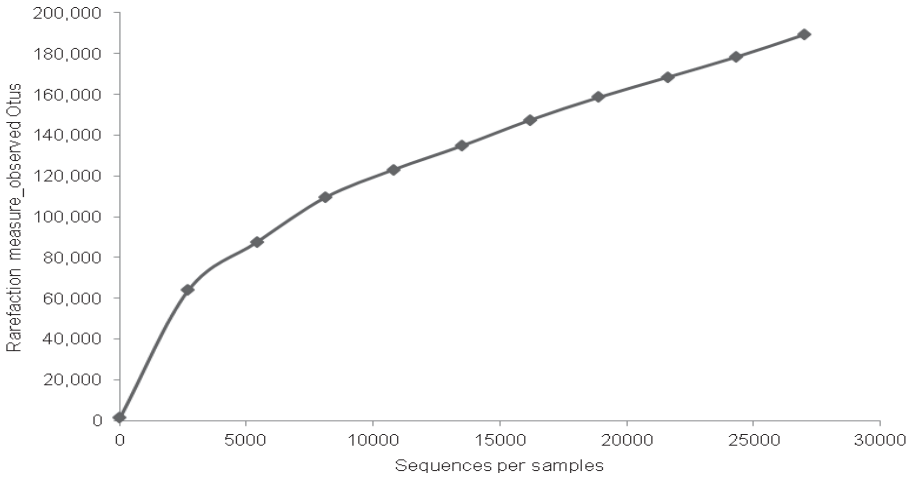
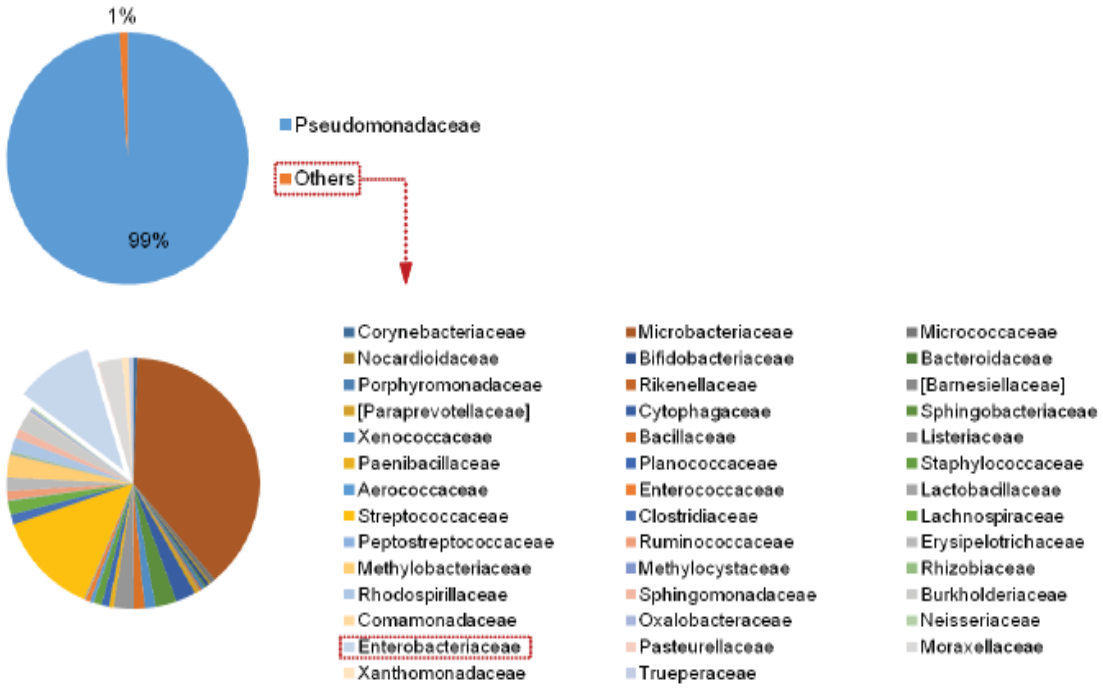


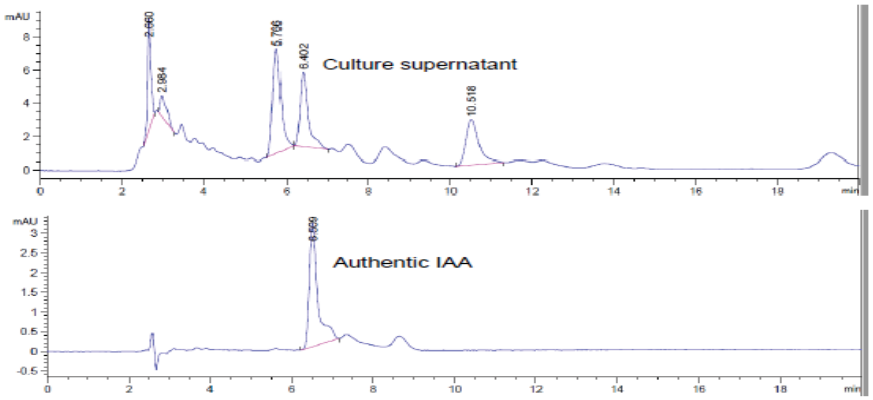
FIGURE 19 – Bacterial diversity in *L. deliciosus* fruiting body, at family level



5.4.3 IAA production

The IAA production was detected by colorimetric method then confirmed by HPLC analysis. Chromatograms of HPLC analysis are shown in FIGURE 20. Strong peak was observed at 6.402min in the chromatogram corresponding to culture supernatant. The authentic IAA was recorded at the retention time 6.509min.

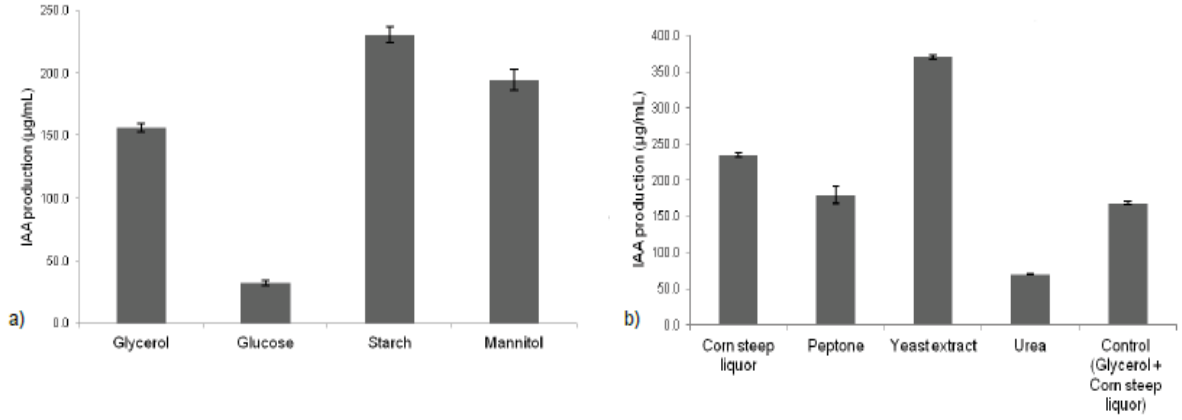
FIGURE 20 – HPLC analysis



5.4.4 One-Factor-At-Time

The effect of different C and N sources on the IAA production is shown in FIGURE 21. Among C and N sources tested, starch and yeast extract were found the best sources for IAA production.

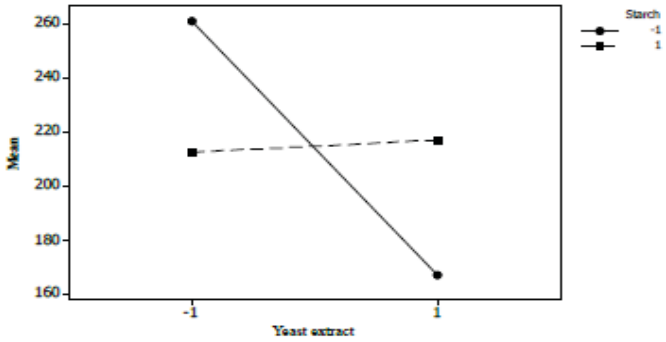
FIGURE 21 – a) Screening of C-sources for IAA production. b) Screening of N-sources for IAA production. Each value is mean of 3 replications. Vertical bars represent standard error.



5.4.5 Two-level Factorial Design

The interaction effect between starch and yeast extract for IAA production is shown in FIGURE 22, and can be observed an antagonistic effect between these input factors, being maximum the IAA production when both factors were at low levels (-1).

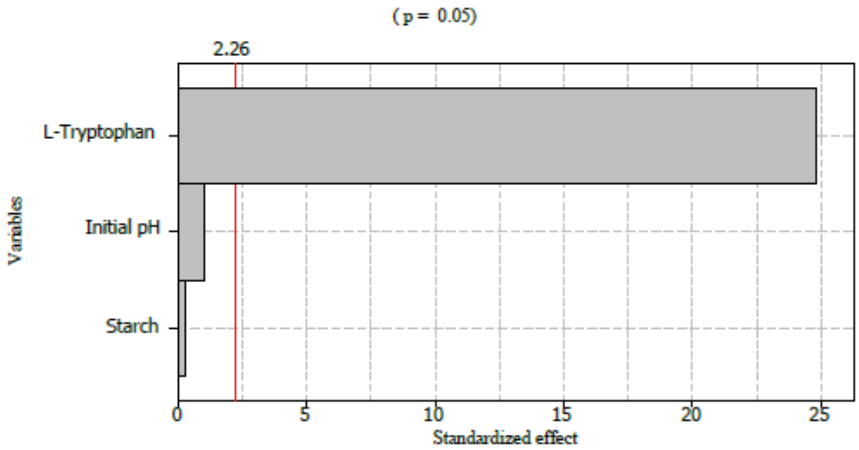
FIGURE 22 – Effect of starch and yeast extract on IAA production



5.4.6 Plackett-Burman Design

Results of PBD are presented through Pareto chart (FIGURE 23). The Pareto chart shows the effect of all variables on IAA production, where variables with statistical confidence level higher than 95% were considered significant to IAA production.

FIGURE 23 – Pareto chart of standardized effects



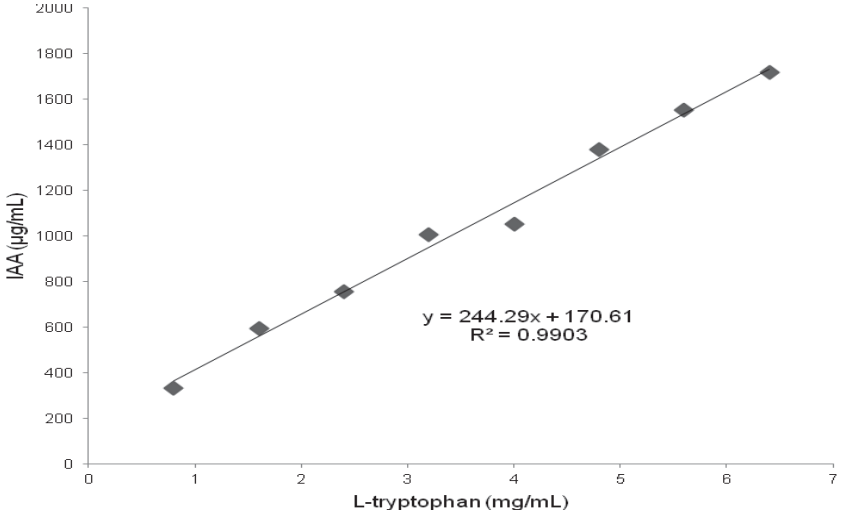
5.4.7 Effect of L-tryptophan on IAA production

The relationship between L-tryptophan and IAA production (FIGURE 24) is described by following simple linear regression model:

$$y=244.29x+170.61 \quad (8)$$

Where, y is the predicted response (IAA production), X is L-tryptophan concentration.

FIGURE 24 – Relationship between L-tryptophan and IAA production



5.4.8 Model validation

Results of model validation are shown in TABLE 13.

