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Antagonistic activity of *Enterobacter* species against phytopathogenic fungal pathogen

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ABSTRACT

This review focuses on the effective bio-control of plant diseases by microorganisms instead of chemical control methods. Chemical pesticides have been used excessively to treat or prevent plant diseases, harming people and polluting the environment. Numerous traits of *Enterobacter* spp. have been identified, including their ability to fix nitrogen, solubilize soil phosphorus, create antibiotics, secrete siderophores, chitinase, ACC deaminase, and hydrolytic enzymes in addition to exopolysaccharides, and increase soil porosity. Genus *Enterobacter* is a facultative anaerobic, straight rod-shaped, motile bacterium and has peritrichous flagella. Scores of *Enterobacter* strains have these traits, which support plant growth and inhibit pathogenic fungi on plants. The *Enterobacter* genus is crucial for halting the spread of fungi that cause plant illnesses.

Figures : 02 References : 49 Table : 00
KEY WORDS : *Enterobacter* spp., Fungal pathogens, Plant diseases, PGP

Introduction

Agriculture, India's main industry, is key to expanding the country's overall economy. A plant's health can be harmed by more than 10,000 different types of fungi. Plasmodiophoromycetes, Oomycetes, Zygomycetes, Ascomycetes, Basidiomycetes, and Deuteromycetes are classes of fungi that frequently cause infection in crops, including clubroot of crucifers, root disease of cereals, and powdery scab of potatoes. Ascomycetes and Deuteromycetes also commonly cause leaf spots, blights, cankers, fruit spots, fruit rots, and anthracnose. To treat plant diseases, agrochemicals are regularly used, endangering human health and devastating the environment. In the Western world, many synthetic fungicides have lately been banned because of their unfavorable traits, namely their high and acute toxicity. Due to the harmful outcomes of synthetic fungicides on life-sustaining systems, there is an urgent need of substitute for the treatment of pathogenic microbes[31]. Utilizing microorganisms, which are naturally occurring adversaries of plant diseases, biological control is a safe and efficient method of preventing or controlling plant disease outbreaks. As bio-control agents against many plant diseases, bacteria from the prokaryotic groups like *Agrobacterium*, *Bacillus*, *Burkholderia*, *Enterobacter*, *Erwinia*, *Lysobacter*, *Pseudomonas*, and *Serratia* have been used successfully[46].

The genus *Enterobacter* still contains 13 species, such as *Enterobacter amnigenus*, *Enterobacter cowanii, Enterobacter gergoviae, Enterobacter intermedius, Enterobacter pyrinus,* and *Enterobacter sakazakii.* Today, it is believed that *Enterobacter aerogenes* and *Klebsiella mobilis* are homotypic synonyms, and *Enterobacter agglomerans* has been moved to the *Pantoea* genus. The so-called *Enterobacter cloacae* complex is made up of seven different species, including *Enterobacter asburiae*, *E. cloacae, E. dissolvens*, *E. hormaechei, E. kobei, E.nimipressuralis*, and *E. cancerogenus*, which is the more senior synonym of *E. taylorae*. The isolates of the genus *Enterobacter* NII-0907 (*E. aerogenes*), NII-0929 (*E. aerogenes*), NII-0931 (*E. cloacae*), and NII-0934 (*E. asburiae*) were tested for their PGP potential[32].

All 4 *Enterobacter* species are superior phosphate solubilizers (60.1-79.5 mg/ml/day after ten days of incubation), outstanding producers of indole acetic acid (23.8-104.8 mg/ml/day after 48 hrs of incubation), HCN, and siderophore[28]. Therefore, the focus of this review is to give an overview on concepts in plant growth promotion and fungal disease control by *Enterobacter* species.

The ability of *Enterobacter* species to prevent illness and enhance plant growth

Enterobacter species are known to exhibit a variety of PGP traits such as the capacity to fix nitrogen, solubilize soil phosphorus, and secrete siderophore products, antibiotics, chitinase, hydrolytic enzymes, ACC deaminase, exopolysaccharides, and increase soil porosity[10]. The establishment of sustainable agriculture systems may benefit from *Enterobacter*. Generally speaking, *Enterobacter* produces certain chemicals for plants, aids in the assimilation of specific nutrients from the soil, and defend plants from disease. Chloramphenicol acetyltransferases, which confer resistance to 20 mg/ml chloramphenicol, are encoded by the *Enterobacter* sp. 638 genome. It is also known that 4-hydroxybenzoate (precursor of the crucial electron transporter ubiquinone) has antimicrobial properties. *Enterobacter* sp. 638 has the ubiC (Ent638 0243) gene, which codes for the supposed enzyme thought to be capable of performing the process where chorismate is broken down into 4-hydroxybenzoate and pyruvate, in contrast to *E. coli* K12, which is unable to do so[42].

