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# Endophytic Bacteria Associated with Rice: Role in Biotic and Abiotic Stress Protection and Plant Growth Promotions

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## ABSTRACT

Rice (Oryzae sativa) is an edible starchy grain for a significant population of the World, and it supplies more than 50% of calories consumed by the entire human population. Regarding rice production, India holds the second position next to China. The threat to rice yield is encouraged by various biotic and abiotic factors. Increased rice production needed to satisfy global demand results in excessive use of chemical fertilizers and pesticides and, in the end, toxicity to human and environmental health. Endophytic microbes have potentials to combat various biotic and abiotic stress causes to the rice production. Endophytic microorganisms also utilized as biocontrol due to its properties such as antibacterial, antifungal and plant growth promotions which make theseas one of the safe and alternative approaches to chemical fertilizers in sustainable agricultural practices. This review, briefly summarised the endophytic bacteria from rice plants with their biocontrol potential, plant growth-promoting attributes and their prospects with special reference to north east India.

Keywords: Endophytic bacteria, Biocontrol agents, Phytopathogens, Plant growth promoting activity, Sustainable agriculture

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## INTRODUCTION

Rice is an important cereal food crop of global significance that belongs to the family Graminae and genus Oryza, including twenty wild species and two cultivated species, namely, Oryza sativa (cultivated throughout the World) and Oryza glaberrima (cultivated mainly in Africa) (Pareja et al., 2011). The monocotyledonous plant is mainly grown in humid tropical and subtropical climates. Rice (Oryza sativa) is a staple food grain for more than one-half of the World's population, and it provides more than 20% of the daily calorie intake (Ray et al., 2013; Habib et al., 2017; Gull & Kausar, 2018). Rice is commonly grown in plain areas and near rivers, but it is difficult to discuss the specific condition for rice cultivation. It can be grown in almost all kinds of environments depending on the nature of the cultivars. Variations in rice yield occur according to latitude. The regions located at the latitude of 40° S and 45°N are recognized for extensive rice cultivation. The highest rice yield is also seen between 30° N and 45° N of equators. Rice can also be grown below sea level, i.e., in Kerala, and at 1979 m altitudes, i.e., in Jammu and Kashmir. The deep-water rice varieties are favourably grown in flood-prone areas during the rainy season (Chang et al., 1987). Almost thousands of varieties of O. sativa are found to grow in more than 100 countries. They can be grouped into three wide ecological varieties: (a) the longgrained indica variety grown in tropical and subtropical Asia, (b) the short or medium-grained rice variety cultivated in temperate regions, and (c) the medium-grained javonica variety grown in the Philippines and the mountainous area of Madagascar and Indonesia (Muthayya et al., 2014). Asian countries, including India, China, Japan, Philippines, Thailand, Indonesia, Sri Lanka, etc., account for 90% of the total World's total rice production, while other non-Asian rice-producing countries include Brazil, Egypt, Nigeria, Madagascar, the United States which account for 5% of the rice produced globally. China and India account for more than 50% of rice production (Muthayya et al., 2012). India is the second largest producer (42.9 million hectares) and 27.1% of the total Rice growing area next to China (Singh et al., 2012). In India, the lower and middle Ganga plains, the east and west Coastal plains, the Brahmaputra valley, and parts of the Peninsular plateau are known as major rice-producing areas (Mahajan et al., 2017). Northeast India has diverse geographical regions with varied climatic conditions for rice cultivation (Singh et al., 2006). India has five rice-growing regions: the northern region, the northeastern region, the eastern region, the southern region, and the western region. Assam, one of the eight states of northeast India located between 24°N and 28°18/N latitudes and 89°4/E and 96°0/E longitudes, is considered an essential contributor of rice to the economy of India (Singh et al., 2003).