TABLE 13 – Model validation

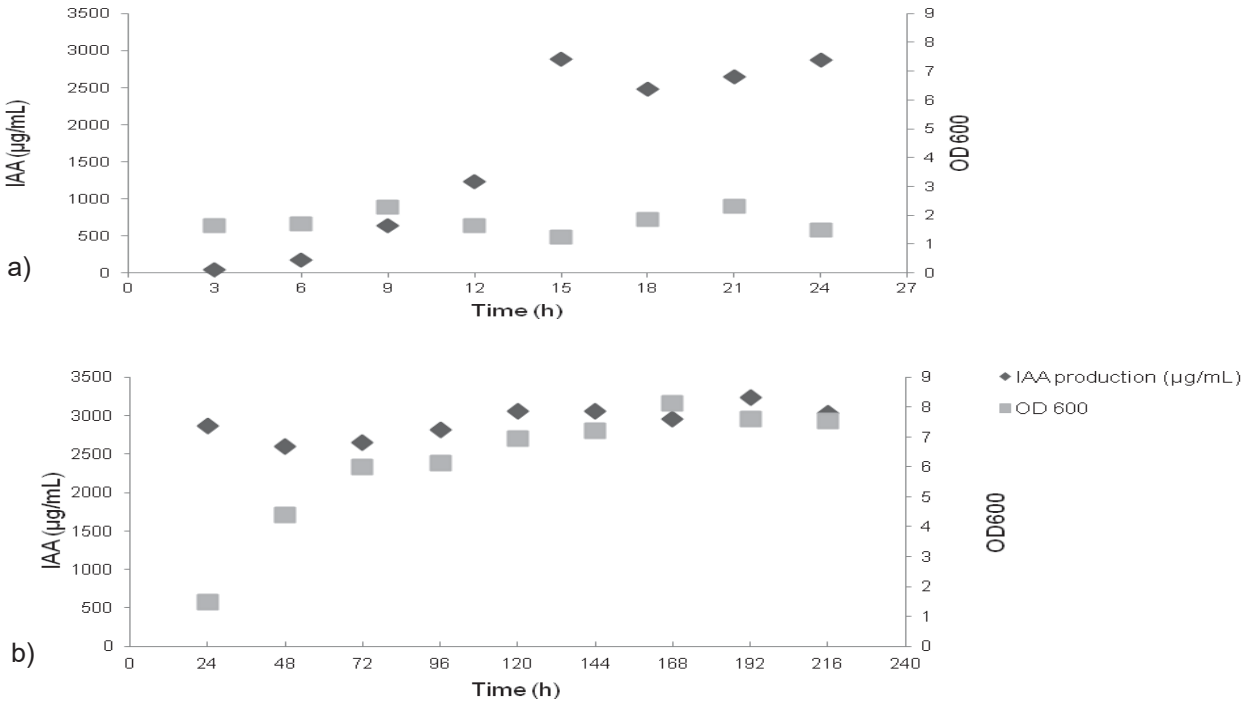
	IAA production(µg/mL)		
L-Tryptophan (mg/mL)	Actual*	Predicted	Error %
3	957±82	903	5.6
6	1796±47	1636	8.9
9	2554±109	2369	7.2

* mean of 3 replications

5.4.9 Effect of incubation time on IAA production

The effect of different incubation time on IAA production by bacterial strain is shown in FIGURE 25.

FIGURE 25 – Effect of incubation time on IAA production. a) Aliquot taken at ever 3h interval during 24h of incubation. b) Aliquot taken at ever 24h.



5.4.10 Other plant growth promotion traits

Result of plant growth promotion traits are summarized in the TABLE 14.

TABLE 14 – Plant growth promotion traits

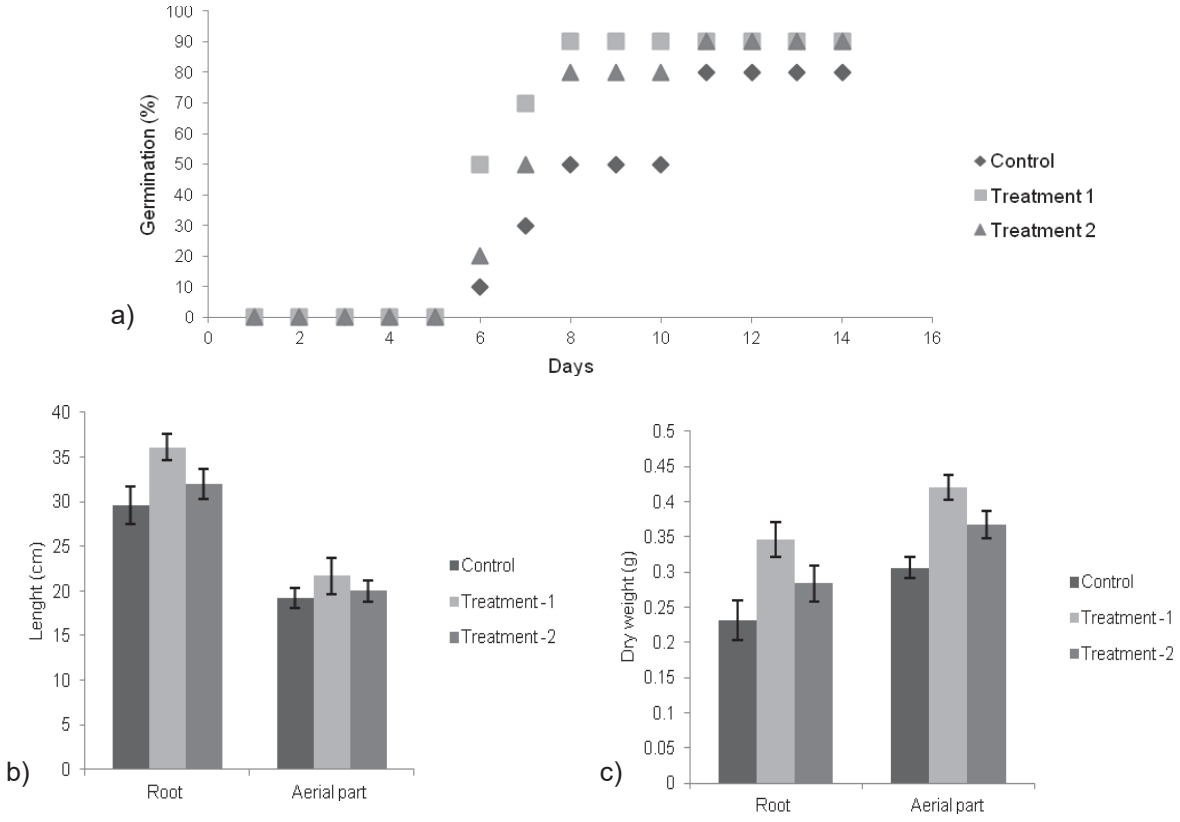
Plant growth traits	
Phosphate Solubilization	5mm *
Nitrogen fixation	+
Ammonia Production	+
HCN Production	+
Aspergillus niger	0% **
Gibberella sp.	12.82 %**
Fusarium moniliforme	8.57%**

* Width
** Relative growth inhibition

5.4.11 Effect of bacterial suspension containing IAA on germination and growth of *Glycine max* (soybean)

The effect of bacterial suspension containing IAA in relation to germination, length and dry weigh of soybean plants is shown in FIGURE 26.

FIGURE 26 – Effect of bacterial suspension containing IAA on soybean crop. a) Germination. b) Plant length. c) Plant weight



5.5 DISCUSSION

Based on 16S rRNA gene sequences, the isolated bacterial strain showed 98.37% similarity with *Providencia* species. The genus *Providencia* belongs to the phylum Proteobacteria, class Gammaproteobacteria, order Enterobacterales, family Enterobacteriaceae, and consists of eight species (*P. alcalifaciens, P. burhodogranariea*, *P. heimbachae*, *P. rettgeri, P. rustigianii*, *P. sneebia*, *P. stuartii* and *P. vermicola),* isolated from various environments (Khunthongpan et al. 2013; Aish et al. 2019). The family Enterobacteriaceae was detected inside the fruiting

body of the mushroom *Lactarius deliciosus* by metagenomic analysis, indicating that the isolated bacterial strain can be a true endophyte. It matches with a genus presenting clear capabilities to promote plant growth through IAA production and phosphate solubilization (Susilowati et al. 2015; Jiang et al. 2018). To corroborate this result, Gautam et al. (2016) reported the isolation of an endophytic bacteria *Enterobacter* sp. from the edible basidiomycete fungus *Agaricus bisporus*, with ability to produce bioactive compounds.

The IAA production was colorimetrically detected using Salkowski´s reagent (Gordon and Weber 1950) and quantified by UV-Vis spectrophotometer. The Salkowski´s reagent is widely used for colorimetric detection of IAA (Ali et al. 2009), because is simple, rapid and cheap, therefore is used for routine analysis. However, Salkowski reagent is not specific for IAA alone, can react with IAA intermediaries present in liquid culture supernatants such as IPyA and IAM, and therefore contributing to color absorption in spectrophotometer quantification (Glickmann and Dessaux 1995; Ali et al. 2009). On other hand, HPLC is more accurate than colorimetric method, but is costly and time-consuming, and therefore is not recommended for daily assays (Galdiano Junior et al. 2011). Thus, HPLC was only used to confirm the IAA production.

The synthesis of secondary metabolites occurs naturally (Okada and Seyedsayamdost 2017), but is greatly influenced by culture conditions. Genes that trigger the biosynthesis of secondary metabolites can be overexpressed by the change or manipulation of cultural conditions such as medium composition (carbon, nitrogen, and phosphorus sources, trace elements), salinity, temperature, pH, and by addition of precursor (Bode et al. 2002; Netzker et al. 2015).

The OFAT revealed that the bacterial strain can use all carbon sources tested for IAA production, but starch was preferred over the other carbon sources tested, yielding 230.6±6.6µg/mL of IAA. Stimulation of IAA production was also observed when mannitol was used as C-source, whereas glucose had repressive effect on the IAA production when compared to glycerol. Early studies demonstrated that several carbon sources can be used for growth and secondary metabolites production, but their preference varies among species (Bharucha et al. 2013; Bhutani et al. 2018). The rapidly metabolized carbon, particularly glucose (hexose), although supports rapid biomass production, is the most effective in repression of the synthesis of secondary metabolites, and such regulation can take place at enzymatic

and/or transcriptional levels (Neidhardt and Magasanik 1957; Ruiz et al. 2010; Sánchez et al. 2010; Romero-Rodríguez et al. 2018). Whereas, polysaccharides, oligosaccharides, and oils are often found to be better than glucose as carbon sources for secondary metabolites production (Demain 1986).

In addition, the type and availability of nitrogen sources also regulate the secondary metabolites production. In the present study, the analysis of N-sources by OFAT revealed maximum IAA production (369.8±3.3µg/mL) when yeast extract was used as N-source. When compared to control, either yeast extract or corn steep liquor stimulated higher IAA production whereas opposite effect was observed with urea.

Results of 2^2 Factorial Design revealed that there was antagonistic interaction between starch and yeast extract. The change in mean response for yeast extract at level (-1) was noticeably higher than at level (+1), this is, yeast extract was less sensitive to variation in mean response at level (+1) of starch. The maximum IAA production was observed when both factors were at low levels (-1), which corresponded to a starch/yeast extract ratio of 24.17/1.68 (g/L) or simply 14.38.

PBD was used to identify variables that significantly affected IAA production, and L-tryptophan was the only factor found significant at 95% confidence level. The effect of different concentrations of L-tryptophan on IAA production was described by simple linear regression model. L-tryptophan greatly influenced the IAA production, for each unit increase in L-tryptophan concentration there was an increase in 244.29 units in response (244.29µg/mL of IAA). This result corroborates with several studies that demonstrated that L-tryptophan is a precursor for IAA production as its addition to the medium greatly increases IAA production (Costacurta et al. 1994; Bharucha et al. 2013; Hasuty et al. 2018).

The effect of incubation time revealed gradual increase in IAA production from the start of the analysis, at 3h, until 15h of incubation (2880±11 µg/mL). After 15h, the increase in incubation period did not result in significant increase in IAA production. On other hand, in the first 24h of incubation, very little bacterial growth was observed. In agreement with our result, Ruiz et al. (2010) found that one distinctive characteristic of secondary metabolism is its association with low growth level.

The IAA concentration achieved by the optimized cultivation of *Providencia* sp. (2880±11 µg/mL) is comparable to the best results reported in literature, i.e. the

study of Apine and Jadhav (2011) that reported maximum IAA production of 2191µg/mL by *Pantoea agglomerans* strain PVM at 48h incubation, as well as Srisuk et al. (2018) that reported high-yield production of IAA (3963 µg/mL) by *Enterobacter* sp. DMKU-RP206 after 3 days of incubation. However, the results of the present study were obtained after 15h of incubation, corresponding to a productivity of 192±0.7 µg/mL.h, which is more than three times higher than those of the previously reported studies (45.65 µg/mL.h and 55.04 µg/mL.h, respectively).

The isolated bacterial strain was found positive for almost all plant growth promoting traits tested *in vitro*, namely phosphate solubilization, N_2 fixation, NH_3 production, HCN production and antifungal activity against *Gibberella* sp. and *Fusarium moniliforme.* Bacterial strains with multiple traits are rare, being phosphate solubilization and N_2 fixation, the direct beneficial traits for plant growth. Many studies have reported stimulation of plant growth through inoculation of bacteria with phosphate solubilization and/or N_2 fixation activity. For example, Afzal et al. (2010) reported higher soybean productivity due to the inoculation of bacteria with phosphate solubilization and N_2 fixation activities, as well as Singh et al. (2010) observed significant increase on soybean growth inoculated with phosphate solubilizing bacteria.

In greenhouse studies, seeds treated with bacterial suspension containing IAA (treatments 1 and 2) achieved efficiency of almost 100% of germination with 3 days less than the control. At 6 to 7 days, 50% of seeds submitted to treatments 1 and 2 were already germinated, while 50% germination of the untreated seeds (control) was observed in 8 days after planting. The higher germination rate of seeds treated with bacterial suspension containing IAA (treatments 1 and 2) could be attributed to the positive effect of bacterial IAA that penetrates the tegument during seed treatment. The IAA is able to activate the initial growth phase of the plant, which includes stimulation of embryo development and root initiation (Möller and Weijers 2009; Sauer et al. 2013) enabling the plant to take water and essential nutrients (e.g. N and P).

Compared to the control, an increase in the length of roots of the plants submitted to treatments 1 and 2 was observed, which means greater capacity for absorbing water and nutrient. In addition, an increase of more than 30% in the dry mass of roots and aerial part of the plants submitted to treatments 1 and 2 was observed. Therefore, the plants submitted to treatments 1 and 2 were more structured, and probably with

greater productive potential, if it closed the growth cycle. The increase in the length and dry mass of the roots and aerial parts could be attributed not only to the positive effect of the bacterial IAA, but also to the capacity of the bacterial strain *Providencia* sp. to make P and N available through phosphate solubilization and atmospheric nitrogen fixation mechanisms.

