JMBFS
Journal of Microbiology, Biotechnology and Food Sciences
International peer-reviewed scientific online journal
Published by Faculty of Biotechnology and Food Sciences

DECIPHERING THE OILSEED BACTERIAL ENDOPHYTES AND STUDY OF THEIR ROLE AND TRIPARTITE INTERACTIONS WITH PLANTS AND PATHOGEN

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https://doi.org/10.55251/jmbfs.9482

ARTICLE INFO

Received 23. 9. 2022
Revised 13. 5. 2024
Accepted 3. 7. 2024
Published 1. 10. 2024

Review

OPEN ACCESS

ABSTRACT

Endophytes colonize interior regions of oilseeds depending on water, nutrient, space availability and microbial competition. Oilseeds are filled with oil globules making it difficult for microbes to obtain direct uptake of water and nutrient for growing into colonies. Recent research shows very less areas explored in endophytic study in oilseeds and as endophytes play a vital part in managing diseases in plants by releasing genes encoding jasmonate and salicylic acid which induces defense against pathogens and modify plant regions structurally adding to their fitness and increased productivity, their study is indispensable. This review focuses on the bacterial endophytes in oilseeds, the challenges faced in their isolation, their colonization and interactions with host and pathogens and their applications. Oilseed endophytic study is essential for understanding the benefits of oilseeds consumed as vegetable oil or used as fuel by people all over the world.

Keywords: Oilseeds, endophytes, pathogen, colonization, biocontrol

INTRODUCTION

Human population is increasing day by day and to cope up with the increasing population, agricultural practices are increasing too for more production of crops and plants for human consumption. Every plant is surrounded by millions of microbes in air, soil and exposed regions of plant parts. But there is another section of microbes that are away from the environmental stress residing inside the plants. The microbes residing inside the plants, colonizing the different tissues and regions of plant interior without causing harm to the plant are known as endophytes. They may be bacteria, fungi or algae. In this review we will be discussing about bacterial endophytes. These endophytes live a part of or all of their life cycle inside plant without causing disease symptoms in their host **(Le Cocq *et al*.,2017;Schulz & Boyle,2005)**. Endophytes reside in plant regions such as roots, stem, leaf, flowers and seeds. Seed is a basic and fundamental part of the plant through which an entire plant develops using nutrients and water available inside the plant and from outer areas like soil, air and water available to plant conferred by bacteria present in those areas. Examples are phosphate solubilising bacteria, nitrogen fixation bacteria etc. Endophytes presence inside seeds is a boon as bacteria derived volatile organic compounds (VOCs) helped the plant in its growth promotion and development, hence improving the plant health **(Raza *et al*.,2016)**.
Vegetable oils produced from oilseed crops are oils or fats in the form of liquid and is oily and fatty. They can be used as oil for cooking or as fuel or diesel. As per market survey, since 2013 till 2011, palm oil followed by soybean oil and rapeseed oil are the most consumed oil in the world. They are followed by palm oil and peanut oil. Olive oil is the least consumed oil among global population. Oilseeds have been one of the crops that though being essential have garnered less interest among researchers especially on its microbiome. Abundant literature is available on the internet on endophytes in different crops such as cereals, legumes, but very less work has been reported in oilseeds. Therefore, a brief study on oilseed endophytes is requisite and points out to the need for identifying questions that can lead to more research on this subject. This study focuses on the bacterial endophytes in oilseeds; their isolation, colonization, effect on phytopathogens and importance of their study. A brief explanation of the process performed for isolation and application of the endophytes has been shown in **Fig. 1** taking mustard-rapeseed as an example.

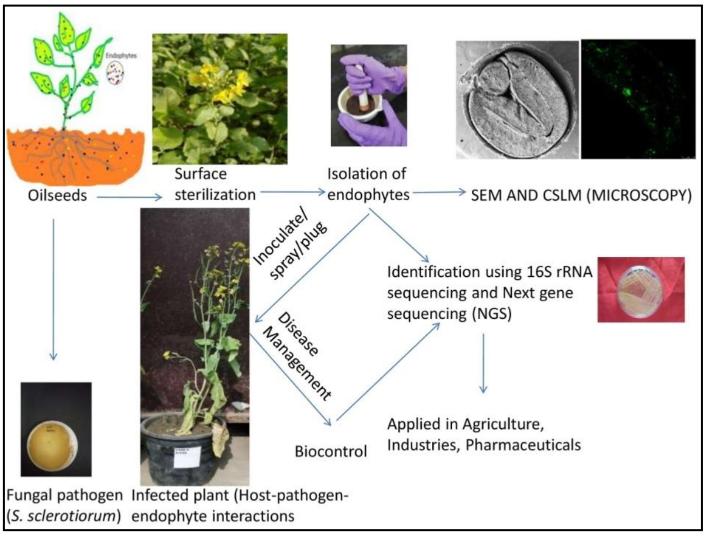

Figure 1 A schematic diagram showing oilseed endophytes isolation, their observation under confocal laser scanning microscope (CLSM) and scanning electron microscopy (SEM) and its applications. An example of *S.Sclerotiorum* as an example of fungal phytopathogen causing disease in mustard crops and its management is shown above.