It is estimated that 800 million tons of rice production will be essential to meet global hunger by 2025. However, increased rice production results in higher costs and excessive use of chemical fertilizers and pesticides. Abiotic conditions like flood, drought, and salinity affect rice productivity (Jana *et al.*, 2022). Furthermore, rice diseases can cause devastating economic loss by decreasing yield production and by disturbing the stable food

supply chain (Kim et al., 2021). Diseases caused by pathogens become a significant threat to rice yield, which causes 20-100% yield losses (Shivappa et al., 2021). Almost 70% of the diseases in rice plants are caused by bacteria, fungi, and nematodes (Etesami, 2019). It is found that during heavy rainfall, the brown spot disease of rice becomes a bulging threat in areas such as the Himalayas, Malabar coast, West Bengal, and Assam (Chakrabarti, 2001). The disease was also known for contributing a significant factor to the "Great Bengal Famine, 1942" by decreasing 50-90% yield losses and causing the death of two million people (Chakrabarti, 2001). In 1910, sheath blight of rice was reported in Japan first time and subsequently spread across temperate and tropical regions (Willocquet & Savary, 2011). Sheath rot disease occurs in all Rice cultivating areas, and now the disease has been recognized as a major threat to rice production (Bigirimana et al., 2015). The most common fungal diseases of rice include sheath blight, sheath rot, brown spot, etc. While in DWR, sheath blight (ShB), sheath rot (ShR), and stem borer (SB) are found (Islam et al., 2004). To control and suppress the total yield loss caused by biotic and abiotic factors, local farmers have used commercially available fungicides and other chemicals, severely affecting human and environmental health. Therefore, there is an urgent need to take action to minimize the negative effect of chemically synthesized fertilizers and fungicides and search for an alternative method that sustainably enhances agricultural practices. The application of beneficial microorganisms having biocontrol potential is considered a safe and an alternative approach to chemical fertilizers and fungicides (Widiantini et al., 2017; Etesami, 2019).

In 1898, Vogl reported the presence of endophytes for the first time, and several reports have studied endophytes isolated from tissues of different plant parts since 1940 (Mano & Morisaki, 2008). Endophytes are defined as microorganisms (fungi and bacteria) that live inside the plant host tissue without producing any symptoms or causing any harm to the host plant (Laskar et al., 2012). Many findings suggested that endophytic bacteria (both gram-positive and gram-negative) have also been extracted from different plants like soybean, wheat, corn, sorghum, cucumber, sugar beet, and Rice (Misaghi & Donndelinger, 1990; Stoltzfus et al., 1997; Zinniel et al., 2002; Chandrashekhara et al., 2007). Endophytes can enter the plant primarily through root tips and aerial portions of the plants, such as stems, flowers, and leaves, and systematically spread over the whole plant body (Kandel et al., 2017). Bacterial endophytes colonize plant tissue by creating beneficial relationships with host plant via the synthesis of phytohormones, production of enzymes, mobilization of nutrients, and nitrogen fixation (Hassan, 2017; Naseem et al., 2018; Hassan et al., 2020). Endophytes are also vital in the plant's physiological activities, such as enhancing resistance to diseases and stress and improving productivity and they also synthesize secondary metabolites of plant importance (Gouda et al., 2016). The biocontrol of plant pathogens using endophytic bacteria has been evaluated for rice plants and other plants (Mano & Morisaki, 2008). Commonly found endophytic bacteria like Pseudomonas, Azospirillum, and Bacillus are known to play a significant role in the growth of crop plants by synthesizing required metabolites (Chandrashekhara et al., 2007; Waqas et al., 2014). Endophytes can exert plant growth-promoting activities in various ways, such as by producing plant growth

hormones like Indole Acetic Acid (IAA), through solubilization of phosphate, Production of siderophores, and providing vitamins and nitrogen to plants (Bandara *et al.*, 2006). They can also accelerate plant growth and nitrogen-fixing capabilities of the host plant (Verma *et al.*, 2001; Rahman & Saiga, 2005).

## Biocontrol mechanisms of endophytic bacteria

The biological interactions provide several benefits to the involved species and have a positive impact on the integrity and sustainability of the agroecosystem (Brusamarello-Santos *et al.*, 2017). The endophytic bacteria can directly act on plant growth enhancement by the production of growth regulators and other lytic enzymes, phosphorous solubilization, and acceleration of digestion which confer resistance to biotic factors. Endophytes can also indirectly transmit beneficial traits by producing bioactive compounds for controlling pathogens, cell wall degrading enzymes, and stimulating systematic resistance (Kandel *et al.*, 2021). **Figure 1** is drawn to explain the mechanisms of action of biocontrol agents and to understand the interaction between endophytes and host plants.

