Research Progress of Promoting Biocontrol Bacteria in Rice Rhizosphere

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Abstract

The irrational application of rice chemical fertilizer and the reduction of rice quality and yield caused by rice diseases have seriously affected agricultural production and the environment. To grow rice more scientifically and greenly, plant growth-promoting rhizobacteria (PGPR) has developed rapidly as a new control method for plant diseases. The discovery of PGPR is an essential process in biological control research. PGPR has many species, excellent biological traits, diverse antagonistic mechanisms, and a wide range of parasitism, which is an essential biological control method to solve the excessive application of chemical fertilizers and pesticides in rice production and has broad development prospects. In this paper, we summarize the research progress of PGPR application to rice and other plants in recent years, expound on the direct and indirect mechanisms, and look forward to the development trend of follow-up research in order to provide a theoretical reference for the in-depth study and better application of biocontrol bacteria.

Keywords: Plant growth-promoting rhizobacteria; rice; biocontrol; Mechanism of action.

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1. INTRODUCTION

Rice is a staple food in Asia and some African countries, with 90% of the world's rice being grown in Asia. China is a large country with a large population and massive demand for food crops such as rice. Increasing rice production and achieving self-sufficiency in staple foods is an essential strategy for China. In 2022, China's rice planting area reached 29.45 million hectares, and the yield was as high as 20.85 million tons. The rice planting area in Heilongjiang Province was about 3.61 million hectares, and the yield was as high as 27.18 million tons. Ensuring the sustainable development of the rice industry is the key to stable agricultural development in Heilongjiang Province and is also crucial to ensuring national food security in China.

However, in actual rice production, insufficient soil fertility and the invasion of diseases greatly hinder rice production and development. At this stage, in addition to selecting and breeding rice varieties with resistance, the main measures are still using chemical fertilizers and pesticides. The excessive use of these two substances will inevitably cause agricultural non-point source pollution. In fact, at this stage, agricultural non-point source pollution has become a significant obstacle to the sustainable development of agriculture, society, and the environment in China. Therefore, reducing the use of chemical fertilizers and pesticides is crucial for the sustainable development of China's rice industry, and plant growth-promoting rhizobacteria (PGPR) is one of the good strategies.

The rice rhizosphere soil is rich in nutrients surrounding the rice root system. It contains various compounds, facilitating microbial migration to the rice rhizosphere, promoting microbial reproduction, and indirectly promoting rice root growth and nutrient acquisition [2-4]. PGPR is a particular group of beneficial microorganisms that directly affect rice or interact with rice and microorganisms that indirectly affect rice [5]. The mechanism of rice-microbial interactions successfully promotes plant growth and PGPR reproduction [6, 7]. Successful PGPRs have three inherent characteristics: (1) They are adept at colonizing the surface of rice roots; (2) They survive, multiply, and compete with other microbial populations for at least the time necessary to express their activity promoting rice growth; (3) They promote rice growth.

Many strains have been reported as good PGPRs, including Bacteria such as Pseudomonas, Bacillus, Enterobacter, Arthrobacter, Azospirillium, Azotobacter, Burkholderia, Klebsiella, Rhizobium, and Serratia; Fungi such as Trichoderma, Coniothyrium, etc. The discovery of PGPR is an essential process in biological control research. PGPR has many species, excellent biological traits, diverse antagonistic mechanisms, and a wide range of parasitism, which is an essential biological control method to solve the excessive application of chemical fertilizers and pesticides in rice production and has broad development prospects. In this paper, we summarize the research progress of PGPR application to rice and other plants in recent years, expound on the direct and indirect mechanisms, and look forward to the development trend of follow-up research in order to provide a theoretical reference for the in-depth study and better application of biocontrol bacteria.
2. PROMOTING MECHANISM OF PGPR

PGPR-promoting rice growth includes direct and indirect mechanisms. The direct mechanism includes producing various plant hormones and activating soil nutrients to provide nitrogen, phosphorus, potassium, and other elements needed for plant growth. The indirect mechanism mainly reduces plant disease by producing antibiotics and other substances. Many studies at home and abroad have shown that these mechanisms often do not act alone but are likely to be coordinated and interact with each other to promote the healthy growth of rice.

2.1 DIRECT MECHANISM

2.1.1 ACTIVATING SOIL NUTRIENTS

Nitrogen (N) is an essential raw material for rice growth and development, mainly used to form proteins, nucleic acids, amino acids, and other substances. In nature, nitrogen exists in various forms, including gaseous nitrogen, organic nitrogen, ammonium nitrogen, and nitrate nitrogen, of which many forms are not directly usable by rice. The primary nitrogen source of rice comes from biological nitrogen fixation, and nitrogen-fixing bacteria are the leading biological group. At present, the isolated nitrogen-fixing bacteria mainly include Acinetobacter, Mycobacterium, Rhizobium, Aerobaeter, Klebsiella, Beijerinckia, Bacillus, Pseudomonas and so on [11, 12]. Their nitrogen fixation mechanism can be mainly divided into three forms:

1. Strains with BNFNIF-related genes can convert nitrogen gas into ammonia through enzyme-catalyzed reactions [13].
2. Some strains can convert nitrate as an electron receptor through denitrification and ultimately be reduced to NH₄⁺ [14].
3. Some strains can also convert organic nitrogen into inorganic nitrogen by releasing hydrolytic enzymes [15].