5.6 CONCLUSION

The bacterial strain *Providencia* sp., isolated from the mushroom *L. deliciosus*, has enormous potential for application in agriculture due to its high rates of production of IAA never reported in the literature, more equally due to its capacity to solubilize phosphate and fix nitrogen. Results of preliminary studies conducted on soybean crop attest to this potential, as well as its promising contributions to the development of more sustainable agriculture.

ACKNOWLEDGEMENTS

The author is thankful to CAPES (Coordenação de Aperfeiçoamento de Pessoal de Nível Superior) for the research scholarship.

CONFLICT OF INTEREST

The author declares no conflict of interest.

AUTHOR CONTRIBUTION STATEMENT

All authors contributed to the study´s conceptualization. Marcelino Lourenço Xavier performed the experiments, data analysis, validation and the writing of manuscript. Susan Grace Karp performed supervision, discussions and reviewed the manuscript. Dão Pedro de Carvalho Neto and Manuel Hospinal-Santiani performed molecular biology analyses. Vanete Thomaz-Soccol provided resources and supervised the experimental work. Carlos Ricardo Soccol performed supervision, project and resources administration, and final manuscript approval.

REFERENCES

Aish AA, Sulaiman MM, Youssef SA, Massoud SI (2019) *Providencia vermicola* mediated growth alteration and inhibited gall formation on tomato plants infected with the root knot nematode *Meloidogyne javancia*. Plant Arch 19:3865–3873

Ali B, Sabri AN, Ljung K, Hasnain S (2009) Auxin production by plant associated bacteria: Impact on endogenous IAA content and growth of *Triticum aestivum* L. Lett Appl Microbiol 48:542–547. https://doi.org/10.1111/j.1472-765X.2009.02565.x

Amaresan N, Velusamy J, Thajuddin N (2014) Isolation and characterization of endophytic bacteria associated with chilli (*Capsicum annuum*) grown in coastal agricultural ecosystem. Indian J Biotechnol 13:247–255

Apine OA, Jadhav JP (2011) Optimization of medium for indole-3-acetic acid production using *Pantoea agglomerans* strain PVM. J Appl Microbiol 1235–1244. https://doi.org/10.1111/j.1365-2672.2011.04976.x

Bharucha U, Patel K, Trivedi UB (2013) Optimization of indole acetic acid production by *Pseudomonas putida* UB1 and its effect as plant growth-promoting rhizobacteria on mustard (*Brassica nigra*). Agric Res 2:215–221. https://doi.org/10.1007/s40003-013-0065-7

Bhutani N, Maheshwari R, Negi M, Suneja P (2018) Optimization of IAA production by endophytic *Bacillus* spp. from *Vigna radiata* for their potential use as plant growth promoters. Isr J Plant Sci 65:83–96. https://doi.org/10.1163/22238980-00001025

Bode HB, Bethe B, Höfs R, Zeeck A (2002) Big effects from small changes: Possible ways to explore nature's chemical diversity. ChemBioChem 3:619–627. https://doi.org/10.1002/1439-7633(20020703)3:7<619::AID-CBIC619>3.0.CO;2-9

Caporaso JG, Kuczynsk J, Stombaugh J, et al (2010) QIIME allows analysis of high-throughput community sequencing data. Nat Methods 7:335–336. https://doi.org/10.1038/nmeth.f.303

Caporaso JG, Lauber CL, Walters WA, et al (2012) Ultra-high-throughput microbial community analysis on the Illumina HiSeq and MiSeq platforms. ISME J 6:1621–1624. https://doi.org/10.1038/ismej.2012.8

Cheng H, Jiang N (2006) Extremely rapid extraction of DNA from bacteria and yeasts. Biotechnol Lett 55–59. https://doi.org/10.1007/s10529-005-4688-z

Costacurta A, Keijers V, Vanderleyden J (1994) Molecular cloning and sequence analysis of an *Azospirillum brasilense* indole-3-pyruvate decarboxylase gene. Mol Gen Genet 243:463–472. https://doi.org/10.1007/BF00280477

Da Costa PB, Granada CE, Ambrosini A, et al (2014) A model to explain plant growth promotion traits: A multivariate analysis of 2,211 bacterial isolates. PLoS One 9:1–25. https://doi.org/10.1371/journal.pone.0116020

Das MP, Devi PV, Yasmine Y (2015) A study on antagonistic potential of bacteria against phytopathogenic fungi. Int J Pharm Sci Rev Res 34:191–193

de Souza R, Ambrosini A, Passaglia LMP (2015) Plant growth-promoting bacteria as inoculants in agricultural soils. Genet Mol Biol 38:401–419. https://doi.org/10.1590/S1415-475738420150053

Demain AL (1986) Regulation of secondary metabolism in fungi. Pure Appl Chem 58:219–226. https://doi.org/10.1351/pac198658020219

Frank A, Saldierna Guzmán J, Shay J (2017) Transmission of bacterial endophytes. microorganisms 5:70. https://doi.org/10.3390/microorganisms5040070

Galdiano Junior RF, Nascimbem Pedrinho EA, Luque Castellane TC, de Macedo Lemos EG (2011) Auxin-producing bacteria isolated from the roots of *Cattleya walkeriana*, an endangered Brazilian orchid, and their role in acclimatization. Rev Bras Cienc Do Solo 35:729–737. https://doi.org/10.1590/S0100-06832011000300008

Gautam CK, Madhav M, Sinha A, Jabez Osborne W (2016) VIT-CMJ2: Endophyte of *Agaricus bisporus* in production of bioactive compounds. Iran J Biotechnol 14:19–24. https://doi.org/10.15171/ijb.1287

Glickmann E, Dessaux Y (1995) A critical examination of the specificity of the Salkowski reagent for indolic compounds produced by phytopathogenic bacteria. Appl Environ Microbiol 61:793–796. https://doi.org/10.1128/aem.61.2.793-796.1995

Gordon SA, Weber RP (1950) Colorimetric estimation of indoleacetic acid. 192–195

Goswami D, Parmar S, Vaghela H, et al (2015) Describing *Paenibacillus mucilaginosus* strain N3 as an efficient plant growth promoting rhizobacteria (PGPR). Cogent Food Agric 163:1–13. https://doi.org/10.1080/23311932.2014.1000714

Hagaggi NSA, Mohamed AAA (2020) Enhancement of *Zea mays* (L .) growth performance using indole acetic acid producing endophyte *Mixta theicola* isolated from Solenostemma. South African J Bot 000:1–8. https://doi.org/10.1016/j.sajb.2020.02.034

Hasegawa M, Kishino H, Yano T aki (1985) Dating of the human-ape splitting by a molecular clock of mitochondrial DNA. J Mol Evol 22:160–174. https://doi.org/10.1007/BF02101694

Hasuty A, Choliq A, Hidayat I (2018) Production of Indole Acetic Acid (IAA) by *Serratia marcescens* subsp. marcescens and *Rhodococcus* aff. qingshengii. Int J Agric Technol 14:299–312

Hussain K, Hameed S, Shahid M, et al (2015) First report of *Providencia vermicola* strains characterized for enhanced rapeseed growth attributing parameters. Int J Agric Biol 17:1110–1116. https://doi.org/10.17957/IJAB/15.0033

Islam F, Yasmeen T, Ali Q, et al (2016) Copper-resistant bacteria reduces oxidative stress and uptake of copper in lentil plants: potential for bacterial bioremediation. 220–233. https://doi.org/10.1007/s11356-015-5354-1

Jiang H, Qi P, Wang T, et al (2018) Isolation and characterization of halotolerant phosphate-solubilizing microorganisms from saline soils. 3 Biotech 8:1–8. https://doi.org/10.1007/s13205-018-1485-7

Khunthongpan S, Sumpavapol P, Tanasupawat S, et al (2013) *Providencia thailandensis* sp. nov., isolated from seafood processing wastewater. J Gen Appl Microbiol 59:185–190. https://doi.org/10.2323/jgam.59.185

Kumar S, Stecher G, Li M, et al (2018) MEGA X: Molecular evolutionary genetics analysis across computing platforms. Mol Biol Evol 35:1547–1549. https://doi.org/10.1093/molbev/msy096

Lahlali R, Bajii M, Jijakli MH (2007) Isolation and evaluation of bacteria and fungi as biological control agents. Commun Agric Appl Biol Sci 973–982

Li M, Guo R, Yu F, et al (2018) Indole-3-acetic acid biosynthesis pathways in the plant-beneficial bacterium *Arthrobacter pascens* zz21. Int J Mol Sci 19:. https://doi.org/10.3390/ijms19020443

Macedo-Raygoza GM, Valdez-Salas B, Prado FM, et al (2019) *Enterobacter cloacae*, an endophyte that establishes a nutrient-transfer symbiosis with banana plants and protects against the black sigatoka pathogen. Front Microbiol 10:1–15. https://doi.org/10.3389/fmicb.2019.00804

Manjunath M, Taylor P, Prasanna R, Sharma P (2013) Archives of Agronomy and Soil Science Developing PGPR consortia using novel genera *Providencia* and *Alcaligenes* along with Cyanobacteria for wheat. Arch Agron Soil Sci 57:873–887

Nautiyal CS (1999) An efficient microbiological growth medium for screening phosphate solubilizing microorganisms. FEMS Microbiol Lett 170:265–270. https://doi.org/10.1016/S0378-1097(98)00555-2

Neidhardt FC, Magasanik B (1957) Reversal of the glucose inhibition of histidase biosynthesis in *Aerobacter aerogenes*. J Bacteriol 73:253–259. https://doi.org/10.1128/jb.73.2.253-259.1957

Netzker T, Fischer J, Weber J, et al (2015) Microbial communication leading to the activation of silent fungal secondary metabolite gene clusters. Front Microbiol 6:1–13. https://doi.org/10.3389/fmicb.2015.00299

Okada BK, Seyedsayamdost MR (2017) Antibiotic dialogues: Induction of silent biosynthetic gene clusters by exogenous small molecules. FEMS Microbiol Rev 41:19–33. https://doi.org/10.1093/femsre/fuw035

Panigrahi S, Mohanty S, Rath CC (2019) Characterization of endophytic bacteria *Enterobacter cloacae* MG00145 isolated from *Ocimum sanctum* with Indole Acetic Acid (IAA) production and plant growth promoting capabilities against selected crops. South African J Bot 000:1–10. https://doi.org/10.1016/j.sajb.2019.09.017

Quast C, Pruesse E, Yilmaz P, et al (2013) The SILVA ribosomal RNA gene database project: Improved data processing and web-based tools. Nucleic Acids Res 41:590–596. https://doi.org/10.1093/nar/gks1219

Rana A, Saharan B, Joshi M, et al (2011) Identification of multi-trait PGPR isolates and evaluating their potential as inoculants for wheat. Ann Microbiol 61:893–900. https://doi.org/10.1007/s13213-011-0211-z

Romero-Rodríguez A, Maldonado-Carmona N, Ruiz-Villafán B, et al (2018) Interplay between carbon, nitrogen and phosphate utilization in the control of secondary metabolite production in *Streptomyces*. Antonie van Leeuwenhoek, Int J Gen Mol Microbiol 111:761–781. https://doi.org/10.1007/s10482-018-1073-1

Ruiz B, Chávez A, Forero A, et al (2010) Production of microbial secondary metabolites: Regulation by the carbon source. Crit Rev Microbiol 36:146–167. https://doi.org/10.3109/10408410903489576

Saharan BS (2015) Evaluation of rhizospheric bacteria from *Ocimum* sp. as potential PGPR. J Microb Biochem Technol 07:88–95. https://doi.org/10.4172/1948-5948.1000187

Sánchez S, Chávez A, Forero A, et al (2010) Carbon source regulation of antibiotic production. J Antibiot (Tokyo) 63:442–459. https://doi.org/10.1038/ja.2010.78

Santoyo G, Moreno-Hagelsieb G, del Carmen Orozco-Mosqueda M, Glick BR (2016) Plant growth-promoting bacterial endophytes. Microbiol Res 183:92–99. https://doi.org/10.1016/j.micres.2015.11.008

Sauer M, Robert S, Kleine-Vehn J (2013) Auxin: Simply complicated. J Exp Bot 64:2565–2577. https://doi.org/10.1093/jxb/ert139

Sheikhian L, Bina S (2016) Simultaneous extraction and HPLC determination of 3-indole butyric acid and 3-indole acetic acid in pea plant by using ionic liquid-modified silica as sorbent. J Chromatogr B Anal Technol Biomed Life Sci 1010:34–43. https://doi.org/10.1016/j.jchromb.2015.11.047

Srisuk N, Sakpuntoon V, Nutaratat P (2018) Production of indole-3-acetic acid by *Enterobacter* sp. DMKU-rp206 using sweet whey as a low-cost feed stock. J Microbiol Biotechnol 28:1511–1516. https://doi.org/10.4014.jmb/1805.04043

Susilowati DN, Sudiana IM, Mubarik NR, Suwanto A (2015) Species and functional diversity of rhizobacteria of rice plant in the Coastal Soils of Indonesia. Indones J Agric Sci 16:39. https://doi.org/10.21082/ijas.v16n1.2015.p39-50

Yulianti E, Rakhmawati A (2018) Screening and characterization of phosphate solubilizing bacteria from isolate of thermophilic bacteria. Biophys Rev 090015: https://doi.org/10.1063/1.4995207

6 FINAL CONCLUSION

This study demonstrated that wild mushroom harbor in its interior diverse cultivable endophytic bacteria, with important plant growth promotion traits such as IAA production, phosphate solubilization and atmospheric N_2 fixing activities.

Among 71 endophytic bacterial isolates, two were found efficient IAA producers and identified by molecular technique as *Enterobacter* sp. and *Providencia* sp. The conditions for IAA production by each of these two isolates was successfully optimized, and the result of *in vivo* study which assessed the exogenous application of IAA produced in promoting plant growth particularly in soybean plant, indicated that has positive role in promoting soybean growth.