ISOLATION OF ENDOPHYTES FROM PARTS OF OILSEED PLANTS

Culture based techniques permit selection of strains tailored to soil environment as well as for the characterization of microbial diversity and knowledge about which microorganism is able to establish a relationship with the host plant **(Ambrosini *et al*.,2012)**. First step to isolate endophytes is surface sterilization of different parts of oilseed crops considered for study. This step involves washing the parts in sterile distilled water followed by immersion in 70% ethanol for 1 min and in sodium hypochlorite solution (4%, v/v) for 2 min for roots of sunflower **(Ambrosini *et al*.,2012)**. Some parts like seeds of peanut were surface sterilized in 70 % Ethanol for 4 mins followed by dipping in 1 % Sodium hypochlorite for 5 min with addition of 100μL/L of Tween 20 **(Sobolev *et al*.,2013)**. Seeds of pre germinated seeds of

Brassica napus (oilseed rape) were surface-sterilized in 70% ethanol for 1 min followed by thorough wash in sterile distilled water (SDW) and a rinse in sterile phosphate buffer saline solution (0.14 M NaCl, 0.003 M KCl and 0.01 M phosphate buffer, pH 7.4) **(Granér *et al.*,2003)**. Different varieties of mustard seeds were surface sterilized as per methods described by **Sinha and Talukdar (2022)** and bacterial endophytes were isolated culturally using new developed methods using centrifugation approach and surfactant. Surfactant was used to separate oil from water in oil-water seed suspension and then used for bacterial colony isolation. Stems and roots of soybean pants were washed in distilled water and rinsed in 70% ethanol for 30 seconds followed by sterilization with 0.1% HgCl2 for 3 minutes for roots and nodules and 5 minutes for stems. The tissues were then washed ten times with sterile water. Immediately after disinfection the parts of crops are checked for sterility by immersing them in 0.85% of sterile saline solution or plating them in agar in triplicates followed by incubation at 28° C for 48-72 hrs **(Hung & Annapurna, 2004)**. Surface sterilized parts were aseptically macerated or crushed with homogenizers. Macerated tissues were diluted into 10^{-1} dilution by adding 9 volumes of SDW. Serial dilution was made up to 10^{-6} dilution by taking 1 ml of well-shaken suspension and adding to 9 ml water blank tubes **(Hung & Annapurna, 2004)**. 100 µl from appropriate dilutions were spread plated on media plates in triplicates followed by incubation at 30° C for 72 hrs. Observed colonies were streaked onto fresh agar plates for isolation and stored in glycerol at −80 °C for later identification **(Sobolev *et al.*,2013)**. Different bacterial species have been isolated from oilseeds as listed in **Table 1**. In our previous study, bacteria of rod shape have been observed inside mustard seed and germinated seed under scanning electron microscope (SEM) (**Fig.2**).

In comparison to seeds of cereals and legumes, isolation of bacterial endophytes from seeds of oilseed crops is challenging as observed in our previous study where bacterial endophytes were isolated from mustard seeds of four varieties **(Sinha and Talukdar,2022)**. On plating crushed seed suspension on nutrient media, bacterial colonies did not appear as the oil present in the suspension did not allow the bacteria to settle on media and develop colonies. New methods were developed for successful isolation. One such method is by performing centrifugation. Since oil is immiscible in nature, if it can be removed from the seed suspension containing seeds crushed in water by centrifugation process, then the bacteria present in the suspension can develop colonies in media when the pellet is plated. The supernatant containing oil can be discarded. This principle has been successfully used for isolation of bacterial endophytes from mustard seeds. Since removal of oil from oilseeds is a challenging task therefore the oil separation principle can be used to isolate bacterial colonies from different sections of oilseed crops. There are more oilseed crops which are yet to be explored for endophytic study.

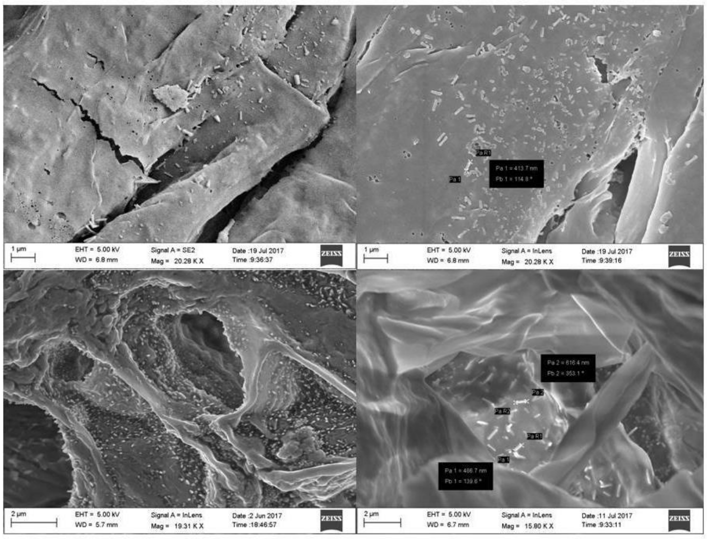

Figure 2 Mustard seed bacterial endophytes observed under Scanning electron microscope (SEM).

COLONIZATION OF ENDOPHYTES IN OILSEED CROPS

Microbes face stress and competition for space and nutrients from surrounding microbes. To avoid this, they tend to enter plants and colonize the interior regions. Moreover, root exudates lure these microbes and the continuous flow of water and nutrients through the plant vascular system forms a good source for microbial survival by their moving into different regions inside and colonizing in those regions. Microbes enter the plant through openings in roots where root hair emerges, stomata, wounds and hydathodes in the shoot regions **(Hardoim *et al.*,2015)**. Microbes colonize different regions and tissues inside the plant systematically **(Compant,Clément & Sessitsch,2010)**. After entry inside the plant, microbes face oxidative environments and have to survive that. Endophytes benefit from host plants as they receive protective shed, organic nutrients and assured transmission to next host generation **(Mengistu, 2020)**. Endophyte colonization depends on various factors such as type of microbe and strain, type of plant genotype and tissue, its age **(Bamisile *et al.*,2018;Hardoim *et al.*,2015)**.