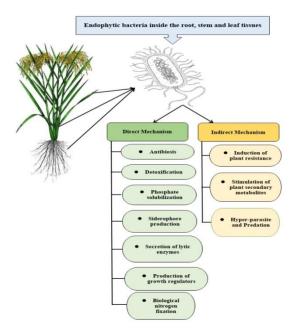


Figure 1. Biocontrol mechanism of actions of endophytic bacteria

# Detoxification of virulent factors

Detoxification and degradation of virulent pathogen factors is a unique mechanism of endophytic bacteria. The mechanism depends on the protein production that binds reversibly and irreversibly to the toxins produced by pathogens (Compant *et al.*, 2005). For example, strains of *B. 2azandar* and *R. solanacearum* can hydrolyze fusaric acid produced by *Fusarium* species (Toyoda & Utsumi, 1991). In addition, it is reported that biocontrol agents such as *Pseudomonas sp* and *Pantoea sp* can detoxify the albicidin toxin produced by *Xanthomonas sp* (Zhang & Birch, 1996; Walsh *et al.*, 2001).

## Competition for iron and siderophore production

Iron is an essential element for the metabolic processes of almost all living organisms. That is why competition may arise

in soil due to scarcity of available ions to the soil microbiota and plant (Mazhar et al., 2016). Thus, microorganisms have adapted several mechanisms. Low molecular weight compounds such as "siderophores" are released by endophytic microbes to absorb iron (Fe+3) (Miethke & Marahiel, 2007). Pedraza et al. (2007) reported that siderophore production could be considered a biocontrol mechanism that showed antagonism towards pathogenic fungi for iron elements. Tian et al. (2009) isolated some gram-negative bacteria like Bacillus, Pseudomonas, and Enterobacter genera which can secret siderophores under iron-limiting conditions.

#### Antibiosis

Antibiotics are low molecular weight heterogenous compounds that inhibit the metabolic and growth functions of pathogens and can enhance the plant defense system. Compant *et al.*, 2005 reported that antibiotics and antibiotic-related compounds such as kanamycin, oligomycin A, xanthobaccin, and zwittermicin A are produced by *Streptomyces, Bacillus*, and *Stenotrophomonas* spp. Haas and Défago, 2005 found six antibiotic groups, including phloroglucinols, pyrrolnitrin, pyoluteorin, phenazines, cyclic lipopeptide, and hydrogen cyanide, can act as inhibitors of root diseases.

## Production of growth regulators

The main growth hormones produced by endophytes are auxin (indole acetic acid- IAA), gibberellic acid (GA), cytokinin, ethylene, etc. IAA is responsible for cell elongation, differentiation, and lateral and adventitious root growth, whereas GA is for seed germination and delaying plant aging. Cytokinin controls cell division and ethylene production responses during environmental stress (Jana et al., 2022). Bacillus, Microbacterium, Micrococcus, and Pseudomonas, etc. are some well-known groups of rice-associated endophytic bacteria that lead synthesis of auxin and GA, seedling growth, and other PGP activity (Ji et al., 2014; Krishnamoorthy et al., 2020; Borah et al., 2021).

## Biological nitrogen fixation

Biological nitrogen-fixing is an environment-friendly key process to fix about 60% of atmospheric nitrogen on the Earth. The nitrogen fixation mechanism is governed by the enzyme nitrogenase (encoded by *nif* genes), which converts atmospheric nitrogen into ammonia for plant uptake (Wang *et al.*, 2013). Endophytic organisms having nitrogen fixation

activity have a significant role in fixing atmospheric nitrogen in an available form for their host species (Puri *et al.*, 2017). Several Rice endophytic bacteria have been reported to help increase nitrogen fixation among the host plant, such as *Azoarcus sp, Burkholderia sp, Herbaspirillum seropedicae*, and *Gluconacetobacter diazotrophicus* (Bhattacharjee *et al.*, 2008; Zhou *et al.*, 2020).

## Induction of plant resistance

Certain bacteria can activate chemicals that fortify plant cell wall strengths and metabolic responses of the host plant, leading to an enhanced plant defense system against pathogenic substances and/ or abiotic stress factors (Compant *et al.*, 2005). *Bacillus* is well known for induced systematic resistance (ISR) under abiotic stress conditions (Chakraborty *et al.*, 2006). Viswanathan and Samiyappan (1999) found that *P. fluorescence* triggered ISR against the red rot disease of sugarcane caused by *Colletotrichum falcatum*.