These two bacterial isolates, in particular, owe other plant growth traits including phosphate solubilization, atmospheric N_2 fixing, and antifungal activities. Therefore, these two isolates have potential to support plant growth and having superior IAA biosynthesis capability would certainly give an idea not only to use them for industrial production of IAA but also for the development of bio-based products to be used as biofertilizer in agriculture.

For industrial purposes of IAA production, we recommend the use of bacterial strain *Providencia* sp., due to its higher production of IAA, higher rate of production of IAA (2880±11µg/mL in 15h of incubation), and lower nutritional requirement, this is, requires lower concentrations of L-tryptophan (9mg/mL) and starch (24.17g/L) as well as yeast extract (1.68g/L). In addition, the production of IAA by *Providencia* sp. is influenced by a single variable (L-tryptophan), making the process of IAA production easy.

7 REFERENCES

Afzal I, Khan Z, Sikandar S, Shahzad S (2019) Plant beneficial endophytic bacteria : Mechanisms , diversity , host range and genetic determinants. Microbiol Res J 221:36–49. https://doi.org/10.1016/j.micres.2019.02.001

Aharonowitz Y, Demain AL (1979) Nitrogen nutrition and regulation of cephalosporin production in *Streptomyces clavuligerus*. Can J Microbiol 25:61–67. https://doi.org/10.1139/m79-010

Ahemad M, Khan MS (2010) Plant growth promoting activities of phosphate-solubilizing *Enterobacter asburiae* as influenced by fungicides. EurAsian J Biosci 88–95. https://doi.org/10.5053/ejobios.2010.4.0.11

Ahsan T, Chen J, Wu Y, Irfan M (2017) Application of response surface methodology for optimization of medium components for the production of secondary metabolites by *Streptomyces diastatochromogenes* KX852460. AMB Express 7:1–10. https://doi.org/10.1186/s13568-017-0388-z

Aish AA, Sulaiman MM, Youssef SA, Massoud SI (2019) *Providencia vermicola* mediated growth alteration and inhibited gall formation on tomato plants infected with the root knot nematode *Meloidogyne javancia*. Plant Arch 19:3865–3873

Aktar W, Sengupta D, Chowdhury A (2009) Impact of pesticides use in agriculture: Their benefits and hazards. Interdiscip Toxicol 2:1–12. https://doi.org/10.2478/v10102-009-0001-7

Aktuganov G, Melentjev A, Galimzianova N, et al (2008) Wide-range antifungal antagonism of *Paenibacillus ehimensis* IB-X-b and its dependence on chitinase and b-1, 3-glucanase production. Can J Microbiol 54:577–587. https://doi.org/10.1139/w08-043

Alam M, Khan MA, Imtiaz M, et al (2020) Indole-3-acetic acid rescues plant growth and yield of salinity stressed tomato (*Lycopersicon esculentum* L.). Gesunde Pflanz 72:87–95. https://doi.org/10.1007/s10343-019-00489-z

Ali B, Hayat S, Aiman Hasan S, Ahmad A (2008) A comparative effect of IAA and 4-Cl-IAA on growth, nodulation and nitrogen fixation in *Vigna radiata* (L.) Wilczek. Acta Physiol Plant 30:35–41. https://doi.org/10.1007/s11738-007-0088-4

Ali B, Sabri AN, Ljung K, Hasnain S (2009) Auxin production by plant associated bacteria: Impact on endogenous IAA content and growth of *Triticum aestivum* L. Lett Appl Microbiol 48:542–547. https://doi.org/10.1111/j.1472-765X.2009.02565.x

Ali MM, Vora D (2014) Potential of Endophytic Bacteria Isolated from *Vitex negundo* L . to Produce Auxin. Res J Recent Sci 3:38–42

Alijani Z, Amini J, Ashengroph M, Bahramnejad B (2019) Antifungal activity of volatile compounds produced by *Staphylococcus sciuri* strain MarR44 and its potential for the biocontrol of *Colletotrichum nymphaeae* , causal agent strawberry anthracnose. Int J Food Microbiol 307:108276. https://doi.org/10.1016/j.ijfoodmicro.2019.108276

Alori ET, Glick BR, Babalola OO (2017) Microbial phosphorus solubilization and its potential for use in sustainable agriculture. Front Microbiol. https://doi.org/10.3389/fmicb.2017.00971

Amaresan N, Velusamy J, Thajuddin N (2014) Isolation and characterization of

endophytic bacteria associated with chilli (*Capsicum annuum*) grown in coastal agricultural ecosystem. Indian J Biotechnol 13:247–255

Anjum N, Chandra R (2015) Endophytic bacteria : Optimizaton of isolation procedure from various medicinal plants and their preliminary characterization. Asian J Pharm Clin Res 8:233–238

Anushi J, Jain A, Mudaliyar V, Durve-Gupta A (2017) Isolation, characterization and application of endophytic bacteria isolated from medicinal plants. Int Res J Nat Appl Scieneces 4:3–20

Apine OA, Jadhav JP (2011) Optimization of medium for indole-3-acetic acid production using *Pantoea agglomerans* strain PVM. J Appl Microbiol 1235–1244. https://doi.org/10.1111/j.1365-2672.2011.04976.x

Aswathy AJ, Jasim B, Jyothis M, Radhakrishnan EK (2013) Identification of two strains of *Paenibacillus* sp. as indole 3 acetic acid-producing rhizome-associated endophytic bacteria from Curcuma longa. 3 Biotech 3:219–224. https://doi.org/10.1007/s13205-012-0086-0

Aziz K, Nawaz M, Nazir J, et al (2015) Isolation, characterization and effect of auxin producing bacteria on growth of *Triticum aestivum*. J Anim Plant Sci 25:1003–1007

Balaji N, Lavanya SS, Muthamizhselvi S, Tamilarasan K (2012) Optimization of fermentation condition for indole acetic acid production by *Pseudomonas* species . Journal, Int Biotechnol 3:797–803

Baldi BG, Maher BR, Slovin JP, Cohen JD (1991) Stable isotope labeling, *in vivo*, of D- and L-tryptophan pools in *Lemna gibba* and the low incorporation of label into indole-3-acetic acid. Plant Physiol 95:1203–1208. https://doi.org/10.1104/pp.95.4.1203

Bandara WMMS, Seneviratne G, Kulasooriya SA (2006) Interactions among endophytic bacteria and fungi: Effects and potentials. J Biosci 31:645–650. https://doi.org/10.1007/BF02708417

Barışçı S, Turkay O (2016) Optimization and modelling using the response surface methodology (RSM) for ciprofloxacin removal by electrocoagulation. Water Sci Technol 73:1673–1679. https://doi.org/10.2166/wst.2015.649

Bartel B (1997) Auxin Biosynthesis. Annu Rev Plant Physiol Plant Mol Biol 48:51–66. https://doi.org/10.1146/annurev.arplant.48.1.51

Bartel B, Fink GR (1995) ILR1, an amylohydrolase that releases active Indole-3- acetic acid from conjugates. Science (80-) 268:1745–1748. https://doi.org/10.1126/science.7792599

Beneduzi A, Ambrosini A, Passaglia LMP (2012) Plant growth-promoting rhizobacteria (PGPR): Their potential as antagonists and biocontrol agents. Genet Mol Biol 4:1044–1051

Bezerra MA, Santelli RE, Oliveira EP, et al (2008) Response surface methodology (RSM) as a tool for optimization in analytical chemistry. Talanta 76:965–977. https://doi.org/10.1016/j.talanta.2008.05.019

Bharucha U, Patel K, Trivedi UB (2013) Optimization of indole acetic acid production by *Pseudomonas putida* UB1 and its effect as plant growth-promoting rhizobacteria on Mustard (*Brassica nigra*). Agric Res 2:215–221. https://doi.org/10.1007/s40003-013-0065-7

Bhutani N, Maheshwari R, Negi M, Suneja P (2018) Optimization of IAA production by endophytic *Bacillus* spp. from *Vigna radiata* for their potential use as plant growth promoters. Isr J Plant Sci 65:83–96. https://doi.org/10.1163/22238980-00001025

Bialek K, Meudt WJ, Cohen JD (1983) Indole-3-acetic Acid (IAA) and IAA conjugates applied to bean stem sections: IAA content and the growth response. Plant Physiol 73:130–134. https://doi.org/10.1104/pp.73.1.130

Bloemberg G V., Lugtenberg BJJ (2001) Molecular basis of plant growth promotion and biocontrol by rhizobacteria. Curr Opin Plant Biol 4:343–350. https://doi.org/10.1016/S1369-5266(00)00183-7

Bode HB, Bethe B, Höfs R, Zeeck A (2002) Big effects from small changes: Possible ways to explore nature's chemical diversity. ChemBioChem 3:619–627. https://doi.org/10.1002/1439-7633(20020703)3:7<619::AID-CBIC619>3.0.CO;2-9

Bononi L, Chiaramonte JB, Pansa CC, et al (2020) Phosphorus-solubilizing *Trichoderma* spp . from Amazon soils improve soybean plant growth. Sci Rep 1–13. https://doi.org/10.1038/s41598-020-59793-8

Brzonkalik K, Hümmer D, Syldatk C, Neumann A (2012) Influence of pH and carbon to nitrogen ratio on mycotoxin production by *Alternaria alternata* in submerged cultivation. AMB Express. https://doi.org/10.1186/2191-0855-2-28

Bunch AW, Harris RE (1986) The manipulation of micro-organisms for the production of secondary metabolites. Biotechnol. Genet. Eng. Rev. 4:117–144

Bunsangiam S, Sakpuntoon V, Srisuk N, et al (2019) Biosynthetic pathway of indole-3-acetic acid in basidiomycetous yeast *Rhodosporidiobolus fluvialis*. Mycobiology 47:292–300. https://doi.org/10.1080/12298093.2019.1638672

Cao X, Yang H, Shang C, et al (2019) The roles of auxin biosynthesis *YUCCA* gene family in plants. Int J Mol Sci 20:8–10. https://doi.org/10.3390/ijms20246343

Capobianchi MR, Giombini E, Rozera G (2013) Next-generation sequencing technology in clinical virology. Clin Microbiol Infect 19:15–22. https://doi.org/10.1111/1469-0691.12056

Caporaso JG, Kuczynsk J, Stombaugh J, et al (2010) QIIME allows analysis of high-throughput community sequencing data. Nat Methods 7:335–336. https://doi.org/10.1038/nmeth.f.303

Caporaso JG, Lauber CL, Walters WA, et al (2012) Ultra-high-throughput microbial community analysis on the Illumina HiSeq and MiSeq platforms. ISME J 6:1621–1624. https://doi.org/10.1038/ismej.2012.8

Caulier S, Nannan C, Gillis A, Licciardi F (2019) Overview of the antimicrobial compounds produced by members of the *Bacillus subtilis* group. 10:1–19. https://doi.org/10.3389/fmicb.2019.00302

Cerboneschi M, Decorosi F, Biancalani C, et al (2016) Indole-3-acetic acid in plant–pathogen interactions: a key molecule for in planta bacterial virulence and fitness. Res Microbiol 167:774–787. https://doi.org/10.1016/j.resmic.2016.09.002

Chen B, Luo S, Wu Y, et al (2017) The effects of the endophytic bacterium *Pseudomonas fluorescens* Sasm05 and IAA on the plant growth and cadmium uptake of *Sedum alfredii* hance. Front Microbiol 8:1–13. https://doi.org/10.3389/fmicb.2017.02538

Chen XC, Bai JX, Cao JM, et al (2009) Medium optimization for the production of cyclic

adenosine 3′,5′-monophosphate by *Microbacterium* sp. no. 205 using response surface methodology. Bioresour Technol 100:919–924. https://doi.org/10.1016/j.biortech.2008.07.062

Cheng CS (2006) Projection properties of factorial designs for factor screening. Screen Methods Exp Ind Drug Discov Genet 156–168. https://doi.org/10.1007/0-387-28014-6_7

Cheng H, Jiang N (2006) Extremely rapid extraction of DNA from bacteria and yeasts. Biotechnol Lett 55–59. https://doi.org/10.1007/s10529-005-4688-z

Clarridge Jill E. (2004) Impact of 16S rRNA gene sequence analysis for identification of bacteria on Clinical Microbiology and Infectious Diseases. Clin Microbiol Rev 17:840–862. https://doi.org/10.1128/CMR.17.4.840

Cohen JD, Bandurski RS (1982) Chemistry and Physiology of the bound auxins. Ann Rev Plant Physlol 403–430. https://doi.org/10.1016/S1876-0813(08)60299-7

Comai L, Kosuge T (1980) Involvement of plasmid deoxyribonucleic acid in indoleacetic acid synthesis in *Pseudomonas savastanoi*. J Bacteriol 143:950–957. https://doi.org/10.1128/jb.143.2.950-957.1980

Cook SD, Nichols DS, Smith J, et al (2016) Auxin biosynthesis: Are the indole-3-acetic acid and phenylacetic acid biosynthesis pathways mirror images? Plant Physiol 171:1230–1241. https://doi.org/10.1104/pp.16.00454

Cook SD, Ross JJ (2016) The auxins, IAA and PAA, are synthesized by similar steps catalyzed by different enzymes. Plant Signal Behav 11:1–4. https://doi.org/10.1080/15592324.2016.1250993

Costacurta A, Keijers V, Vanderleyden J (1994) Molecular cloning and sequence analysis of an *Azospirillum brasilense* indole-3-pyruvate decarboxylase gene. Mol Gen Genet 243:463–472. https://doi.org/10.1007/BF00280477