Microbes generally colonize in regions where food, easy transportation is available as observed under Scanning electron microscopy in our previous study using mustard seeds (**Fig.2**). Bacteria were found to colonize in the vascular bundle's region and near the root regions. These regions provide nutrients and easy transport throughout the plant interior regions. Bacteria were not observed in regions containing oil globules indicating that colonization and growing in those regions was not convenient for them as oil does not provide an easy substrate for their growth and multiplication. When glucosinolates inside oilseeds hydrolyze, they form bioactive products which are bactericidal **(Brown and Morra,1997)** and may prevent colonization of microbes, but if the bacteria are established early in the young plants, then the effect of glucosinolates is nil. The amount of glucosinolates inside plants can be monitored using bacterial reporter gene assay **(O'Callaghan *et al*., 2000)**. This assay can be used to study the necessary phytochemicals which influence colonization of bacteria in oilseed crops. Colonization patterns in different oilseed crops can be studied to understand the bacterial movement pattern and the core bacterial species present in certain regions inside plant. As movement through oil is challenging, these regions can be studied to identify the presence or absence of microbial species in these oily regions.

Different oilseed bacterial endophytes have been studied previously as mentioned in **Table 1**.

Table 1 Endophytic bacteria isolated and colonized in oilseeds

Oilseed crops	Phylum obtained from different parts of the crops	References
Sunflower	*Enterobacter, Klebsiella, Burkholderia*	**(Ambrosini *et al.*, 2012)**
Mustard	*Proteobacteria, Firmicutes, Actinobacteria*	**(Granér *et al.*, 2003; Sinha & Talukdar,2024)**
Peanut	*Firmicutes, Proteobacteria*	**(Sobolev *et al.*, 2013)**
Soybean	***Deinococcota, Firmicutes, Actinomycetota***	**(Hung & Annapurna, 2004)**
Groundnut	*Firmicutes, Alphaproteobacteria, Betaproteobacteria, Gammaproteobacteria*	**(Preyanga *et al*., 2021)**
Canola	*Actinobacteria, Firmicutes, Bacteroidetes*	**(Germida *et al*., 1998)**

EFFECT OF ENDOPHYTES ON PATHOGENS OF OILSEED CROPS

Endophytes have the ability to inhibit pathogens and resist their growth in the host plant. When they invade into the host plant, they compete with each other as their patterns of colonization are similar. This makes the endophytes, potential biocontrol agents. Endophytes may use mechanism for protecting plants like producing antibiotics, production of lytic enzymes, siderophore, can solubilize phosphate, phytohormones, competing with pathogens, producing secondary metabolites and inducing plant systemic resistance. Oilseeds such as peanut, rapeseed–mustard, sunflower and soybean are subject to attack by numerous pathogens (**Table 2**). The loss in yield of these crops may be different depending upon the nature of pathogen and severity of attack **(Chattopadhyay *et al.*, 2015)**. One of the oilseed crops, mustard contains glucosinolates inside the seeds. Glucosinolates derivatives such as Isothyacyanates are formed when myrosinase present in myrosin cells comes in contact with glucosinolates which are antioxidant, antibacterial and antifungal **(Ratzka *et al.*, 2002)**. Some diseases formed in oilseeds are rusts, downy mildews, leaf spots and blights.

Among different strains of endophytic bacteria, those from genera *Bacillus*, *Pseudomonas* and *Agrobacterium* play the most vital role in biological control. Medicinal plants are carriers of *Bacillus* and *Paenibacillus* strains for the production of novel antimicrobial agents **(Ghiasvand *et al.*, 2020)**. *Pseudomonas fluorescens* and *Pseudomonas aeruginosa* produce compounds such as 2, 4-diacetylphloroglucinol, penazine-1-carboxylic acid, py-oleutirin, pyrrolnitrin, or hydrogen cyanide, which suppresses pathogenic fungal growth **(Lashin *et al.*, 2021)**. Bacillus group contains some important molecules such as circular lipopeptides from surfactin, iturin and fengycin families which affect by hydrolyzing the hyphal membrane of target cells of pathogens affecting their growth and also induces plant systemic resistance **(Fira *et al.*, 2018)**. This hydrolyzing of hyphal membrane of fungi promotes nutrient leakage reducing the virulence of fungi **(Lashin *et al.*, 2021)**. The endophytes compete for nutrients, space and ecological niches with the pathogens affecting the host plant. An example is of *Arabidopsis thaliana*, whose bacterial endophyte; *Bacillus subtilis BSn5* produces subtilomycin which affects flg22-induced plant defense. It binds with flagellin enhancing its ability to colonize plant interior **(Deng *et al.*, 2019)**. When the fungal pathogen is bacteria-treated, it causes swelling of tip, leakage of cytoplasm and shrinking of fungal hyphae. Higher vacuoles may be present in those hyphal cells than those non-treated. Leakage of cytoplasm leads to reduction of biomass thus reducing infection and its suppression **(Chen *et al.*, 2014)**. Endophytes induce plant systemic resistance (PSR) by up regulation of genes inducing jasmonic acid and ethylene pathways (Pangesti et al., 2016). Structural

modifications such as lignin and callose deposition in tissues colonized by endophytes (**Constantin *et al.*, 2019)** and biochemical responses such as reactive oxygen species (ROS) synthesis and production of bioactive metabolites **(Samain *et al.*, 2017)** and volatile compounds by endophytes are all as part of plant systemic resistance.
Endophyte-pathogen interaction can be studied using marker approach using fluorescent tags and later observed under microscope. Their pattern of interaction can be studied using the same. The regions of interaction can be extracted to explore the compounds and products involved in their interaction process and these compounds can be studied for their importance. The biochemistry and mechanisms involved can be explored for use in other crops and applications in agricultural and pharmaceutical industry.