## Hyperparasite and predation

The bacterial endophytes can adopt hyperparasitism and/or predation by synthesizing lytic enzymes such as chitinase, glucanase, cellulose, etc., which can degrade the cell wall of fungal pathogens. The extracellular chitinase and laminariase produced by *Pseudomonas stutzeri* digest and lyse mycelia of *F. solani* (Lim et al., 1991). B-1,3 glucanase enzyme synthesized by *B. 3azandar* can destroy the integrity of *R. solani*, *S. rolfsii*, and *Pythium ultimum* (Fridlender et al., 1993).

# Rice endophytes against phytopathogens

Endophytic microorganisms that live inside the plant parts have a significant role in plant growth and defense response. The application of endophytic organisms and their bioactive compound is considered an alternative strategy to control phytopathogens. Mukhopadhyay *et al.* (1996) isolated bacterial endophytes from the seedling of rice exhibiting antagonistic effects against fungal pathogens *R. solani, P. myriotylum, G. graminis, H. annosum* by secreting various antifungal compounds. Endophytic bacteria isolated from rice plants have potential control for rice seedling disease and plant growth promotion (Adhikari *et al.,* 2001). Isolation of endophytic fungi and actinomycetes from rice cultivars showed an antagonistic effect against rice pathogens (Tian *et al.,* 2004). **Table 1** is presented to describe the application of rice endophytic bacteria against phytopathogens exclusively.

**Table 1**. Application of rice endophytic bacteria against phytopathogens

Rice variety	Isolated endophytic bacteria	Biocontrol potential against phytopathogens	References
Oryza sativa L. var. Morelos and Apatzingan (parts used- seeds)	Corynebacterium avescens and Bacillus pumilus	Inhibited the growth of <i>Azospirillum brasilense</i> in rice seedlings.	Bacilio-Jiménez et al., 2001
Oryza sativa L. (parts used- stems)	Bacillus sp. CHM1	Antifungal activities against Fusarium oxysporum, Rhizoctonia solani, Colletotrichum gossypii, Gibberella zeae, Botrytis cinerea pers, and Dothiorella grgaria.	Wang <i>et al.</i> , 2009
Oryza sativa L. Bacillus sp. (parts used- roots)		Antibacterial activities against <i>Xanthomonas oryzae</i> and <i>Burkholderia glumae</i> cause bacterial blight and panicle blight disease of rice.	Chung et al., 2015

Oryza sativa L. cv. Katy and MH86 (parts used- seeds)	Bacillus amyloliquefaciens B.methylotrophicus and B. subtilis	Antagonistic activities against <i>Xanthomonas oryzae</i> , cause the bacterial leaf blight disease of rice.	El-shakh <i>et al.,</i> 2015
Oryza sativa L. (parts used- roots)	Rhizobium sp., Azospirillum sp.	Antagonistic activities against Rhizoctonia solani, Fusarium oxysporum, and Pythium sp.	Sev <i>et al.,</i> 2016
<i>Oryza sativa</i> L. (parts used- roots)	Bacillus	Suppression of the development of sheath blight disease and bacterial panicle blight disease of rice.	Shrestha et al. (2016)
Oryza sativa L. cv. Gohar (parts used- seeds)	Stenotrophomonas maltophilia SEN1	Antifungal activity against <i>Magnaporthe grisea</i> , by secretion of fungistatic metabolites.	Etesami and Alikhani, 2016
Oryza sativa L. cv. Gohar (parts used- roots)	Bacillus cereus	Showed inhibition of mycelial growth against Fusarium proliferum, F. verticillioides, F. fujikuroi, Magnaporthe 4azandar, and Magnaporthe grisea.	Etesami and Alikhani, 2017
Oryza sativa L (parts used- rhizosphere)	Bacillus sp MBRL-576	Anti-microbial potential against fungal pathogens such as Curvularia oryzae, Rhizoctonia solani, and Fusarium oxysporum, by producing diffusing and volatile compounds and fungal cell wall degrading enzymes.	Tamreihao <i>et al.,</i> 2018
Oryza sativa L. var. indica cv. RD41 and s, O. sativa L. var. indica cv. Pathumthani 1 (parts used- roots)	Bacillus subtilis, Bacillus kochii, Bacillus altitudinis	Antifungal activity against Alternaria, Bipolaris, Cercospora, Curvularia, Fusarium, and Sarocladium, which causes dirty panicle disease (DPD) OF RICE	Rangjaroen <i>et al.,</i> 2019
<i>Oryza sativa</i> L. (parts used- roots)	Bacillus, Fictibacillus, Lysinibacillus, Paenibac illus, Cupriavidus,and Microbacterium	Resistance against fungal pathogens, including <i>M. oryzae</i> , <i>R.solani, F. graminearum, F. moniliforme,</i> by synthesizing different bioactive compounds	Khaskheli <i>et al.,</i> 2020
Oryza sativa L.	Paenibacillus polymyxa	Shown promising activity against phytopathogens such as Fusarium oxysporum, P. aphanidermatum, P. myriotylum, P. infestans, C. acutatum, and S. rolfsii	Radhakrishnan et al., 2021
Oryza sativa L. (Parts used- roots)	Burkholderia sp	The isolate inhibits infection of Magnaporthe oryzae, which causes rice blast disease by the production of small molecules of antifungal compounds.	Xue <i>et al.</i> , 2022