Costacurta A, Vanderleyden J (1995) Synthesis of phytohormones by plant-associated bacteria. Crit Rev Microbiol 21:1–18. https://doi.org/10.3109/10408419509113531

Da Costa PB, Granada CE, Ambrosini A, et al (2014) A model to explain plant growth promotion traits: A multivariate analysis of 2,211 bacterial isolates. PLoS One 9:1–25. https://doi.org/10.1371/journal.pone.0116020

Das MP, Devi PV, Yasmine Y (2015) A study on antagonistic potential of bacteria against phytopathogenic fungi. Int J Pharm Sci Rev Res 34:191–193

Dasri K, Kaewharn J, Kanso S, Sangchanjiradet S (2014) Optimization of indole-3-acetic acid (IAA) production by Rhizobacteria isolated from epiphytic orchids. KKU Res J 19:268–275

Datta KS, Varma SK, Angrish R, et al (1997) Alleviation of salt stress by plant growth regulators in *Triticum aestivum* L. Biol. Plant. 40:269–275

de Souza R, Ambrosini A, Passaglia LMP (2015) Plant growth-promoting bacteria as inoculants in agricultural soils. Genet Mol Biol 38:401–419. https://doi.org/10.1590/S1415-475738420150053

Delgado M, Mendez J, Rodríguez-Herrera R, et al (2014) Characterization of phosphate-solubilizing bacteria isolated from the arid soils of a semi-desert region of north-east Mexico. Biol Agric Hortic 30:211–217. https://doi.org/10.1080/01448765.2014.909742

Demain AL (1986) Regulation of secondary metabolism in fungi. Pure Appl Chem 58:219–226. https://doi.org/10.1351/pac198658020219

Demain AL (1998) Induction of microbial secondary metabolism. Int Microbiol 1:259–264. https://doi.org/10.2436/im.v1i4.26

Demain AL (2006) Overproduction of microbial metabolites and enzymes due to alteration of regulation. Adv Biochem Eng Vol 1 I:113–142. https://doi.org/10.1007/bfb0044732

Deurenberg RH, Bathoorn E, Chlebowicz MA, et al (2017) Application of next generation sequencing in clinical microbiology and infection prevention. J Biotechnol 243:16–24. https://doi.org/10.1016/j.jbiotec.2016.12.022

Dinarvand M, Rezaee M, Masomian M, et al (2013) Effect of C/N ratio and media optimization through Response Surface Methodology on simultaneous productions of intra- and extracellular inulinase and invertase from *Aspergillus niger*. Biomed Res Int. https://doi.org/10.1155/2013/508968

Donoso R, Leiva-Novoa P, Zúñiga A, et al (2016) Biochemical and genetic basis of indole-3-acetic acid (auxin phytohormone) degradation by the plant growth promoting rhizobacterium *Paraburkholderia phytofirmans* PsJN. Appl Environ Microbiol. https://doi.org/10.1128/AEM.01991-16

Doull JL, Vining LC (1990) Nutritional control of actinorhodin production by *Streptomyces coelicolor* A3(2): suppressive effects of nitrogen and phosphate. Appl Microbiol Biotechnol 32:449–454. https://doi.org/10.1007/BF00903781

Drew SW, Demain AL (1977) Effect of primary metabolites on secondary metabolism. Annu Rev Microbiol 31:343–356. https://doi.org/10.1146/annurev.mi.31.100177.002015

Dubey MK, Meena M, Aamir M, et al (2019) Regulation and role of metal ions in secondary metabolite production by microorganisms. Elsevier B.V.

Duca D, Lorv J, Patten CL, et al (1996) Indole-3-acetic acid in plant – microbe interactions. Can J Microbiol. https://doi.org/10.1139/m96-032

Duca D, Rose DR, Glick BR (2014) Characterization of a nitrilase and a nitrile hydratase from *Pseudomonas* sp. strain UW4 that converts indole-3-acetonitrile to indole-3-acetic acid. Appl Environ Microbiol 80:4640–4649. https://doi.org/10.1128/AEM.00649-14

Durakovic B (2017) Design of experiments application, concepts, examples: State of the art. Period Eng Nat Sci 5:421–439. https://doi.org/10.21533/pen.v5i3.145

Dutta D, Puzari KC, Gogoi R, Dutta P (2014) Endophytes: Exploitation as a tool in plant protection. Brazilian Arch Biol Technol 57:621–629. https://doi.org/10.1590/S1516-8913201402043

Egamberdieva D, Ahmad P (2018) Plant Microbiome: Stress Response, Microorgan. Springer Nature Singapore Pte Ltd, Singapore

Erica de OA (2015) Rizobacteria in the control of pest insects in agriculture. African J Plant Sci 9:368–373. https://doi.org/10.5897/ajps2015.1318

Espeso EA, Tilburn J, Arst HN, Peñalva MA (1993) pH regulation is a major determinant in expression of a fungal penicillin biosynthetic gene. EMBO J 12:3947–3956. https://doi.org/10.1002/j.1460-2075.1993.tb06072.x

Etminani F, Harighi B (2018) Isolation and identification of endophytic bacteria with plant growth promoting activity and biocontrol potential from wild pistachio trees. Plant Pathol J 34:208–217. https://doi.org/10.5423/PPJ.OA.07.2017.0158

Failla LJ, Niehaus WG (1986) Regulation of Zn^{2+} uptake and versicolorin A synthesis in a mutant strain of *Aspergillus parasiticus*. Exp Mycol 10:35–41. https://doi.org/10.1016/0147-5975(86)90028-9

Figueiredo M do VB, Bonifacio A, Rodrigues AC, Araujo FF de (2016) Plant growth-promoting rhizobacteria: Key Mechanisms of Action. Microbial-Mediated Induc Syst Resist Plants 1–226. https://doi.org/10.1007/978-981-10-0388-2

Forget G (1994) Balancing the need for pesticides with the risk to human health. Impact Pestic Use Heal Dev Ctries 2–16

Frank A, Saldierna Guzmán J, Shay J (2017) Transmission of bacterial endophytes. Microorganisms 5:70. https://doi.org/10.3390/microorganisms5040070

Frank A, Saldierna Guzmán J, Shay J (2017) Transmission of bacterial endophytes. microorganisms 5:70. https://doi.org/10.3390/microorganisms5040070

Frey DD, Engelhardt F, Greitzer EM (2003) A role for “one-factor-at-a-time” experimentation in parameter design. Res Eng Des 14:65–74. https://doi.org/10.1007/s00163-002-0026-9

Fu SF, Wei JY, Chen HW, et al (2015) Indole-3-acetic acid: A widespread physiological code in interactions of fungi with other organisms. Plant Signal Behav 10:. https://doi.org/10.1080/15592324.2015.1048052

Fukuda IM, Pinto CFF, Moreira CDS, et al (2018) Design of experiments (DoE) applied to pharmaceutical and analytical quality by design (QbD). Brazilian J Pharm Sci 54:1–16. https://doi.org/10.1590/s2175-97902018000001006

Galdiano Junior RF, Nascimbem Pedrinho EA, Luque Castellane TC, de Macedo Lemos EG (2011) Auxin-producing bacteria isolated from the roots of *Cattleya walkeriana*, an endangered Brazilian orchid, and their role in acclimatization. Rev Bras Cienc Do Solo 35:729–737. https://doi.org/10.1590/S0100-06832011000300008

Gana, A S (2011) The role of synthetic growth hormones in crop multiplication and improvement. African J Biotechnol 10:10330–10334. https://doi.org/10.5897/AJB10.301

Ganeshan G, Kumar AM (2007) *Pseudomonas fluorescens*, a potential bacterial antagonist to control plant diseases. J Plant Interact 9145:123–134. https://doi.org/10.1080/17429140600907043

Gardiner DM, Osborne S, Kazan K, Manners JM (2009) Low pH regulates the production of deoxynivalenol by *Fusarium graminearum*. Microbiology 155:3149–3156. https://doi.org/10.1099/mic.0.029546-0

Garza DR, Dutilh BE (2015) From cultured to uncultured genome sequences : metagenomics and modeling microbial ecosystems. Cell Mol Life Sci 72:4287–4308. https://doi.org/10.1007/s00018-015-2004-1

Gaspar T, Kevers C, Faivre-Rampant O, et al (2003) Changing concepts in plant hormone action. Vitr Cell Dev Biol - Plant 39:85–106. https://doi.org/10.1079/IVP2002393

Gautam CK, Madhav M, Sinha A, Jabez Osborne W (2016) VIT-CMJ2: Endophyte of

Agaricus bisporus in production of bioactive compounds. Iran J Biotechnol 14:19–24. https://doi.org/10.15171/ijb.1287

Giordano W, Avalos J, Cerda-Olmedo E, Domenech CE (1999) Nitrogen availability and production of bikaverin and gibberellins in *Gibberella fujikuroi*. FEMS Microbiol Lett 173:389–393. https://doi.org/10.1111/j.1574-6968.1999.tb13530.x

Glick BR (2012) Plant growth-promoting bacteria: Mechanisms and Applications. Scientifica (Cairo) 2012:. https://doi.org/http://dx.doi.org/10.6064/2012/963401

Glickmann E, Dessaux Y (1995) A critical examination of the specificity of the Salkowski reagent for indolic compounds produced by phytopathogenic bacteria. Appl Environ Microbiol 61:793–796. https://doi.org/10.1128/aem.61.2.793-796.1995

Gordon SA, Weber RP (1950) Colorimetric estimation of indoleacetic acid. 192–195

Goswami D, Parmar S, Vaghela H, et al (2015) Describing *Paenibacillus mucilaginosus* strain N3 as an efficient plant growth promoting rhizobacteria (PGPR). Cogent Food Agric 163:1–13. https://doi.org/10.1080/23311932.2014.1000714

Gouda S, Das G, Sen SK, et al (2016) Endophytes: A treasure house of bioactive compounds of medicinal importance. Front Microbiol 7:1–8. https://doi.org/10.3389/fmicb.2016.01538

Gray WM (2004) Hormonal regulation of plant growth and development. PLoS Biol 2:. https://doi.org/10.1371/journal.pbio.0020311

Grossmann K (2010) Auxin herbicides: Current status of mechanism and mode of action. Pest Manag Sci 66:113–120. https://doi.org/10.1002/ps.1860

Gunatilaka AAL (2006) A.A.L. Gunatilaka. Natural products from plant-associated microorganisms: Distribution, structural diversity, bioactivity and implication of their occurrence. J.Net.Prod . 2006, 69: 509-526. J Nat Prod 69:509–526

Guo H, Glaeser SP, Alabid I, et al (2017) The abundance of endofungal bacterium *Rhizobium radiobacter* (syn. *Agrobacterium tumefaciens*) increases in its fungal host *Piriformospora indica* during the tripartite sebacinalean symbiosis with higher plants. Front Microbiol 8:1–13. https://doi.org/10.3389/fmicb.2017.00629

Hagaggi NSA, Mohamed AAA (2020) Enhancement of *Zea mays* (L .) growth performance using indole acetic acid producing endophyte *Mixta theicola* isolated from Solenostemma. South African J Bot 000:1–8. https://doi.org/10.1016/j.sajb.2020.02.034

Halmann M (1990) Synthetic plant growth regulators. Adv Agron 43:47–105. https://doi.org/10.1016/S0065-2113(08)60476-9

Hasegawa M, Kishino H, Yano T aki (1985) Dating of the human-ape splitting by a molecular clock of mitochondrial DNA. J Mol Evol 22:160–174. https://doi.org/10.1007/BF02101694

Hasuty A, Choliq A, Hidayat I (2018) Production of Indole Acetic Acid (IAA) by *Serratia marcescens* subsp. marcescens and *Rhodococcus* aff. qingshengii. Int J Agric Technol 14:299–312

Hata H, Natori T, Mizuno T, et al (2016) Phylogenetics of family Enterobacteriaceae and proposal to reclassify *Escherichia hermannii* and *Salmonella subterranea* as *Atlantibacter hermannii* and *Atlantibacter subterranea* gen. nov., comb. nov. Microbiol Immunol 303–311. https://doi.org/10.1111/1348-0421.12374

Hegde S, Bhadri G, Narsapur K, et al (2013) Statistical optimization of medium components by Response Surface Methodology for enhanced production of bacterial cellulose by *Gluconacetobacter persimmonis*. Bioprocess Biotech 4:1–5. https://doi.org/10.4172/2155-9821.1000142

Herlina L, Pukan KK, Mustikaningtyas D (2017) The endophytic bacteria producing IAA (Indole Acetic Acid) in *Arachis hypogaea*. 1:31–35. https://doi.org/10.13057/cellbioldev/t010106

Hernández-Soberano C, Ruíz-Herrera LF, Valencia-Cantero E (2020) Endophytic bacteria *Arthrobacter agilis* UMCV2 and *Bacillus methylotrophicus* M4-96 stimulate achene germination, *in vitro* growth, and greenhouse yield of strawberry (Fragaria × ananassa). Sci Hortic (Amsterdam) 261:109005. https://doi.org/10.1016/j.scienta.2019.109005

Herrero A, Muro-pastor AM, Flores E (2001) MINIREVIEW Nitrogen control in Cyanobacteria. 183:411–425. https://doi.org/10.1128/JB.183.2.411

Hobbs G, Frazer CM, Gardner DCJ, et al (1990) Pigmented antibiotic production by *Streptomyces coelicolor* A3(2): Kinetics and the influence of nutrients. J Gen Microbiol 136:2291–2296. https://doi.org/10.1099/00221287-136-11-2291