Table 2 Oilseed crop diseases and their causal organism

Diseases in oilseeds	Causal organism	Symptoms	Reported works
Alternaria Black spot	*Alternaria brassicae*	Lesions on pods and stems.	**Reshu & Khan, 2012**
Sclerotinia stem rot	*Sclerotinia sclerotiorum*	Cottony growth	**Chen *et al.*, 2014; de Almeida Lopes *et al.*, 2018; Massawe *et al.*, 2018**
Alternaria blight	*Alternaria brassicae*	Lesion on leaves and shattering of pods	**Yasin *et al.*, 2017**
Phoma stem Canker	*Leptosphaeria maculans*	Damage on stem base canker	**Fitt *et al*.,2006**
Club root	*Plasmodiophora brassicae*	Stunted, yellow and wilted plants	**Liao *et al*.,2022**

PLANT-HOST MICROBE INTERACTIONS

The rhizosphere serves as a core for plant-endophyte communication during the initial stages of the colonization progression and facilitates admission to the inside of the plant tissues through openings in the plant. Some bacterial endophytes have the potential to colonize every one of the plant parts and interact beneficially with the host plant. Endophytic bacteria can promote growth in plant by synthesizing plant hormones such as indole-3-acetic acid (IAA), cytokinin and gibberellin or by regulating hormone levels inside plant body **(Santoyo *et al.*, 2016; Spaepen & Vanderleyden, 2011)**. When plants were inoculated with bacterial endophytes capable of producing auxin, plants showed growth **(Barra *et al.*, 2016; Z. Khan *et al.*, 2016; Santoyo *et al.*, 2016; Shi *et al.*, 2009;Xin *et al.*, 2009)**. Bacteria solubilizing phosphorus can help in plant growth promotion by solubilizing immobile phosphorus in soil making it available for plants to absorb **(Dias *et al.*, 2009; Joe *et al.*, 2016; Oteino *et al.*, 2015;Passari *et al.*, 2015)**. Bacterial endophytes can develop resistance and tolerance in plants from biotic and abiotic stress by producing siderophores, releasing antimicrobial compounds, modulating plant resistance response and by competing for space and nutrient **(Friesen *et al.*, 2011; Mercado-Blanco & JJ Lugtenberg, 2014; Santoyo *et al.*, 2016)**. Protein secretions define plant-microbe interactions. Effector proteins when transferred suppress host defense system thus supporting the parasitic lifestyle of bacteria whereas the host stimulates immune responses triggered by the presence of those effector proteins **(Mengistu, 2020)**. In a study by **Eslamyan *et al.* (2013)**, *Pseudomonas florescence* was inoculated which led to increase in oil content and plant growth. Several other microbes have shown to help in uptake of metals from soil and promote plant biomass increase in oilseed crops **(Sheng *et al.*, 2008, Sheng & Xia, 2006)**.
The topic of plant microbe interactions is vast but very less explored in oilseeds. Oilseed crops have somehow been left behind in this area as oil provides a completely different environment for microbes to associate together and interact among each other. Some microbes specific to such environment form relationship with the host by helping each other mutually but some microbes tend to be removed, mutate or get lost inside such environments. Exploring this section would be interesting and challenging and would unravel answers to various important issues such as their core population, less diversity and population and toxic interior environment.

APPLICATION OF ENDOPHYTES

Phosphorus solubilizing bacteria has applications in agriculture for promoting plant growth **(Hardoim *et al.*, 2015; M. Khan, Zaidi, & Ahmad, 2014)**. Some endophytic strains of *Bacillus*, *Pseudomonas*, *Burkholderia*, *Serratia* and *Enterobacter* were able to suppress the growth of pathogenic microbes in both in vivo and in vitro conditions **(Sinha *et al.*, 2023)**. Alleviation of drought, heat and salt stress in crops were successful by some strains of *Bacillus*, *Streptomyces*, *Pseudomonas*, *Enterobacter*, *Azotobacter*, *Arthrobacter*and *Isoptericola* **(Ali *et al.*, 2014; Naveed *et al.*, 2014; Qin *et al.*, 2014; Rojas-Tapias *et al.*, 2012; Yaish *et al.*, 2015)**. Endophytic bacteria aids plants in metal uptake and maintaining of Na^+ concentration thus helping the plant tolerate excessive salt concentration in soil **(Dodd & Pérez-Alfocea, 2012)**. **Table 3** lists some applications of endophytes in plant-microbe interactions.

1. In Agriculture
   They help in plant growth promotion directly or indirectly by performing the different functions. Endophytes possess biocontrol activity and protect the plant from phytopathogens by inhibiting their growth and colonization inside plants. Therefore, they can be used as an alternative to fertilizers and pesticides.
2. Pharamaceutical applications
   Endophytes can be used as insecticides, antioxidants and antimicrobial agents, produce antiviral, anticancer, immunosuppressive and antidiabetic compounds. They produce compounds which may help against tuberculosis, malaria and cancer **(Kapoor *et al.*, 2019)**. Many metabolites acting as anticancer or antimicrobial agents targets plants, animals, and human pathogens and offer many scopes in veterinary and medical therapy. Some bacteria are even considered eco-safe **(Ek-Ramos *et al.*, 2019)**.
3. Industrial applications
   Some bacteria produce nanoparticles and act as antimicrobial, antifungal and antibacterial, anti-multidrug resistant **(Baker & Satish, 2015; El-Moslamy, 2018; Ibrahim *et al.*, 2019; Monowar *et al.*, 2018; Rajabairavi *et al.*, 2017)**

Table 3 Applications of endophytes and their role in plant-microbe interactions

Type of Applications	Role of endophytes	References
Agricultural Applications	Production of phytohormones Plant nutrient availability Alleviating abiotic stress Suppressing plant diseases Induction of plant systematic resistance Protect the plant from insects and pests Help in phytoremediation	**Baker & Satish, 2015; Eid *et al.*, 2021; Lugtenberg *et al.*, 2013; Mercado-Blanco & JJ Lugtenberg, 2014; Sinha *et al.*,2023**
Pharmaceutical Applications	Insecticides, antioxidants and antimicrobial agents, produce antiviral, anticancer, immunosuppressive and antidiabetic compounds and antibiotics Anti-malarial, anti-tuberculosis, anti-cancer compounds	**Mercado-Blanco & JJ Lugtenberg, 2014; Ryan *et al.*, 2008; Sinha *et al.*,2023**
Industrial Applications	Nanoparticles acing as antimicrobial, antifungal and antibacterial, anti-multidrug resistant	**Eid *et al*., 2021; Mercado-Blanco & JJ Lugtenberg, 2014; Ryan *et al.*, 2008**