Rice endophytic bacteria for plant growth promotion

Endophytic plant growth-promoting (PGP) bacteria utilize many direct and indirect mechanisms to enhance plant growth and productivity. Due to its environment-friendly nature, the application of endophytes has been considered an alternative

biocontrol strategy in agricultural practices (Ali *et al.*, 2017). The application of rice endophytic bacteria for plant growth-promoting activity and their potentiality as biocontrol approaches are discussed below **(Table 2)**.

Table 2. Application of rice endophytic bacteria for plant growth promotion activity

Rice variety name	Parts used	Isolated endophytic bacteria	PGP activity	Reference
Oryza sativa L.	Roots, stems, and leaves	Methylobacterium sp, Curtobacterium sp	Showed osmotic resistance, nitrogen-fixing ability, and cellulase activity.	Elbeltagy <i>et al.,</i> 2000
Oryza sativa L.	Roots	P. Pseudoalcaligenes, B. pumilus	Showed better responses against the adverse effects of salinity.	Jha <i>et al.,</i> 2011
<i>Oryza sativa</i> L. cv. KDML105	Roots, stems, and leaves	Streptomyces sp	PGP activity by siderophore production.	Rungin et al., 2012
Oryza sativa L.	Roots	Rhizobium sp., Azospirillum sp.	Exhibited plant growth enhancement by IAA production, phosphate solubilizing activity, and nitrogen fixation capacity	Sev <i>et al.,</i> 2016
<i>Oryza sativa</i> L. ssp. Indica	Seeds	Flavobacterium sp., Microbacterium sp., and Xanthomonas sp	Performed PGP activities such as hormone modulation, nitrogen fixation, siderophore production, and phosphate solubilization	Walitang <i>et al.,</i> 2017

Oryza sativa L.	Leaf, stem, and root	Pseudomonas aeruginosa , Bacillus megaterium, Sphingobacterium siyangensis, Stenotrophomonas pavanii and Curtobacterium plantarum	PGP trait by Production of IAA and siderophore and secretion of phosphate solubilization and ACC deaminase. These isolates are promising bioinoculants for the detoxification of chlorpyrifos (cp) residues in rice plants and grains.	Feng <i>et al.,</i> 2017
Oryza sativa L.	Leaf, stem, and root	Lysinibacillus sphaericus	The isolates showed Nitrogen-fixing activity	Shabanamol et al., 2018
Oryza sativa L.	Shoots and roots	Mycobacterium, Bacillus, Pseudacidovorax, Rhizobium, Sphingomonas, Flavobacterium, Pseudomonas	Isolates showed nitrogen fixation potential, IAA production ability, and tolerance towards etridiazole and metalaxyl application	Shen <i>et al.,</i> 2019
Oryza sativa L.	Roots	Rhizobium sp.	PGP traits by Production of siderophore, ACC deaminase, and IAA. Also produced some secondary metabolites.	Zhao <i>et al.</i> , 2020
Oryza sativa L.	Roots	Bacillus tequilensis and Bacillus aryabhattai	The isolates were found to be tolerant at high salt concentrations and could be used as a good potential for salinity mitigation practice for coastal rice cultivation	Shultana et al., 2020
Oryza sativa L.	Roots	Pseudomonas, Ralstonia, Burkholderia, Bradyrhizobium, Clostridium, Sideroxydans, Kineosporia, Bacillus	Endophytic bacteria from Cadmium- contaminated rice roots display high Cd resistance and may promote plant growth, suggesting their potential in reducing high metal stress on the plant	Chu <i>et al.</i> , 2021
Oryza meridionalis	Roots, stems, and leave <b>s</b>	Bacteroides,Prevotella, Alistipes,Rhodanobacter, Brevundimonas, Lactobacillus, Haliangium, Faecalibacterium, Alloprevotella, Terriglobu	Isolates could be applied as a good potential to reduce phthalates (PAEs) accumulation in crops and to increase yield	Liu <i>et al.,</i> 2020