Huang J, Lu C, Qian X, et al (2011) Effect of salinity on the growth, biological activity and secondary metabolites of some marine fungi. Acta Oceanol Sin 30:118–123. https://doi.org/10.1007/s13131-011-0126-3

Hurst CJ, Crawford RL, Garland JL, et al (2016) Manual of environmental microbiology, Third edit. American Society for Microbiology Press, Washington, DC. 20036–2904, USA

Hussain K, Hameed S, Shahid M, et al (2015) First report of *Providencia vermicola* strains characterized for enhanced rapeseed growth attributing parameters. Int J Agric Biol 17:1110–1116. https://doi.org/10.17957/IJAB/15.0033

Islam F, Yasmeen T, Ali Q, et al (2016) Copper-resistant bacteria reduces oxidative stress and uptake of copper in lentil plants: potential for bacterial bioremediation. 220–233. https://doi.org/10.1007/s11356-015-5354-1

Janda JM, Abbott SL (2007) 16S rRNA gene sequencing for bacterial identification in the diagnostic laboratory: Pluses , Perils , and Pitfalls. J Clin Microbiol 45:2761–2764. https://doi.org/10.1128/JCM.01228-07

Jiang H, Qi P, Wang T, et al (2018) Isolation and characterization of halotolerant phosphate-solubilizing microorganisms from saline soils. 3 Biotech 8:1–8. https://doi.org/10.1007/s13205-018-1485-7

Johnson TR, Case CL (2014) Laboratory Experiments in Microbiology, Tenth Edit. Pearson, Harlow

Kalayu G (2019) Phosphate solubilizing microorganisms: Promising approach as biofertilizers. Int J Agron 2019:. https://doi.org/10.1155/2019/4917256

Kandel S, Joubert P, Doty S (2017) Bacterial endophyte colonization and distribution within plants. Microorganisms 5:77. https://doi.org/10.3390/microorganisms5040077

Kasahara H (2016) Current aspects of auxin biosynthesis in plants. Biosci Biotechnol Biochem 80:34–42. https://doi.org/10.1080/09168451.2015.1086259

Katoh K, Rozewicki J, Yamada KD (2018) MAFFT online service: Multiple sequence alignment, interactive sequence choice and visualization. Brief Bioinform 20:1160–1166. https://doi.org/10.1093/bib/bbx108

Keller NP, Nesbitt C, Sarr B, et al (1997) pH regulation of sterigmatocystin and aflatoxin biosynthesis in *Aspergillus* spp. Phytopathology 87:643–648. https://doi.org/10.1094/PHYTO.1997.87.6.643

Khalifa AYZ, Alsyeeh AM, Almalki MA, Saleh FA (2016) Characterization of the plant growth promoting bacterium, *Enterobacter cloacae* MSR1, isolated from roots of non-nodulating *Medicago sativa*. Saudi J Biol Sci 23:79–86. https://doi.org/10.1016/j.sjbs.2015.06.008

Khan AL, Halo BA, Elyassi A, et al (2016a) Indole acetic acid and ACC deaminase from endophytic bacteria improves the growth of *Solanum lycopersicum*. Electron J Biotechnol 21:58–64. https://doi.org/10.1016/j.ejbt.2016.02.001

Khan Z, Rho H, Firrincieli A, et al (2016b) Growth enhancement and drought tolerance of hybrid poplar upon inoculation with endophyte consortia. Curr Plant Biol 6:38–47. https://doi.org/10.1016/j.cpb.2016.08.001

Khunthongpan S, Sumpavapol P, Tanasupawat S, et al (2013) *Providencia thailandensis* sp. nov., isolated from seafood processing wastewater. J Gen Appl Microbiol 59:185–190. https://doi.org/10.2323/jgam.59.185

Kochar M, Upadhyay A, Srivastava S (2011) Indole-3-acetic acid biosynthesis in the biocontrol strain *Pseudomonas fluorescens* Psd and plant growth regulation by hormone overexpression. Res Microbiol 162:426–435. https://doi.org/10.1016/j.resmic.2011.03.006

Kopittke PM, Menzies NW, Wang P, et al (2019) Soil and the intensification of agriculture for global food security. Environ Int 132:105078. https://doi.org/10.1016/j.envint.2019.105078

Kumar S, Khapre A (2017) Phytocapping technology for sustainable management of landfill sites, Environmen. Springer

Kumar S, Stecher G, Li M, et al (2018) MEGA X: Molecular evolutionary genetics analysis across computing platforms. Mol Biol Evol 35:1547–1549. https://doi.org/10.1093/molbev/msy096

Kumla J, Suwannarach N, Matsui K, Lumyong S (2020) Biosynthetic pathway of indole-3-acetic acid in ectomycorrhizal fungi collected from northern Thailand. PLoS One 15:1–15. https://doi.org/10.1371/journal.pone.0227478

Kunamneni A, Ellaiah P (2002) Response surface optimization of the critical medium components for the production of alkaline protease by a newly isolated *Bacillus* sp. J Pharm Pharm Sci 3:272–278

Kunkel BN, Harper CP (2018) The roles of auxin during interactions between bacterial plant pathogens and their hosts. J Exp Bot 69:245–254. https://doi.org/10.1093/jxb/erx447

Lacava PT, Li W, Araujo WL, et al (2007) The endophyte *Curtobacterium flaccumfaciens* reduces symptoms caused by xylella fastidiosa in *Catharanthus roseus*. Jounal Microbiol 45:388–393

Lahlali R, Bajii M, Jijakli MH (2007) Isolation and evaluation of bacteria and fungi as biological control agents. Commun Agric Appl Biol Sci 973–982

Lahlali R, Bajii M, Jijakli MH (2007) Isolation and evaluation of bacteria and fungi as biological control agents. Commun Agric Appl Biol Sci 973–982

Lalit MS (2002) Plant Growth and Development. Hormones and Environment. 155–168

LeClere S, Tellez R, Rampey RA, et al (2002) Characterization of a family of IAA-amino acid conjugate hydrolases from *Arabidopsis*. J Biol Chem 277:20446–20452. https://doi.org/10.1074/jbc.M111955200

Lefebvre O, Ha Nguyen TT, Al-Mamun A, et al (2010) T-RFLP reveals high - Proteobacteria diversity in microbial fuel cells enriched with domestic wastewater. J Appl Microbiol 109:839–850. https://doi.org/10.1111/j.1365-2672.2010.04735.x

Li M, Guo R, Yu F, et al (2018) Indole-3-acetic acid biosynthesis pathways in the plant-beneficial bacterium *Arthrobacter pascens* zz21. Int J Mol Sci 19:. https://doi.org/10.3390/ijms19020443

Lind AL, Smith TD, Saterlee T, et al (2016) Regulation of secondary metabolism by the velvet complex is temperature-responsive in *Aspergillus*. G3 Genes, Genomes, Genet 6:4023–4033. https://doi.org/10.1534/g3.116.033084

Ljung K, Hull AK, Kowalczyk M, et al (2002) Biosynthesis, conjugation, catabolism and homeostasis of indole-3-acetic acid in *Arabidopsis thaliana*. Plant Mol Biol 50:309–332. https://doi.org/10.1023/A:1016024017872

Lodewyckx C, Vangronsveld J, Porteous F, et al (2015) Critical reviews in plant sciences endophytic bacteria and their potential applications. 2689:37–41. https://doi.org/10.1080/0735-260291044377

López JLC, Pérez JAS, Sevilla JMF, et al (2003) Production of lovastatin by *Aspergillus terreus*: effects of the C:N ratio and the principal nutrients on growth and metabolite production. Enzyme Microb Technol 33:270–277. https://doi.org/10.1016/S0141-0229(03)00130-3

Lounès A, Lebrihi A, Benslimane C, et al (1996) Regulation of spiramycin synthesis in *Streptomyces ambofaciens*: Effects of glucose and inorganic phosphate. Appl Microbiol Biotechnol 45:204–211. https://doi.org/10.1007/s002530050671

Luchese RH, Harrigan WF (1993) Biosynthesis of aflatoxin-the role of nutritional factors. J Appied Bacteriol 5–14

Ludwig-Müller J (2011) Auxin conjugates: Their role for plant development and in the evolution of land plants. J Exp Bot 62:1757–1773. https://doi.org/10.1093/jxb/erq412

Macedo-Raygoza GM, Valdez-Salas B, Prado FM, et al (2019) *Enterobacter cloacae*, an endophyte that establishes a nutrient-transfer symbiosis with banana plants and protects against the black sigatoka pathogen. Front Microbiol 10:1–15. https://doi.org/10.3389/fmicb.2019.00804

Machackova I, Zazimalova E, George EF (2008) Plant Growth Regulators: Auxins, their Analogues and Inhibitors, Plant Prop. Springer Netherlands, Netherlands

Madhaiyan M, Poonguzhali S, Lee JS, et al (2010) *Enterobacter arachidis* sp. nov., a plant-growth-promoting diazotrophic bacterium isolated from rhizosphere soil of groundnut. Int J Syst Evol Microbiol 60:1559–1564. https://doi.org/10.1099/ijs.0.013664-0

Maela PM, Serepa-dlamini MH (2019) Current understanding of bacterial endophytes , their diversity, colonization and their roles in promoting plant growth. Appl Microbiol Open Access 5:1–12. https://doi.org/10.4172/2471-9315.1000157

Malik DK, Sindhu SS (2011) Production of indole acetic acid by *Pseudomonas* sp.: Effect of coinoculation with *Mesorhizobium* sp. Cicer on nodulation and plant growth of chickpea (*Cicer arietinum*). Physiol Mol Biol Plants 17:25–32. https://doi.org/10.1007/s12298-010-0041-7

Manjunath M, Taylor P, Prasanna R, Sharma P (2013) Archives of Agronomy and Soil Science Developing PGPR consortia using novel genera *Providencia* and *Alcaligenes* along with Cyanobacteria for wheat. Arch Agron Soil Sci 57:873–887

Mano Y, Nemoto K (2012) The pathway of auxin biosynthesis in plants. J Exp Bot 63:2853–2872. https://doi.org/10.1093/jxb/ers091

Martín JF (2004) Phosphate control of the biosynthesis of antibiotics and other secondary metabolites is mediated by the PhoR-PhoP system: An unfinished story. 186:5197–5201. https://doi.org/10.1128/JB.186.16.5197

Martin JF, Demain AL (1980) Control of antibiotic biosynthesis. Microbiol Rev 44:230–251

Masclaux-Daubresse C, Daniel-Vedele F, Dechorgnat J, et al (2010) Nitrogen uptake, assimilation and remobilization in plants: Challenges for sustainable and productive agriculture. Ann Bot 105:1141–1157. https://doi.org/10.1093/aob/mcq028

Mashiguchi K, Tanaka K, Sakai T, et al (2011) The main auxin biosynthesis pathway in *Arabidopsis*. Pnas 108:18512–18517. https://doi.org/10.1073/pnas.1108434108/-/DCSupplemental.www.pnas.org/cgi/doi/10.1073/pnas.1108434108

Masuma R, Tanaka Y, Ōmura S, Tanaka H (1986) Production of nanaomycin and other antibiotics by phosphate-depressed fermentation using phosphate-trapping agents. J Antibiot (Tokyo) 39:1557–1564. https://doi.org/10.7164/antibiotics.39.1557

Mateo-Sagasta J, Marjani S, Turral H, Burke J (2017) Water pollution from agriculture: a global review. FAO y IWMI 35. https://doi.org/http://www.fao.org/3/a-i7754e.pdf

Miche L, Balandreau J (2001) Effects of rice seed surface sterilization with hypchlorite on iniculated *Burkholderia vietnamiesis*. Appl Environ Microbiol 67:3046–3052. https://doi.org/10.1128/AEM.67.7.3046-3052.2001

Mohite B (2013) Isolation and characterization of indole acetic acid (IAA) producing bacteria from rhizospheric soil and its effect on plant growth. J Soil Sci Plant Nutr 13:638–649. https://doi.org/10.4067/S0718-95162013005000051

Morales-lópez S, Yepes JA, Prada-herrera JC, Torres-jiménez A (2019) *Enterobacteria* in the 21 st century : a review focused on taxonomic changes. J Infect Dev Ctries. https://doi.org/10.3855/jidc.11216

Muzzamal H, Sarwar R, Sajid I, Hasnain S (2012) Isolation, identification and screening of endophytic bacteria antagonistic to biofilm formers. Pak J Zool 44:249–257

Myo EM, Ge B, Ma J, et al (2019) Indole-3-acetic acid production by *Streptomyces fradiae* NKZ-259 and its formulation to enhance plant growth. BMC Microbiol 1–14. https://doi.org/https://doi.org/10.1186/s12866-019-1528-1

Nalini G, Rao YRKVT (2014) Effect of different carbon and nitrogen sources on growth and indole acetic acid production by *Rhizobium* species Isolated from cluster bean [*Cyamopsis tetragonoloba* (L .)]. Br Microbiol Researh J 4:1189–1197

Nannipieri P, Giagnoni L, Landi L, Renella G (2011) Phosphorus in action, Phosphorus. Springer, New York

Nasrabadi MRN, Razavi SH (2010) Enhancement of canthaxanthin production from *Dietzia natronolimnaea* HS-1 in a fed-batch process using trace elements and statistical methods. Brazilian J Chem Eng 27:517–529. https://doi.org/10.1590/s0104-66322010000400003

Nautiyal CS (1999) An efficient microbiological growth medium for screening phosphate solubilizing microorganisms. FEMS Microbiol Lett 170:265–270. https://doi.org/10.1016/S0378-1097(98)00555-2

Neidhardt FC, Magasanik B (1957) Reversal of the glucose inhibition of histidase biosynthesis in *Aerobacter aerogenes*. J Bacteriol 73:253–259. https://doi.org/10.1128/jb.73.2.253-259.1957