For fungicide forbearance and the generation of PGP attribute in both the presence and absence of fungicides, some workers investigated *Enterobacter*. Even in the presence of fungicides, strain PS2 exhibited PGP activities, although these activities gradually declined as fungicide concentration increased. Others studied the PGP potential of strains NII0907 (*E. aerogenes*), NII-0929 (*E. aerogenes*), NII-0931(*E. cloacae*), and NII-0934 (*E. asburiae*) members of the genus *Enterobacter*. Treatments with *E. cloacae* isolates greatly enhanced the growth of rice seedlings, where the plant height, root length, and a number of roots all significantly (p0.05) influence the plant height[6].

Characterization of *Enterobacter cloacae* MSR1, a plant growth-promoting bacterium reported in the roots of *Medicago sativa* that does not nodulate. The sole supplies of nitrogen for *Enterobacter cloacae* were arginine, tryptophan, and ornithine, while the sole sources of carbon for the organism were glycerol, d-xylose, d-maltose, and esculin melibiose. Through the synthesis of antimicrobial substances, *Enterobacter* species demonstrated as antiphytophathogenic agents. *Lycopersicon esculentum* tomato wilt caused by the bacterium *Ralstonia solanacearum* was more effectively controlled by *Enterobacter* species[48].

Mechanism to stop fungus-causing illnesses from spreading

These bacteria fight plant diseases using a variety of strategies, including parasitism, cross-protection, antibiosis, induced resistance, and competition. The