Exploration of endophytic bacteria from rice varieties of north-

Thakuria et al., 2004 isolated different groups of bacteria (Azospirilla, Bacillus, and Pseudomonas) from rice cultivated in the acidic nature of the soil in Assam and evaluated them for phosphate solubilizing activity, IAA production level, nitrogenase activity, and antibiotic resistance profile. Laskar et al. (2012) isolated endophytes from rice plants in the Barak valley of Assam. The results revealed that the stems and leaves region of rice plants contain the maximum diversity of endophytes. Roy et al. (2021) investigated fungi that live inside the seeds of indigenous varieties of rice plants in Northeast India and determined IAA activity in vitro, and Fusarium sp showed the highest antifungal activity against the rice pathogen Magnaporthe grisea. The research concluded that seed-borne endophytes could be used as bioinoculants for crop improvement. Saikia and Bora (2021) explored actinomycetes and endophytes isolated from rice cultivation of the Jorhat and Lakhimpur districts of Assam and found effective management for rice bacterial blight. Borah et al. (2018) investigated the endophytic microbial diversity of wild and cultivated rice varieties and concluded that rice endophytes could be applied as efficient plant growth promoters and biofertilizers. Kumar et al. (2020) isolated and characterized endophytic bacteria from six rice varieties grown in central, eastern, and northeast India. The findings suggested that Bacillus subtilis isolate exhibiting antibacterial and antifungal activity in their study may be utilized for the development of bio formulations for controlling multiple biotic stress. Shepra et al. (2021) isolated and

characterized rhizobacteria from a paddy field in Sikkim, India. The results indicated their plant growth-promoting attributes in rice plants and biocontrol potential against phytopathogen *Colletotrichum gloeosporioides* of large cardamom (*Amomum subulatum*). Borah *et al.* (2021) studied endophytic bacteria isolated from wild and cultivated rice varieties of Assam and their utility as growth-promoting factors to plants. The result indicated that rice endophytes have the potential as an effective bioinoculant.

# CONCLUSION

The application of biocontrol agents satisfies the goal of a sustainable agricultural system. Understanding the mechanism of interaction between antagonist and pathogen is one of the critical key steps of sustainable agriculture as it provides correct hints for the selection of effective biocontrol agents. Unfortunately, it is estimated that only less than 10% of the overall global crop protection market is covered by biocontrol agents (McDougall, 2018). Therefore, there is an urgent need for more comprehensive biocontrol research. The foremost step in the development of effective commercial biological controlbased products is the screening for appropriate candidates (Raymaekers et al., 2020). Since the current scenario is facing the urge for food to satisfy the hunger of the increasing human population, developing biocontrol agents with high productivity impact in rice crops is a tough challenge. One crucial point is that one particular endophyte might not offer all the beneficial characteristics to the host. Thus, depth research for searching and finding bacteria with potential growth-promoting characteristics, stress tolerance, and biocontrol features is essential.

Most of the experiments and studies mentioned in this paper have only been done at a laboratory scale. Therefore, further research should be carried out to provide more knowledge on the mechanisms of biological control agents. The research should be performed at a commercial scale to largely occupy the global market of crop protection. It is expected that in the future, the biological control-based product will be commercially available to farmers worldwide, and this will 6azand to achieve higher and better yields in farming practices.

Recent studies reveal that only limited rice varieties have rarely been analyzed for endophytic biology till today. Since each 300,000 plant species on the Earth harbors one or more endophytes (Borah *et al.*, 2021); thus, there are more chances of discovering novel endophytic bacteria from indigenous rice varieties of different parts of the World.

There are many examples of endophytic organisms that can help their host plant to compete and overcome various biotic and abiotic stress. Thus, an innovative strategy for the development of biofertilizers and beneficial microbes may create a new era in future agriculture. Moreover, this could be an effective tool for the enhancement of crop yield in an environment-friendly manner.

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