Nemoto K, Hara M, Suzuki M, et al (2009) The *NtAMI1* gene functions in cell division of tobacco BY-2 cells in the presence of indole-3-acetamide. FEBS Lett 583:487–492. https://doi.org/10.1016/j.febslet.2008.12.049

Netzker T, Fischer J, Weber J, et al (2015) Microbial communication leading to the activation of silent fungal secondary metabolite gene clusters. Front Microbiol 6:1–13. https://doi.org/10.3389/fmicb.2015.00299

Nor NM, Mohamed MS, Loh TC, et al (2017) Comparative analyses on medium optimization using one-factor-at-a-time, response surface methodology, and artificial neural network for lysine – methionine biosynthesis by *Pediococcus pentosaceus* RF-1. Biotechnol Biotechnol Equip 5:935–947. https://doi.org/10.1080/13102818.2017.1335177

Normanly J, Cohen JD, Fink GR (1993) *Arabidopsis thaliana* auxotrophs reveal a tryptophan-independent biosynthetic pathway for indole-3-acetic acid. Proc Natl Acad Sci U S A 90:10355–10359. https://doi.org/10.1073/pnas.90.21.10355

Novák O, Hényková E, Sairanen I, et al (2012) Tissue-specific profiling of the *Arabidopsis thaliana* auxin metabolome. Plant J 72:523–536. https://doi.org/10.1111/j.1365-313X.2012.05085.x

Nutaratat P, Monprasit A, Srisuk N (2017) High-yield production of indole-3-acetic acid by *Enterobacter* sp . DMKU-RP206 , a rice phyllosphere bacterium that possesses plant growth-promoting traits. 3 Biotech 7:1–15. https://doi.org/10.1007/s13205-017-0937-9

Nutaratat P, Srisuk N, Arunrattiyakorn P, Limtong S (2016) Fed-batch fermentation of indole-3-acetic acid production in stirred tank fermenter by red yeast *Rhodosporidium paludigenum*. Biotechnol Bioprocess Eng 21:414–421. https://doi.org/10.1007/s12257-015-0819-0

Oberhansli T, Defago G, Haas D (1991) Indole-3-acetic acid (IAA) synthesis in the biocontrol strain CHA0 of *Pseudomonas fluorescens*: Role of tryptophan side chain oxidase. J Gen Microbiol 137:2273–2279. https://doi.org/10.1099/00221287-137-10-2273

Ohyama T (2010) Nitrogen as a major essential element of plants. Nitrogen Assim plants 1–17

Okada BK, Seyedsayamdost MR (2017) Antibiotic dialogues: Induction of silent biosynthetic gene clusters by exogenous small molecules. FEMS Microbiol Rev

41:19–33. https://doi.org/10.1093/femsre/fuw035

Olanrewaju OS, Glick BR, Babalola OO (2017) Mechanisms of action of plant growth promoting bacteria. World J Microbiol Biotechnol 33:0. https://doi.org/10.1007/s11274-017-2364-9

Olatunji D, Geelen D, Verstraeten I (2017) Control of endogenous auxin levels in plant root development. Int J Mol Sci 18:. https://doi.org/10.3390/ijms18122587

Palaniyandi SA, Yang SH, Zhang L, Suh JW (2013) Effects of Actinobacteria on plant disease suppression and growth promotion. Appl Microbiol Biotechnol 97:9621–9636. https://doi.org/10.1007/s00253-013-5206-1

Pan CM, Fan YT (2008) Statistical optimization of process parameters on biohydrogen production from glucose by *Clostridium* sp . Fanp2. 99:3146–3154. https://doi.org/10.1016/j.biortech.2007.05.055

Pan R, Bai X, Chen J, et al (2019) Exploring structural diversity of microbe secondary metabolites using OSMAC strategy: A literature review. Front Microbiol 10:1–20. https://doi.org/10.3389/fmicb.2019.00294

Panda BP, Ali M, Javed S (2007) Fermentation process optimization. Res. J. Microbiol. 2:201–208

Panigrahi S, Mohanty S, Rath CC (2019) Characterization of endophytic bacteria *Enterobacter cloacae* MG00145 isolated from *Ocimum sanctum* with Indole Acetic Acid (IAA) production and plant growth promoting capabilities against selected crops. South African J Bot 000:1–10. https://doi.org/10.1016/j.sajb.2019.09.017

Pařízková B, Pernisová M, Novák O (2017) What has been seen cannot be unseen-detecting auxin *in vivo*. Int J Mol Sci 18:1–26. https://doi.org/10.3390/ijms18122736

Park JY, Fox LK, Seo KS, et al (2011) Comparison of phenotypic and genotypic methods for the species identification of coagulase-negative staphylococcal isolates from bovine intramammary infections. Vet Microbiol 147:142–148. https://doi.org/10.1016/j.vetmic.2010.06.020

Patten CL, Glick BR (1996) Bacterial biosynthesis of indole-3-acetic acid. Can J Microbiol 42:207–220. https://doi.org/10.1139/m96-032

Patten CL, Glick BR (2002a) Role of *Pseudomonas putida* indoleacetic acid in development of the host plant root system. Appl Environ Microbiol 68:3795–801. https://doi.org/10.1128/AEM.68.8.3795

Patten CL, Glick BR (2002b) Regulation of indoleacetic acid production in *Pseudomonas putida* GR12-2 by tryptophan and the stationary-phase sigma factor RpoS. Can J Microbiol 48:635–642. https://doi.org/10.1139/w02-053

Peker N, Garcia-Croes S, Dijkhuizen B, et al (2019) A comparison of three different bioinformatics analyses of the 16S-23S rRNA encoding region for bacterial identification. Front Microbiol 10:1–13. https://doi.org/10.3389/fmicb.2019.00620

Petti CA (2007) Detection and identification of microorganisms by gene amplification and sequencing. Clin Infect Dis 44:1108–1114. https://doi.org/10.1086/512818

Petti CA, Polage CR, Schreckenberger P (2005) The role of 16S rRNA gene sequencing in identification. J Clin Microbiol 43:6123–6125. https://doi.org/10.1128/JCM.43.12.6123

Pettit RK (2011) Minireview Small-molecule elicitation of microbial secondary metabolites. 4:471–478. https://doi.org/10.1111/j.1751-7915.2010.00196.x

Pickens LB, Tang Y, Chooi Y-H (2011) Metabolic engineering for the production of natural products. Annu Rev Chem Biomol Eng 23:211–236. https://doi.org/10.1038/jid.2014.371

Plackett RL, Burman JP (1946) The design of optimum multifactorial experiments. Oxford Journals 33:305–325

Pollmann S, Düchting P, Weiler EW (2009) Tryptophan-dependent indole-3-acetic acid biosynthesis by “IAA-synthase” proceeds via indole-3-acetamide. Phytochemistry 70:523–531. https://doi.org/10.1016/j.phytochem.2009.01.021

Pollmann S, Neu D, Weiler EW (2003) Molecular cloning and characterization of an amidase from *Arabidopsis thaliana* capable of converting indole-3-acetamide into the plant growth hormone, indole-3- acetic acid. Phytochemistry 62:293–300

Poza-Carrión C, Jiménez-Vicente E, Navarro-Rodríguez M, et al (2014) Kinetics of *nif* gene expression in a nitrogen-fixing bacterium. J Bacteriol 196:595–603. https://doi.org/10.1128/JB.00942-13

Prinsen E, Costacurta A, Michiels K, et al (1993) *Azospirillum brasilence* indole-3-acetic acid biosynthesis: Evidence for a non-tryptophan dependent pathway. 609–615

Puri A, Padda KP, Chanway CP (2017) Beneficial effects of bacterial endophytes on forest tree species, Sustainabl. Springer International Publishing

Quast C, Pruesse E, Yilmaz P, et al (2013) The SILVA ribosomal RNA gene database project: Improved data processing and web-based tools. Nucleic Acids Res 41:590–596. https://doi.org/10.1093/nar/gks1219

Rahimi M, Mokhtari J (2018) Modeling and Optimization of Waterproof-Breathable Thermo- Regulating Core-Shell Nanofiber / Net Structured Membrane for Protective Clothing Applications. https://doi.org/10.1002/pen.24776

Rajput KN, Patel KC, Trivedi UB (2016) Screening and selection of medium components for cyclodextrin glucanotransferase production by new alkaliphile *Microbacterium terrae* KNR 9 using Plackett-Burman Design. 2016:. https://doi.org/10.1155/2016/3584807

Ramalashmi K, Prasanna VK, Magesh K, et al (2018) A potential surface sterilization technique and culture media for the isolation of endophytic bacteria from *Acalypha indica* and its antibacterial activity. J Med Plants Stud 6:181–184

Rampey RA, LeClere2 S, Kowalczyk M, et al (2004) A family of auxin-conjugate hydrolases that contributes to free indole-3-acetic acid levels during *Arabidopsis* germination. Plant Physiol 135:978–988. https://doi.org/10.1104/pp.104.039677

Rana A, Saharan B, Joshi M, et al (2011) Identification of multi-trait PGPR isolates and evaluating their potential as inoculants for wheat. Ann Microbiol 61:893–900. https://doi.org/10.1007/s13213-011-0211-z

Reilly CO, Turner PD (2003) The nitrilase family of CN hydrolysing enzymes – a comparative study. 1161–1174. https://doi.org/10.1046/j.1365-2672.2003.02123.x

Ribeiro VP, Marriel IE, Sousa SM de, et al (2018) Endophytic *Bacillus* strains enhance pearl millet growth and nutrient uptake under low-P. Brazilian J Microbiol 49:40–46. https://doi.org/10.1016/j.bjm.2018.06.005

Rincón A, Priha O, Sotta B, et al (2003) Comparative effects of auxin transport inhibitors on rhizogenesis and mycorrhizal establishment of spruce seedlings inoculated with *Laccaria bicolor*. Tree Physiol 23:785–791. https://doi.org/10.1093/treephys/23.11.785

Romero-Rodríguez A, Maldonado-Carmona N, Ruiz-Villafán B, et al (2018) Interplay between carbon, nitrogen and phosphate utilization in the control of secondary metabolite production in *Streptomyces*. Antonie van Leeuwenhoek, Int J Gen Mol Microbiol 111:761–781. https://doi.org/10.1007/s10482-018-1073-1

Romero-Rodríguez A, Rocha D, Ruiz-Villafán B, et al (2017) Carbon catabolite regulation in *Streptomyces*: new insights and lessons learned. World J Microbiol Biotechnol 33:0. https://doi.org/10.1007/s11274-017-2328-0

Rosenblueth M, Martínez-Romero E (2006) Bacterial endophytes and their interactions with hosts. Mol Plant-Microbe Interact 19:827–837. https://doi.org/10.1094/MPMI-19-0827

Rückert GVON, Giani A (2004) Effect of nitrate and ammonium on the growth and protein concentration of *Microcystis viridis* Lemmermann (Cyanobacteria). Rev Bras Bot 27:325–331

Ruiz B, Chávez A, Forero A, et al (2010) Production of microbial secondary metabolites: Regulation by the carbon source. Crit Rev Microbiol 36:146–167. https://doi.org/10.3109/10408410903489576

Ruiz Rosquete M, Barbez E, Kleine-Vehn J (2012) Cellular auxin homeostasis: Gatekeeping is housekeeping. Mol Plant 5:772–786. https://doi.org/10.1093/mp/ssr109

Ryan RP, Germaine K, Franks A, et al (2008) Bacterial endophytes: recent developments and applications. FEMS Microbiol Lett 278:1–9. https://doi.org/10.1111/j.1574-6968.2007.00918.x

Saedon JB, Soo SL, Aspinwall DK, et al (2012) Prediction and Optimization of Tool Life in Micromilling AISI D2 (~ 62 HRC) Hardened Steel. Eng Procedia 41:1674–1683. https://doi.org/10.1016/j.proeng.2012.07.367

Saharan BS (2015) Evaluation of rhizospheric bacteria from *Ocimum* sp. as potential PGPR. J Microb Biochem Technol 07:88–95. https://doi.org/10.4172/1948-5948.1000187

Sánchez S, Chávez A, Forero A, et al (2010) Carbon source regulation of antibiotic production. J Antibiot (Tokyo) 63:442–459. https://doi.org/10.1038/ja.2010.78

Sanchez S, Demain AL (2002) Metabolic regulation of fermentation processes. Enzyme Microb Technol 31:895–906. https://doi.org/10.1016/S0141-0229(02)00172-2

Sánchez S, Paniagua L, Mateos RC, et al (1981) Nitrogen regulation of Penicillin G biosynthesis in *Penicillium Chrysogenum*. Ferment Prod 147–154. https://doi.org/10.1016/b978-0-08-025385-5.50031-6

Santi C, Bogusz D, Franche C (2015) Biological nitrogen fixation in non-legume plants. Ann Bot. https://doi.org/10.1093/aob/mct048

Santoyo G, Moreno-Hagelsieb G, del Carmen Orozco-Mosqueda M, Glick BR (2016) Plant growth-promoting bacterial endophytes. Microbiol Res 183:92–99. https://doi.org/10.1016/j.micres.2015.11.008

Sasirekha B, Shivakumar S, Sullia SB (2012) Statistical optimization for improved indole-3-acetic acid (iaa) production by *Pseudomonas aeruginosa* and demonstration of enhanced plant growth promotion. J Soil Sci Plant Nutr 12:863–873. https://doi.org/10.4067/s0718-95162012005000038

Sauer M, Robert S, Kleine-Vehn J (2013) Auxin: Simply complicated. J Exp Bot 64:2565–2577. https://doi.org/10.1093/jxb/ert139