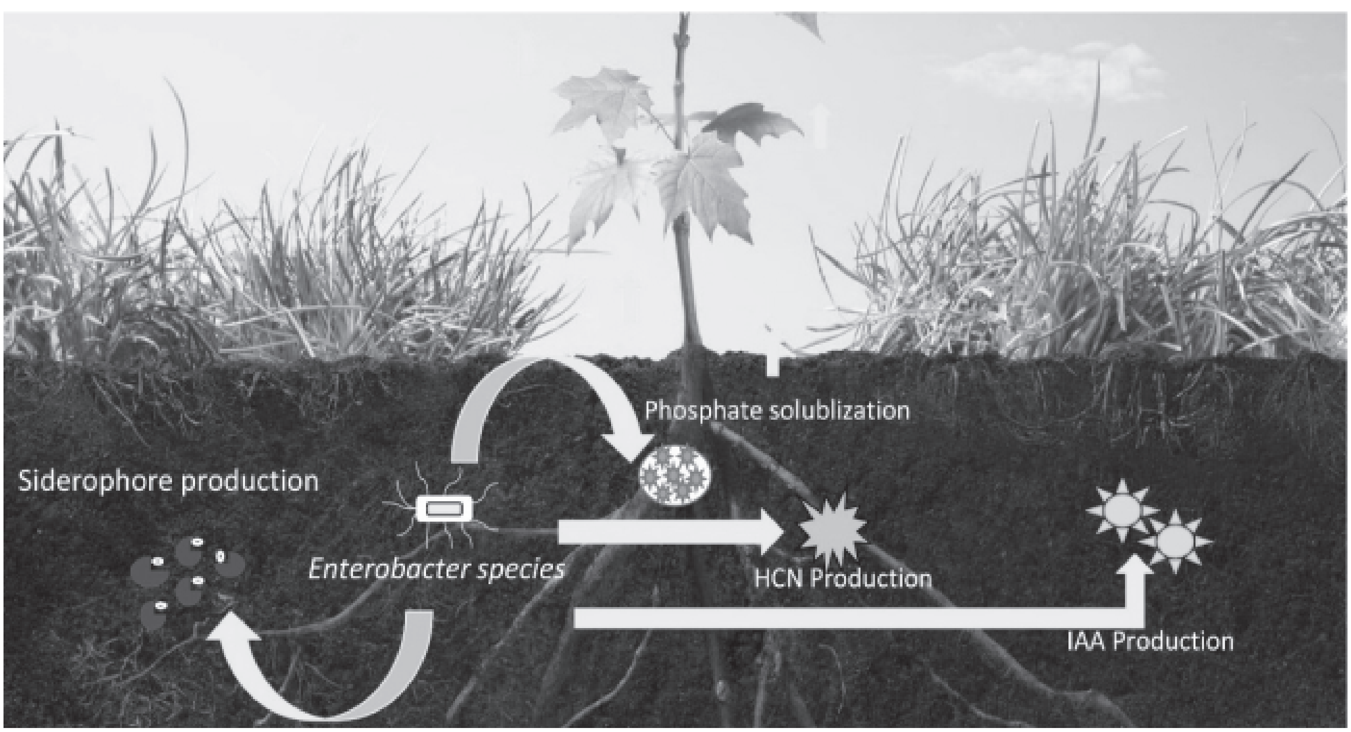

Fig. 1 : *Enterobacter* sp. plays a variety of roles responsible for fostering plant development

microbes compete with one another through nutritional or space conflict. Restricting the incidence and severity of the disease depends on competition between non-pathogens and pathogens for nutrient resources. A mechanism contributing to the inhibition of *Pythium ultimum* by *Enterobacter cloacae*, for instance, has been found to effective degradation of nutrients in the spermosphere. Additionally, these microorganisms create compounds that inhibit infections. The biological control agent *Enterobacter aerogenes* substantially inhibited 35 bacteria and 16 fungi in a dual culture test.When competing with other plant-associated microbes, soil-borne pathogens, such as *Pythium* and *Fusarium* species, are more vulnerable to germination directly on plant surfaces than those that invade through appressoria and infection pegs[45]. The interactions between *Enterobacter cloacae* and *Rhizopus stolonifer* were investigated to determine probable mechanisms by which *E. cloacae* protects peach fruit against postharvest rot caused by *R. stolonifer*. The concentration of the *E. cloacae* as an antagonist affects the capacity to inhibit *Rhizopus* sporangiospores germination. The dosage needed for fruit infection was comparable to the quantity of antagonist (1 x 10^{10}cfu/ mL)) needed to completely inhibit germination. *E. cloacae* prevents *Rhizopus stolonifer* from infecting damaged peach fruit[47].

Siderophores fabricator- Numerous phytopathogens were inhibited by siderophores, the iron-binding compounds made by many rhizobacteria[48].*Enterobacter species* that produce siderophores may indirectly promote plant growth by engaging the antiphytophathogenic actions[35]. Iron chelation is made possible by siderophores, which explains why this process keeps the pathogen away from the iron nutrient[42]. Additionally, siderophores most likely aid in the development of induced systemic resistance in plants. Induced systemic resistance is described as a plant defense system that emerges in response to an infection[5]**.** The siderophore enterobactin can be produced by *Enterobacter* sp. 638, which can also secrete, the iron-enterobactin complex using a ferric siderophore absorption system (ExbDB), and then extract the iron using an enterobactin esterase (Fes). A large group of 17 genes (Ent638 1111-1128) that code for 2- ABC transporters involved in iron uptake (sit ABCD and fep CGDB) are grouped with the genes involved in this enterobactin biosynthesis[17]. Some rhizobacteria, such as strains of *Enterobacter cloacae*, promote plant growth by releasing volatiles. A PGP species of the Enterobacteriaceae family was discovered[19]. This analysis identified AU5 454 *E. radicincitans* as the root-promoting rod[13]. Strain *E. aerogenes* B8 was discovered to be capable of producing an antibiotic-like compound. It was discovered that certain *E. cloacae* and *E. agglomerans* strains produce hydroxamate siderophores as well as several volatile and nonvolatile antifungal compounds[21].

Secondary metabolites producers- A wide range of secondary metabolites, such as antibiotics, hormones, enzymes, pigments, mycotoxins, *etc.*, are produced by microorganisms. Secondary metabolites are produced physiologically, have a high degree of target specificity, and are hence eco-friendly for good creatures. These metabolites are safe for the environment because they are biological in origin, are intrinsically biodegradable, and frequently do not survive in nature[46].

For example **–** All aerobic microorganisms are very hazardous to hydrogen cyanide (HCN) at picomolar concentrations because it disrupts the cytochrome oxidase pathway efficiently. Some volatile substances, including the ammonia generated by *Enterobacter cloacae*, were essential in preventing *Pythium ultimum* caused cotton seedling damping-off[13].