CONCLUSIONS AND FUTURE PERSPECTIVES

Isolation of bacterial endophytes from oilseeds is challenging especially from seeds and those regions with oil globules. Isolation from other parts of plants has been successfully performed as observed in different oilseed crops. The colonization pattern of endophytes and pathogens inside the different regions of the plant needs to be understood for developing isolation techniques for bacterial isolation from difficult regions and advanced biocontrol mechanisms. The movement of bacteria inside the plant can be tracked to understand their colonization patterns and selection of a particular region for forming its niche. Through this study, for the first time, we have provided a detailed study on oilseed endophytes, its colonization and defense against pathogens. We have understood that oil can be separated from certain regions and then bacteria can be isolated using centrifugation techniques. Even though oilseeds contain a different type of environment endophytic study is possible and necessary to understand their functioning, importance and role in oilseeds. Using endophytes as biocontrol agents in its independent form or as microbial inoculum where different beneficial endophytes are mixed together can be a good alternative to chemical fertilizers and pesticides. Tagged endophytes can be reintroduced in host plant to study their pattern of movement and colonization. Furthermore their interaction with other microbes can be studied and thus used as vectors in areas of infection and genetic engineering.

Acknowledgement: I would like to thank Council of Scientific and Industrial Research (CSIR) to fund my research.

DECLARATIONS

Funding: This research was financially supported by The Council of Scientific and Industrial Research (CSIR) File No: 09/835(0018)/2017-EMR-1.
Conflicts of interest: The authors declare that they have no conflict of interests.
Ethics approval: The experiments performed did not involve any animal or human participation.

REFEENCES

Ali, S., Charles, T. C., & Glick, B. R. (2014). Amelioration of high salinity stress damage by plant growth-promoting bacterial endophytes that contain ACC deaminase. *Plant Physiology and Biochemistry*, *80*, 160-167. https://doi.org/10.1016/j.plaphy.2014.04.003

Ambrosini, A., Beneduzi, A., Stefanski, T., Pinheiro, F. G., Vargas, L. K., & Passaglia, L. M. (2012). Screening of plant growth promoting rhizobacteria isolated from sunflower (*Helianthus annuus L.*). *Plant and soil*, *356*(1), 245-264. https://doi.org/10.1007/s11104-011-1079-1

Baker, S., & Satish, S. (2015). Biosynthesis of gold nanoparticles by *Pseudomonas veronii* AS41G inhabiting *Annona squamosa L.Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy*, *150*, 691-695. https://doi.org/10.1016/j.saa.2015.05.080

Bamisile, B. S., Dash, C. K., Akutse, K. S., Keppanan, R., Afolabi, O. G., Hussain, M.,Wang, L. (2018). Prospects of endophytic fungal entomopathogens as biocontrol and plant growth promoting agents: An insight on how artificial inoculation methods affect endophytic colonization of host plants. *Microbiological research*, *217*, 34-50. https://doi.org/10.1016/j.micres.2018.08.016

Barra, P. J., Inostroza, N. G., Acuña, J. J., Mora, M. L., Crowley, D. E., & Jorquera, M. A. (2016). Formulation of bacterial consortia from avocado (*Persea americana Mill.*) and their effect on growth, biomass and superoxide dismutase activity of wheat seedlings under salt stress. *Applied Soil Ecology*, *102*, 80-91. https://doi.org/10.1016/j.apsoil.2016.02.014

Brown, P. D., Morra, M. J. (1997). Control of soil-borne plant pests using glucosinolate-containing plants. *Advances in Agronomy*, *61*:167–231.

Chattopadhyay, C., Kolte, S., & Waliyar, F. (2015). Diseases of edible oilseed crops: *Taylor & Francis*.

Chen, Y., Gao, X., Chen, Y., Qin, H., Huang, L., & Han, Q. (2014). Inhibitory efficacy of endophytic *Bacillus subtilis* EDR4 against *Sclerotinia sclerotiorum* on rapeseed. *Biological Control*, *78*, 67-76. https://doi.org/10.1016/j.biocontrol.2014.07.012

Compant, S., Clément, C., & Sessitsch, A. (2010). Plant growth-promoting bacteria in the rhizo-and endosphere of plants: their role, colonization, mechanisms involved and prospects for utilization. *Soil Biology and Biochemistry*, *42*(5), 669-678. https://doi.org/10.1016/j.soilbio.2009.11.024

Constantin, M. E., De Lamo, F. J., Vlieger, B. V., Rep, M., & Takken, F. L. (2019). Endophyte-mediated resistance in tomato to *Fusarium oxysporum* is independent of ET, JA, and SA. *Frontiers in plant science*, *10*, 979. https://doi.org/10.3389/fpls.2019.00979

de Almeida Lopes, K. B., Carpentieri-Pipolo, V., Fira, D., Balatti, P. A., López, S. M. Y., Oro, T. H., Degrassi, G. (2018). Screening of bacterial endophytes as potential biocontrol agents against soybean diseases. *Journal of Applied Microbiology*, *125*(5), 1466-1481. https://doi.org/10.1111/jam.14041

Deng, Y., Chen, H., Li, C., Xu, J., Qi, Q., Xu, Y., Ruan, L. (2019). Endophyte *Bacillus subtilis* evade plant defense by producing lantibiotic subtilomycin to mask self-produced flagellin. *Communications biology*, *2*(1), 1-12. https://doi.org/10.1038/s42003-019-0614-0