Sentausa E, Fournier P (2013) Advantages and limitations of genomics in prokaryotic taxonomy. Clin Microbiol Infect 19:790–795. https://doi.org/10.1111/1469-0691.12181

Senthil N, Elangovan K, Rajkumar S, Bavya M (2010) Studies on indole acetic acid production and phosphate solubilization from forest soil bacteria. Biotechnology 4:3–6

Senthilraj R, Prasad GS, Janakiraman K (2016) Sequence-based identification of microbial contaminants in non-parenteral products. Brazilian J Pharm Sci 52:329–336. https://doi.org/10.1590/S1984-82502016000200011

Sharma B, Jha DK (2015) Role of nitrogen sources in regulation of fungal secondary metabolism. Fungal Biomol Sources, Appl Recent Dev 213–224. https://doi.org/10.1002/9781118958308.ch16

Sharon JA, Hathwaik LT, Glenn GM, et al (2016) Isolation of efficient phosphate solubilizing bacteria capable of enhancing tomato plant growth. J Soil Sci Plant Nutr 16:525–536

Sheikhian L, Bina S (2016) Simultaneous extraction and HPLC determination of 3-indole butyric acid and 3-indole acetic acid in pea plant by using ionic liquid-modified silica as sorbent. J Chromatogr B Anal Technol Biomed Life Sci 1010:34–43. https://doi.org/10.1016/j.jchromb.2015.11.047

Shi T-Q, Peng H, Zeng S-Y, et al (2017) Microbial production of plant hormones: Opportunities and challenges. Bioengineered 8:124–128. https://doi.org/10.1080/21655979.2016.1212138

Shi Y, Lou K, Li C (2009) Promotion of plant growth by phytohormone-producing endophytic microbes of sugar beet. Biol Fertil Soils 45:645–653. https://doi.org/10.1007/s00374-009-0376-9

Shoebitz M, Ribaudo CM, Pardo MA, et al (2009) Plant growth promoting properties of a strain of *Enterobacter ludwigii* isolated from Lolium perenne rhizosphere. Soil Biol Biochem 41:1768–1774. https://doi.org/10.1016/j.soilbio.2007.12.031

Silva CF, Vitorino LC, Mendonça MAC, et al (2019) Screening of plant growth-promoting endophytic bacteria from the roots of the medicinal plant *Aloe vera*. South African J Bot 000:1–14. https://doi.org/10.1016/j.sajb.2019.09.019

Singh DP, Singh BH, Prabha R (2016) Microbial Inoculants in Sustainable Agricultural Productivity. Springer.

Singh V, Haque S, Niwas R, et al (2017) Strategies for fermentation medium optimization: An in-depth review. Front Microbiol 7:. https://doi.org/10.3389/fmicb.2016.02087

Sitbon F, Östin A, Sundberg B, et al (1993) Conjugation of indole-3-acetic acid (IAA) in wild-type and IAA-overproducing transgenic tobacco plants, and identification of the main conjugates by frit-fast atom bombardment liquid chromatography-mass

spectrometry. Plant Physiol 101:313–320. https://doi.org/10.1104/pp.101.1.313

Soni P, Singh M, Kamble AL, Banerjee UC (2007) Response surface optimization of the critical medium components for carbonyl reductase production by *Candida viswanathii* MTCC 5158. Bioresour Technol 98:829–833. https://doi.org/10.1016/j.biortech.2006.03.008

Spaepen S, Vanderleyden J (2011) Auxin and plant-microbe interactions. Cold Spring Harb Perspect Biol 3:1–13. https://doi.org/10.1101/cshperspect.a001438

Spaepen S, Vanderleyden J, Remans R (2007) Indole-3-acetic acid in microbial and microorganism-plant signaling. FEMS Microbiol Rev 31:425–448. https://doi.org/10.1111/j.1574-6976.2007.00072.x

Spížek J, Tichý P (1995) Some aspects of overproduction of secondary metabolites. Folia Microbiol (Praha) 40:43–50. https://doi.org/10.1007/BF02816527

Srisuk N, Sakpuntoon V, Nutaratat P (2018) Production of indole-3-acetic acid by *Enterobacter* sp. DMKU-rp206 using sweet whey as a low-cost feed stock. J Microbiol Biotechnol 28:1511–1516. https://doi.org/10.4014.jmb/1805.04043

Sugawara S, Mashiguchi K, Tanaka K, et al (2015) Distinct characteristics of indole-3-acetic acid and phenylacetic acid, two common auxins in plants. Plant Cell Physiol 56:1641–1654. https://doi.org/10.1093/pcp/pcv088

Suhandono S, Kusumawardhani MK, Aditiawati P (2016) Isolation and molecular identification of endophytic bacteria from rambutan fruits (Nephelium lappaceum L.) Cultivar Binjai. HAYATI J Biosci 23:39–44. https://doi.org/10.1016/j.hjb.2016.01.005

Susilowati DN, Sudiana IM, Mubarik NR, Suwanto A (2015) Species and functional diversity of rhizobacteria of rice plant in the Coastal Soils of Indonesia. Indones J Agric Sci 16:39. https://doi.org/10.21082/ijas.v16n1.2015.p39-50

Syed MB, Seraman S, Rajendran A, Thangavelu V (2013) Optimization of compactin production by Plackett-Burman Method using *Penicillium citrinum*. Int J ChemTech Res 5:1393–1400

Tamošiūnė I, Stanienė G, Haimi P, et al (2018) Endophytic *Bacillus* and *Pseudomonas* spp. modulate apple shoot growth, cellular redox balance, and protein expression under *in vitro* conditions. Front Plant Sci 9:1–14. https://doi.org/10.3389/fpls.2018.00889

Teale WD, Paponov IA, Palme K (2006) Auxin in action: Signalling, transport and the control of plant growth and development. Nat Rev Mol Cell Biol 7:847–859. https://doi.org/10.1038/nrm2020

Tenorio-salgado S, Tinoco R, Vazquez-duhalt R, et al (2013) Identification of volatile compounds produced by the bacterium *Burkholderia tropica* that inhibit the growth of fungal pathogens. Bioengineered 4:236–243. https://doi.org/http://dx.doi.org/10.4161/bioe.23808

Thi Nguyen HY, Tran GB (2018) Optimization of fermentation conditions and media for production of glucose isomerase from *Bacillus megaterium* using Response Surface Methodology. Scientifica (Cairo) 2018:. https://doi.org/10.1155/2018/6842843

Tilman D, Cassman KG, Matson PA, et al (2002) Agricultural sustainability and intensive production practices. Nature 418:671–677. https://doi.org/10.1038/nature01014

Toldrá F (2008) Meat Biotechnology, Meat Biote. Springer Science + Business Media,

LLC, New York

Torres M, Llamas I, Torres B, et al (2020) Growth promotion on horticultural crops and antifungal activity of *Bacillus velezensis* XT1. Appl Soil Ecol 150:103453. https://doi.org/10.1016/j.apsoil.2019.103453

Tsavkelova EA, Klimova SY, Cherdyntseva TA, Netrusov AI (2006) Microbial producers of plant growth stimulators and their practical use: A review. Appl Biochem Microbiol 42:117–126. https://doi.org/10.1134/S0003683806020013

Tsugafune S, Mashiguchi K, Fukui K, et al (2017) Yucasin DF , a potent and persistent inhibitor of auxin biosynthesis in plants. Sci Rep 1–13. https://doi.org/10.1038/s41598-017-14332-w

Tudzynski B (2014) Nitrogen regulation of fungal secondary metabolism in fungi. Front Microbiol 5:1–15. https://doi.org/10.3389/fmicb.2014.00656

United Nations (2015) The future of food and agriculture: Trends and challenges

United Nations (2019) World population prospects 2019

Varma A, C. Kharkwal A (2009) Symbiotic Fungi, Principles and practice. Springer-Verlag Berlin Heidelberg

Vetrovsky T, Baldrian P (2013) The variability of the 16S rRNA gene in bacterial genomes and its consequences for bacterial community analyses. 8:1–10. https://doi.org/10.1371/journal.pone.0057923

Wade W (2002) Unculturable bacteria - the uncharacterized organisms that cause oral infections. J R Soc Med 95:81–83

Wanderley CDS, Faria RT de, Ventura MU, Vendrame W (2014) The effect of plant growth regulators on height control in potted *Arundina graminifolia* orchids (Growth regulators in *Arundina graminifolia*). Acta Sci Agron 36:489. https://doi.org/10.4025/actasciagron.v36i4.18085

Wang Y, Lu Z, Sun K, Zhu W (2011) Effects of high salt stress on secondary metabolite production in the marine-derived fungus *Spicaria elegans*. Mar Drugs 9:535–542. https://doi.org/10.3390/md9040535

Weinberg ED (1969) Biosynthesis of secondary metabolites: Roles of trace metals. Adv Microb Physiol 4:1–44. https://doi.org/10.1016/S0065-2911(08)60438-5

Weyens N, van der Lelie D, Taghavi S, Vangronsveld J (2009) Phytoremediation: plant-endophyte partnerships take the challenge. Curr Opin Biotechnol 20:248–254. https://doi.org/10.1016/j.copbio.2009.02.012

Widowati T, Nuriyanah, Sukiman H (2019) Production of indole acetic acid by *Enterobacter cloacea* H3 isolated from Mungbean (Vigna radiata) and its potential supporting the growth of soybean seedling. IOP Conf Ser Earth Environ Sci 308. https://doi.org/10.1088/1755-1315/308/1/012040

Wilson D (2020) Endophyte: the evolution of a term, and clarification of its use and definition. Nord Soc Oikos 73:274–276

Woo PCY, Lau SKP, Teng JLL, et al (2008) Then and now: Use of 16S rDNA gene sequencing for bacterial identification and discovery of novel bacteria in clinical microbiology laboratories. Clin Microbiol Infect 14:908–934. https://doi.org/10.1111/j.1469-0691.2008.02070.x

Wright AD, Sampson MB, Neuffer MG, et al (1991) Indole-3-acetic acid biosynthesis in the mutant maize orange pericarp, a tryptophan auxotroph. Science (80-) 254:998–1000. https://doi.org/10.1126/science.254.5034.998

Xin G, Zhang G, Kang JW, et al (2009) A diazotrophic, indole-3-acetic acid-producing endophyte from wild cottonwood. Biol Fertil Soils 45:669–674. https://doi.org/10.1007/s00374-009-0377-8

Yadavalli R, Rao R, Rao CS (2013) Response Surface Methodological Approach to optimize process parameters for the biomass production of *Chlorella pyrenoidosa*. Int J Bio-Technology 3:37–48

Yamada T, Palm CJ, Brooks B, Kosuge T (1985) Nucleotide sequences of the *Pseudomonas savastanoi* indoleacetic acid genes show homology with *Agrobacterium tumefaciens* T-DNA. Proc Natl Acad Sci U S A 82:6522–6526. https://doi.org/10.1073/pnas.82.19.6522

Yamakawa T, Kurahashi O, Ishida K, et al (1979) Stability of indole-3-acetic acid to autoclaving, aeration and light illumination. Agric Biol Chem 43:879–880. https://doi.org/10.1080/00021369.1979.10863551

Yang S, Zhang Q, Guo J, et al (2007) Global effect of indole-3-acetic acid biosynthesis on multiple virulence factors of *Erwinia chrysanthemi* 3937. Appl Environ Microbiol 73:1079–1088. https://doi.org/10.1128/AEM.01770-06

Yulianti E, Rakhmawati A (2018) Screening and characterization of phosphate solubilizing bacteria from isolate of thermophilic bacteria. Biophys Rev 090015: https://doi.org/10.1063/1.4995207

Yulianti E, Rakhmawati A (2018) Screening and characterization of phosphate solubilizing bacteria from isolate of thermophilic bacteria. Biophys Rev 090015: https://doi.org/10.1063/1.4995207

Zazimalová E, Petrasek J, Benková E (2014) Auxin and its role in plant development. Auxin Its Role Plant Dev 1–444. https://doi.org/10.1007/978-3-7091-1526-8

Zeinab AK, Faiza A, Fatma EM (2015) The Plackett-Burman Design to evaluate significant media components for antimicrobial production of *Lactobacillus rhamnosus*. IntJCurrMicrobiolAppSci 4:1082–1096

Zhang CH, Ma YJ, Yang FX, et al (2009) Optimization of medium composition for butyric acid production by *Clostridium thermobutyricum* using Response Surface Methodology. Bioresour Technol 100:4284–4288. https://doi.org/10.1016/j.biortech.2009.03.022

Zhao Y (2010) Auxin biosynthesis and its role in plant development. Annu Rev Plant Biol 61:49–64. https://doi.org/10.1146/annurev-arplant-042809-112308.Auxin

Zhao Y (2012) Auxin biosynthesis: A simple two-step pathway converts tryptophan to indole-3-acetic acid in plants. Mol Plant 5:334–338. https://doi.org/10.1093/mp/ssr104

Zhao Y (2014) Auxin Biosynthesis. Arab B 12:e0173. https://doi.org/10.1199/tab.0173

Zhao Y, Hull AK, Gupta NR, et al (2002) Trp-dependent auxin biosynthesis in *Arabidopsis* : involvement of cytochrome P450s CYP79B2 and CYP79B3. 3100–3112. https://doi.org/10.1101/gad.1035402.5

Zhu F, Qu L, Hong X, Sun X (2011) Isolation and characterization of a phosphate-solubilizing halophilic bacterium *Kushneria* sp . YCWA18 from Daqiao Saltern on the Coast of Yellow Sea of China. Evidence-based Complement Altern Med 2011:. https://doi.org/10.1155/2011/615032