Chitinolytic activity- Most fungal cell walls made up of chitin, an insoluble linear polymer of N-acetylglucosamine (GlcNAc) with a â-1, 4 linkage. Many species of bacteria, fungi, and plants also produce chitinolytic enzymes. It was believed that the attachment to fungus hyphae was one important mechanism in the biocontrol activity of *E. cloacae* strains against *Pythium ultimum*. A wide range of antifungal activity is exhibited by the soil-borne *Enterobacter agglomerans* IC1270. This strain produces and secretes two N-acetyl-b-D-glucosaminidases, an endochitinase, as well as additional chitinolytic enzymes.

***Enterobacter* ability to stick-** Enteric bacteria, such as *E. cloacae* have Type 1 fimbriae on their surfaces, which are made up of proteins that bind only to mannoside residues[2]. These filamentous appendages emanate peritrichously from the bacterial cell surface and play a role in how cells adhere to the surfaces of plant, animal, and yeast cells[8]. Type 1 fimbrial-mediated binding to mammalian cells is typically identified by the *in vitro* agglutination of bacteria to either erythrocytes or yeast (*Saccharomyces cerevisiae*) cells, and it is inhibited by low concentrations of mannose and other mannosides[32]. The adherence of strains of *Enterobacter agglomerans* to plant roots may be mediated by Type 1 fimbriae as well as other purported Type 3 fimbriae. These fimbriae are thought to assist the use of root exudates by these species and to help these species establish in the rhizosphere population[12]. *E. cloacae* bacteria strongly cling to hyphal cell walls, reducing fungal growth in the process. Pretreating *E. cloacae* cell suspensions with different mono-saccharides, di-saccharides, and tri-saccharides, as well as with particular amino sugars or p-linked glucosides, however, prevents cells from attaching to intact hyphae and also eliminates cells' capacity to inhibit fungal growth. On the other hand, pretreating cells with specific monosaccharides or methylated sugars do not affect *E. cloacae's* capacity to cling to hyphae and stop *P. ultimum* from growing. When applied to plant seeds that initially release little in the way of carbohydrates, *Enterobacter cloacae* is an efficient biological seed protectant. However, it is useless when applied to plant seeds that initially release a lot of sugar[7].

An attribute of *Enterobacter spp.* is motility- All of these species, except *Enterobacter asburiae*, are facultative anaerobic and motile *via* peritrichous flagella. *Enterobacter* species are motile by four to six peritrichous flagella. Some are encapsulated. These bacteria typically range in size from 0.6 to 1.0, 2-3 m. When cultivated on nutritive agar, *Enterobacter* species create irregularly edged, spherical, iridescent, flat colonies. Although they may grow in temperatures as high as 44 °C, the ideal growth range is between 30 and 37 °C[7]. An essential quality of endophytes is motility. Endophytic colonization occurs most frequently in the rhizospheric area of root and the *Enterobacter sp*. 638 is also well-suited to this area and actively moves there. In the presence of various carbon sources, *Enterobacter sp*. 638 produces 2-phenyl ethanol. The pleasant floral scent emanating from *Enterobacter sp*. 638 cultures is a result of the antibiotic substance 2-phenylethanol, which is often used in perfumery. Two genes, Ent638 1306 and Ent638 1876, are considered to encode an enzyme that is necessary for converting phenyl-acetaldehyde into 2-phenyl ethanol[42].

***Enterobacter spp.* encodes Superoxide dismutase-** Three superoxide dismutase are encoded on the chromosome of *Enterobacter sp.* 638 are-(1)SodA, and Mn superoxide dismutase, (Ent638 4063); (2) SodB, a Fe superoxide dismutase; and (3) SodC, a Cu/Zn superoxide dismutase, (Ent638 1801). *Enterobacter sp*. 638 appears to be able to detoxify free radical nitric oxide by the presence of a flavohemoprotein nitric oxide dioxygenase (Ent638 3037) and an anaerobic nitrate reduction operon[42].

***Enterobacter spp.* produces antimicrobial lipopeptides-** Typically, the antibacterial lipopeptides have a cyclic or linear oligopeptide connected to a variable-length -hydroxy fatty acid tail. The first study on *Citrobacter* and *Enterobacter* strains that are widely found in food and are a part of the human gut flora that produce antibacterial lipopeptides. The lipopeptides are extremely beneficial compounds with prospective uses in a variety of technological fields, including medicine, cosmetics, food preservation, and dairy products[29].