Dias, A. C., Costa, F. E., Andreote, F. D., Lacava, P. T., Teixeira, M. A., Assumpçao, L. C., . . . Melo, I. S. (2009). Isolation of micropropagated strawberry endophytic bacteria and assessment of their potential for plant growth promotion. *World Journal of Microbiology and Biotechnology*, *25*(2), 189-195. https://doi.org/10.1007/s11274-008-9878-0

Dodd, I. C., Pérez-Alfocea, F. P. (2012) Microbial amelioration of crop salinity stress. *Journal of Experimental Botany*, *63*: 415–428. https://doi.org/10.1093/jxb/ers033

Eid, A. M., Fouda, A., Abdel-Rahman, M. A., Salem, S. S., Elsaied, A., Oelmüller, R., Hassan, S. E.-D. (2021). *Harnessing bacterial endophytes for promotion of plant growth and biotechnological applications: an overview. Plants, 10*(5), 935. https://doi.org/10.3390/plants10050935

Ek-Ramos, M. J., Gomez-Flores, R., Orozco-Flores, A. A., Rodríguez-Padilla, C., González-Ochoa, G., & Tamez-Guerra, P. (2019). Bioactive products from plant-endophytic Gram-positive bacteria. *Frontiers in microbiology*, *10*, 463. https://doi.org/10.3389/fmicb.2019.00463

El-Moslamy, S. H. (2018). Bioprocessing strategies for cost-effective large-scale biogenic synthesis of nano-MgO from endophytic *Streptomyces coelicolor* strain E72 as an anti-multidrug-resistant pathogens agent. *Scientific reports*, *8*(1), 1-22. https://doi.org/10.1038/s41598-018-22134-x

Eslamyan, L., Alipour, Z. T., Beidokhty, S. R., & Sobhanipour, A. (2013). *Pseudomonas fluorescens* and sulfur application affect rapeseed growth and nutrient uptake in calcareous soil. *International Journal of Agriculture and Crop Sciences (IJACS)*, *5*(1), 39-43.

Fira, D., Dimkić, I., Berić, T., Lozo, J., & Stanković, S. (2018). Biological control of plant pathogens by *Bacillus* species. *Journal of biotechnology*, *285*, 44-55. https://doi.org/10.1016/j.jbiotec.2018.07.044

Fitt, B.D.L., Brun, H., Barbetti, M.J., Rimmer, S.R. (2006). World-wide importance of phoma stem canker (*Leptosphaeria maculans* and *L. biglobosa*) on oilseed rape (*Brassica napus*). In: Fitt, B.D.L., Evans, N., Howlett, B.J., Cooke, B.M. (eds) Sustainable strategies for managing Brassica napus (oilseed rape) resistance to Leptosphaeria maculans (phoma stem canker). *Springer Dordrecht*. https://doi.org/10.1007/1-4020-4525-5_1

Friesen, M. L., Porter, S. S., Stark, S. C., von Wettberg, E. J., Sachs, J. L., & Martinez-Romero, E. (2011). Microbially mediated plant functional traits. *Annual Review of Ecology, Evolution and Systematics*, *42*, 23-46. https://doi.org/10.1146/annurev-ecolsys-102710-145039

Germida, J. J., Siciliano, S. D., Renato de Freitas, J., & Seib, A. M. (1998). Diversity of root-associated bacteria associated with field-grown canola (*Brassica napus L.*) and wheat (*Triticum aestivum L.*). *FEMS Microbiology Ecology*, *26*(1), 43-50. https://doi.org/10.1146/annurev-ecolsys-102710-145039

Ghiasvand, M., Makhdoumi, A., Matin, M. M., & Vaezi, J. (2020). Exploring the bioactive compounds from endophytic bacteria of a medicinal plant: *Ephedra foliata* (Ephedrales: Ephedraceae). *Advances in Traditional Medicine*, *20*(1), 61-70. https://doi.org/10.1007/s13596-019-00410-z

Granér, G., Persson, P., Meijer, J., & Alström, S. (2003). A study on microbial diversity in different cultivars of *Brassica napus* in relation to its wilt pathogen, *Verticillium longisporum.FEMS microbiology letters*, *224*(2), 269-276. https://doi.org/10.1016/S0378-1097(03)00449-X

Hardoim, P. R., Van Overbeek, L. S., Berg, G., Pirttilä, A. M., Compant, S., Campisano, A.,Sessitsch, A. (2015). The hidden world within plants: ecological and evolutionary considerations for defining functioning of microbial endophytes. *Microbiology and molecular biology reviews*, *79*(3), 293-320. https://doi.org/10.1128/mmbr.00050-14

Hung, P. Q., & Annapurna, K. (2004). Isolation and characterization of endophytic bacteria in soybean (*Glycine sp.*). *Omonrice*, *12*(4), 92-101.

Ibrahim, E., Fouad, H., Zhang, M., Zhang, Y., Qiu, W., Yan, C., Chen, J. (2019). Biosynthesis of silver nanoparticles using endophytic bacteria and their role in inhibition of rice pathogenic bacteria and plant growth promotion. *RSC advances*, *9*(50), 29293-29299.

Joe, M. M., Devaraj, S., Benson, A., & Sa, T. (2016). Isolation of phosphate solubilizing endophytic bacteria from *Phyllanthus amarus Schum & Thonn*: Evaluation of plant growth promotion and antioxidant activity under salt stress. *Journal of applied research on medicinal and aromatic plants*, *3*(2), 71-77. https://doi.org/10.1016/j.jarmap.2016.02.003

Kapoor, N., Jamwal, V. L., & Gandhi, S. G. (2019). Endophytes as a source of high-value, bioactive metabolites. *Phytochemistry*, 427-458.