***Enterobacter* spp. act as a phosphate solubilizers-** The primary P-solubilizers come from the genera *Enterobacter* .Because they can solubilize inorganic phosphate, *Enterobacter spp*. promote plant development. This capacity is more likely the result of the secretion of organic acids and phosphatases, which increase the solubilization of phosphate and make it easier for plant roots to absorb phosphorus, hence promoting plant growth and production[23].

Sources of 1-aminocyclopropane-1-carboxylate deaminase- The 1-aminocyclopropane-1-carboxylate deaminase enzyme found in Plant growth promoting microorganisms transforms ACC, (precursor of ethylene), to alpha-ketobutyrate and ammonium, reducing the concentration of ethylene under stress and promoting plant growth[49]. Numerous bacterial species that live in rhizospheric soil, such as *Enterobacter species*, can use ACC as their only source of nitrogen. According to one study, ACC deaminase activity from a halotolerant *Enterobacter* species promoted the development of rice seedlings[37]. In studies on wheat and sugarcane plants under salinity stress, it has been demonstrated that *Enterobacter* spp. exhibiting different ACC deaminase activities that boost plant development[22,40].

Nitrogen fixer- *Enterobacter s*pecies have been recovered from numerous plants, including sugarcane (*Saccharum officinarum*), which contains the nitrogen-fixing *E. sacchari* and the nitrogen-fixing *E. oryzae* from the wild rice species *i.e. Oryza latifolia*[34]. In addition, the rhizosphere soil of groundnuts included the diazotrophic bacterium *E. arachidis*, which promotes plant growth. Since nitrogen is the scarcest nutrient for plant growth, nitrogen fixation is a crucial characteristic for relevant *Enterobacter* species that operate as plant growth promoters[27].

Hormones producer- IAA is a well-known hormone that controls plant growth and affects root elongation and cell division. A common component of plant root exudates is tryptophan, a precursor to IAA. *Enterobacter* species[11,30], including *E. asburiae*[17], *E. ludwigii*[31] and *E. cloacae*[45] have been documented to produce IAA.

Bio surfactant producers- A biosurfactant produced by *Enterobacter sp*. MS16- Surfactants are amphiphilic substances with hydrophilic and hydrophobic groups that can lower surface and interfacial tension. When grown on less expensive carbon sources like sunflower oil cake, *Enterobacter sp*. MS16, which was recognised by its 16S rRNA, produced biosurfactants. The biosurfactant was discovered to contain glucose, galactose, and

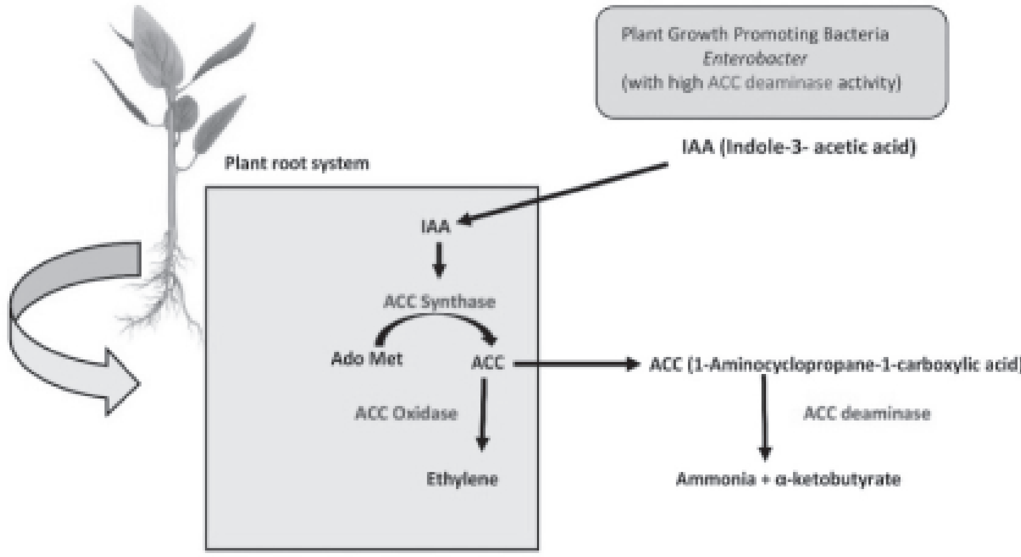

Fig.2 : Function of ACC deaminase producing *Enterobacter species*

arabinose as well as an FA moiety of C16 and C18. It exhibited antifungal properties and prevented the germination of fungus spores[29].