Khan, M., Zaidi, A., & Ahmad, E. (2014). Mechanism of phosphate solubilization and physiological functions of phosphate-solubilizing microorganisms Phosphate solubilizing microorganisms. *Springer*, 31-62. https://doi.org/10.1007/978-3-319-08216-5_2

Khan, Z., Rho, H., Firrincieli, A., Hung, S. H., Luna, V., Masciarelli, O., Doty, S. L. (2016). Growth enhancement and drought tolerance of hybrid poplar upon inoculation with endophyte consortia. *Current Plant Biology*, *6*, 38-47. https://doi.org/10.1016/j.cpb.2016.08.001

Lashin, I., Fouda, A., Gobouri, A. A., Azab, E., Mohammedsaleh, Z. M., & Makharita, R. R. (2021). Antimicrobial and in vitro cytotoxic efficacy of biogenic silver nanoparticles (Ag-NPs) fabricated by callus extract of *Solanum incanum L. Biomolecules*, *11*(3), 341. https://doi.org/10.3390/biom11030341

Le Cocq, K., Gurr, S. J., Hirsch, P. R., & Mauchline, T. H. (2017). Exploitation of endophytes for sustainable agricultural intensification. *Molecular Plant Pathology*, *18*(3), 469-473. https://doi.org/10.1111/mpp.12483

Liao, J., Luo, L., Zhang, L., Wang, L., Shi, X., Yang, H., ...& Mao, Z. (2022). Comparison of the effects of three fungicides on clubroot disease of tumorous stem mustard and soil bacterial community. *Journal of Soils and Sediments*, 1-16. https://doi.org/10.1007/s11368-021-03073-z

Lugtenberg, B. J., Malfanova, N., Kamilova, F., & Berg, G. (2013). Plant growth promotion by microbes. *Molecular microbial ecology of the rhizosphere*, *2*, 561-573. https://doi.org/10.1002/9781118297674.ch53

Massawe, V. C., Hanif, A., Farzand, A., Mburu, D. K., Ochola, S. O., Wu, L.,Gao, X. (2018). Volatile compounds of endophytic *Bacillus spp.* have biocontrol activity against *Sclerotinia sclerotiorum*. *Phytopathology*, *108*(12), 1373-1385. https://doi.org/10.1094/PHYTO-04-18-0118-R

Mengistu, A. A. (2020). Endophytes: colonization, behaviour, and their role in defense mechanism. International Journal of Microbiology, 2020. https://doi.org/10.1155/2020/6927219

Mercado-Blanco, J., & JJ Lugtenberg, B. (2014). Biotechnological applications of bacterial endophytes. *Current Biotechnology*, *3*(1), 60-75.

Monowar, T., Rahman, M. S., Bhore, S. J., Raju, G., & Sathasivam, K. V. (2018). Silver nanoparticles synthesized by using the endophytic bacterium *Pantoea* *ananatis* are promising antimicrobial agents against multidrug resistant bacteria. *Molecules*, *23*(12), 3220. https://doi.org/10.3390/molecules23123220

Naveed, M., Mitter, B., Reichenauer, T. G., Wieczorek, K., & Sessitsch, A. (2014). Increased drought stress resilience of maize through endophytic colonization by *Burkholderia phytofirmans* PsJN and *Enterobacter sp.* FD17. *Environmental and Experimental Botany*, *97*, 30-39. https://doi.org/10.1016/j.envexpbot.2013.09.014

O'Callaghan, K. J., Stone, P. J., Hu, X., Griffiths, D. W., Davey, M. R., Cocking, E.C. (2000). Effects of glucosinolates and flavonoids on colonization of the roots of *Brassica napus* by *Azorhizobium caulinodans* ORS571. *Applied and Environmental Microbiology*, *66*(5):2185-91. https://doi.org/10.1128/aem.66.5.2185-2191.2000

Oteino, N., Lally, R. D., Kiwanuka, S., Lloyd, A., Ryan, D., Germaine, K. J., & Dowling, D. N. (2015). Plant growth promotion induced by phosphate solubilizing endophytic *Pseudomonas* isolates. *Frontiers in microbiology*, *6*, 745. https://doi.org/10.3389/fmicb.2015.00745

Pangesti, N., Reichelt, M., van de Mortel, J. E., Kapsomenou, E., Gershenzon, J., van Loon, J. J., Pineda, A. (2016). Jasmonic acid and ethylene signaling pathways regulate glucosinolate levels in plants during rhizobacteria-induced systemic resistance against a leaf-chewing herbivore. *Journal of Chemical Ecology*, *42*(12), 1212-1225. https://doi.org/10.1007/s10886-016-0787-7

Passari, A. K., Mishra, V. K., Gupta, V. K., Yadav, M. K., Saikia, R., & Singh, B. P. (2015). In vitro and in vivo plant growth promoting activities and DNA fingerprinting of antagonistic endophytic actinomycetes associates with medicinal plants. *PLoS one*, *10*(9), e0139468. https://doi.org/10.1371/journal.pone.0139468

Preyanga, R., Anandham, R., Krishnamoorthy, R., Senthilkumar, M., Gopal, N., Vellaikumar, A., & Meena, S. (2021). Groundnut (*Arachis hypogaea*) nodule Rhizobium and passenger endophytic bacterial cultivable diversity and their impact on plant growth promotion. *Rhizosphere*, *17*, 100309. https://doi.org/10.1016/j.rhisph.2021.100309

Qin, S., Zhang, Y.-J., Yuan, B., Xu, P.-Y., Xing, K., Wang, J., & Jiang, J.-H. (2014). Isolation of ACC deaminase-producing habitat-adapted symbiotic bacteria associated with halophyte *Limonium sinense (Girard) Kuntze* and evaluating their plant growth-promoting activity under salt stress. *Plant and soil*, *374*(1), 753-766. https://doi.org/10.1007/s11104-013-1918-3

Rajabairavi, N., Raju, C. S., Karthikeyan, C., Varutharaju, K., Nethaji, S., Hameed, A. S. H., & Shajahan, A. (2017). Biosynthesis of novel zinc oxide nanoparticles (ZnO NPs) using endophytic bacteria *Sphingobacterium thalpophilum.Recent trends in materials science and applications* (pp. 245-254): Springer. https://doi.org/10.1007/978-3-319-44890-9_23

Ratzka, A., Vogel, H., Kliebenstein, D. J., Mitchell-Olds, T., & Kroymann, J. (2002). Disarming the mustard oil bomb. *Proceedings of the National Academy of Sciences*, *99*(17), 11223-11228. https://doi.org/10.1073/pnas.172112899

Raza, W., Yousaf, S., & Rajer, F. (2016). Plant growth promoting activity of volatile organic compounds produced by biocontrol strains. *Science Letters*, *4*(1), 40-43. https://doi.org/10.1038/srep24856

Reshu, K. M., & Khan, M. (2012). Role of different microbial-origin bioactive antifungal compounds against *Alternaria spp*. causing leaf blight of mustard. *Plant Pathology Journal*, *11*, 1-9.

Rojas-Tapias, D., Moreno-Galván, A., Pardo-Díaz, S., Obando, M., Rivera, D., & Bonilla, R. (2012). Effect of inoculation with plant growth-promoting bacteria (PGPB) on amelioration of saline stress in maize (*Zea mays*). *Applied Soil Ecology*, *61*, 264-272. https://doi.org/10.1016/j.apsoil.2012.01.006

Ryan, R. P., Germaine, K., Franks, A., Ryan, D. J., & Dowling, D. N. (2008). Bacterial endophytes: recent developments and applications. *FEMS microbiology letters*, *278*(1), 1-9. https://doi.org/10.1111/j.1574-6968.2007.00918.x

Samain, E., van Tuinen, D., Jeandet, P., Aussenac, T., & Selim, S. (2017). Biological control of septoria leaf blotch and growth promotion in wheat by *Paenibacillus sp.* strain B2 and *Curtobacterium plantarum* strain EDS. *Biological Control*, *114*, 87-96. https://doi.org/10.1016/j.biocontrol.2017.07.012

Santoyo, G., Moreno-Hagelsieb, G., del Carmen Orozco-Mosqueda, M., & Glick, B. R. (2016). Plant growth-promoting bacterial endophytes. *Microbiological research*, *183*, 92-99. https://doi.org/10.1016/j.micres.2015.11.008

Schulz, B., & Boyle, C. (2005). The endophytic continuum. *Mycological research*, *109*(6), 661-686. https://doi.org/10.1017/S095375620500273X

Sheng, X. F., Xia, J. J. (2006). Improvement of rape (*Brassica napus*) plant growth and cadmium uptake by cadmium- resistant bacteria. *Chemosphere 64*: 1036–1042.

Sheng, X. F., Xia, J. J., Jiang, C. Y., He, L. Y., Qian, M. (2008). Characterization of heavy metal-resistant endophytic bacteria from rape (*Brassica napus*) roots and their potential in promoting the growth and lead accumulation of rape. *Environmental Pollution*, *156*: 1164–1170. https://doi.org/10.1016/j.chemosphere.2006.01.051

Shi, Y., Lou, K., & Li, C. (2009). Promotion of plant growth by phytohormone-producing endophytic microbes of sugar beet. *Biology and Fertility of Soils*, *45*(6), 645-653. https://doi.org/10.1007/s00374-009-0376-9

Sinha, T., Talukdar, C. N. (2022). Determination of mustard seeds endophytic bacterial population and species composition by development of new method. *Journal of Oilseed Brassica*, *13*(2): 119-129.

Sinha, T., Malakar, C., & Talukdar, N. C. (2023). Mustard seed–associated endophytes suppress Sclerotinia sclerotiorum causing Sclerotinia rot in mustard crop. International Microbiology, 26(3), 487-500. https://doi.org/10.1007/s10123-022-00314-0

Sinha, T., & Talukdar, N. C. (2024). Phylum Level Diversity of Plant Interior Bacteria in Seeds, Supernatant and Pellet Phases of Seed Suspension of Mustard Plant. Indian Journal of Microbiology, 1-11. https://doi.org/10.1007/s12088-023-01184-4

Sobolev, V., Orner, V., & Arias, R. (2013). Distribution of bacterial endophytes in peanut seeds obtained from axenic and control plant material under field conditions. *Plant and soil*, *371*(1), 367-376. https://doi.org/10.1007/s11104-013-1692-2

Spaepen, S., & Vanderleyden, J. (2011). Auxin and plant-microbe interactions. *Cold Spring Harbor Perspectives in Biology*, *3*(4). https://doi.org/10.1101/cshperspect.a001438

Xin, G., Zhang, G., Kang, J. W., Staley, J. T., & Doty, S. L. (2009). A diazotrophic, indole-3-acetic acid-producing endophyte from wild cottonwood. *Biology and Fertility of Soils*, *45*(6), 669-674. https://doi.org/10.1007/s00374-009-0377-8

Yaish, M. W., Antony, I., & Glick, B. R. (2015). Isolation and characterization of endophytic plant growth-promoting bacteria from date palm tree (*Phoenix dactylifera L.*) and their potential role in salinity tolerance. *Antonie Van Leeuwenhoek*, *107*(6), 1519-1532. https://doi.org/10.1007/s10482-015-0445-z

Yasin, N., Khan, W. U., Akram, W., Ahmad, A., Ashraf, Y., & Ali, A. (2017). Application of rhizobacteria for induction of systemic resistance in *Brassica campestris L.* against Alternaria leaf spot disease caused by *Alternaria brassicae*. *Research and Reviews: Journal of Microbiology and Biotechnology*, *6*(1), 51-58.