Other enactment of *Enterobacter* spp.

- Different *Enterobacter cloacae* strains were tested for their capacity to reduce the stimulatory activity of plant seed exudates to sporangia of the seed-rotting fungus *P. ultimum*. These microorganisms successfully prevent damping-off and *Pythium* seed rot[45].
- When cultured on culture media containing amino acids, *Enterobacter cloacae* is able to deaminate amino acids in a manner that produce ammonia that is poisonous to *P. ultimum* and other fungi that infect roots[16].
- With the use of *Enterobacter aerogenes* B8, *Phytophtora cactorum*-induced apple crown and root rot infections were significantly reduced[43].
- Certain *Enterobacter agglomerans* strains have been demonstrated to be effective in the management of plant diseases brought on by various bacterial and fungal infections[33].
- *E. cloacae* had a concentration-dependent effect on the inhibition of *Rhizopus* sporangiospore germination. The antagonist concentration (1 x 10^{10} CFU/mL) required to totally suppress germination and required to stop fruit infection. *Rhizopus stolonifer* infection of injured peach fruit is thwarted by *E. cloacae*. Among the 70 bacterial isolates evaluated, this one was the most efficient[47].
- Since many endophytes will aid in the growth, development, and health of their host plant, endophytic colonisation is regarded as an indication of a healthy plant system. Numerous bacterial endophytes from a variety of genera, including *Enterobacter*, can colonise every organ of the plant. Many of these endophytes are closely related to common soil bacteria[25].
- In a recent study, we found that the rhizobacteria *Enterobacter agglomerans*, seemed to inhibit the growth of *Pyricularia oryzae*, the causative agent of rice blast disease. By continuously improving isolation, formulation, and application techniques, particularly in the field, these agent's potential can be increased[41].
- To protect *Lycopersicon esculentum* plants against FORL (*Fusarium oxysporum* f. sp. *radicis-lycopersici*), *Enterobacter species* (AR1.22) cell-free culture extracts were applied to tomato roots. This indicates that antibiosis is a form of biological control action[20].
- Applications of the bacteria *E. cloacae* have also been used to control *Pythium* damping-off. The bacteria make a lectin that binds to hyphae of *Pythium sp*ecies and is likely a key player in the biocontrol action[26].
- *Enterobacter* species produced antimicrobial substances that acted as antiphytophathogenic agents. The tomato (*Lycopersicon esculentum*) bacterial wilt (*Ralstonia solanacearum*) was more effectively controlled by *Enterobacter* species[48].
- Some *Enterobacter species* have been described as plant growth promoters because they manifest a variety of growth-enhancing properties. Some examples are- *Enterobacter cloacae* from maize, citrus trees[3,10]; *Enterobacter asburiae* from sweet potato; *Enterobacter agglomerans* and *Enterobacter sakazakii* from soybean[4]. Recently, *Enterobacter*

cloacae was discovered in the soybean rhizosphere, and it dramatically accelerated the growth of soybean-wheat plants[36].

- Rhizosphere-associated bacteria as *E. oryzendophyticus*[14],*Enterobacter oryziphilus*, *E. oryzae*[30], *E. agglomerans*[1] and *E. cloacae*[38] that can tolerate anaerobic growth environments and have been found to fixing atmospheric nitrogen and encouraging root growth with bacterial phytohormones in rice plants to increase nutrition availability in the rhizosphere[24].

Conclusion

Future outlook for the biological management of plant diseases are promising, and given the growing demand among producers for biocontrol products, it might be a successful strategy. Not to mention, there are bacteria like *Enterobacter spp*. that can be employed to suppress fungal growth. Numerous *Enterobacter species* have different traits that encourage plant growth, and they can do it either directly or indirectly. These include the formation of phytohormones, siderophores, and antibiotics as well as the solubilization of phosphate and the fixation of nitrogen. In light of this, expanding the biotechnological uses of *Enterobacter species* as biostimulants, biocontrol agents, biofertilizers, and biofortifiers is necessary as an environmentally benign measure to promote sustainable agriculture and the production of nutritious food worldwide. The bacterium *Enterobacter* can greatly aid in the prevention of fungal infections and the growth of plants in addition to being a human pathogen.

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