Luo Jiyu et al., studied the application of PGPR Bacillus subtilis and Streptomyces to the rhizosphere soil of Chinese pine. The results showed that the application of these two strains had a significant effect on the rapidly available phosphorus and total nitrogen, total phosphorus, total potassium, organic matter, and rapidly available potassium in the soil [16]; JIN et al., also found that applying efficient nitrogen-fixing bacteria P208 to rice significantly promoted the growth of rice seedling roots [17]; and many heavy metal-resistant bacteria also have specific nitrogen-fixing ability. Pramanik et al., isolated enterobacter K2 from heavy metal-contaminated rice rhizosphere soil with nitrogen-fixing ability. Under heavy metal cadmium stress, the germination rate of rice seeds increased by 40% [18]. Nitrogen-fixing ability may have a promoting effect on stress alleviation.

Phosphorus (P) is an essential component of nucleic acid, nuclear protein, and phospholipid in rice, participating in rice photosynthesis and the synthesis and transformation of chlorophyll and other substances in rice leaves, thereby affecting rice growth [19]. In nature, soil particles readily adsorb phosphorus or combine with calcium, magnesium, and other metal cations to form insoluble complexes. About 40%-90% of phosphorus exists in organic phosphorus, while only 0.1% can be directly absorbed and utilized by rice [20]. PGPR can transform phosphorus that is difficult for rice to use into phosphorus that rice can use. It can be roughly divided into two modes: (1) by secreting organic acids, iron carriers, protons, and some extracellular enzymes, converting insoluble calcium phosphate and hydroxypapatite into soluble phosphate; (2) by the synergistic action of non-specific acid phosphatase, phytase, and lyase, converting organic phosphorus into available phosphate. In rice cultivation, applying phosphorus-solubilizing bacteria can significantly promote rice growth. Qi Xiuxiu et al., added five strains of efficient nitrogen-fixing bacteria and phosphorus-solubilizing bacteria to the rice seedling cultivation substrate: Bacillus safensis, Bacillus amyloliquesciens, Pseudomonas chlororaphis, Bacillus subtilis, Pseudomonas Moraviana. Compared with the non-addition of PGPRs, rice seedlings' root volume, and total root length increased significantly after adding these strains. In addition, the root system structure was improved, and root activity was significantly enhanced, significantly increasing rice seedlings' biomass and strong seedling index above ground [21].

Potassium (K) plays a role in promoting the development of the rice root system, regulating water balance in rice, and participating in the metabolism of organic acids, fats, nitrogen, and other compounds in rice. Although soil contains rich potassium, 80%-90% exists in mineral form [22, 23]. With the ability to release potassium, PGPR can transform the potassium that cannot be used by rice into the one that can be absorbed and utilized by rice. It mainly has two ways of action: (1) Potassium-solubilizing bacteria can dissolve insoluble phosphorus through their own active or passive movement and mineral contact, mainly through various forces; (2) Potassium-solubilizing bacteria can promote the decomposition of potassium-containing minerals through chelation or pH change through secreting inorganic acid, organic acid, protein, and extracellular polysaccharide. When combined with biochar, the application of putrefactive bacteria can increase the content of available potassium in soil by 73.2%, increase the abundance of Gemmatinimonadetes, Actinobacteria, and Bacteroidetes, and reduce the abundance of Chloroflexi and Nitrospira, effectively changing soil microbial community structure [24]. In the research on rice seedling cultivation, Liu et al., found that applying biochar with phosphorus-solubilizing bacteria Bacillus megaterium can significantly increase rice plant height and dry matter accumulation, improve root morphology, and enhance root activity [25].
2.1.2 PRODUCTION OF PLANT HORMONES

Indole-3-acetic acid (IAA) is one of the essential regulatory hormones in rice, which promotes cell growth and seed germination and regulates rice root growth. There is also research showing that IAA plays a crucial role in the response of rice to salt stress [26]. There are different production pathways for IAA. Beneficial microorganisms mainly produce IAA through the pathway of indole-3-pyruvate (IPyA), which is mediated by the critical enzyme IPyA decarboxylase encoded by the pyruvate dehydrogenase E1 component subunit beta gene. This enzyme catalyzes the decarboxylation of IPyA to produce an indole-3-acetaldehyde intermediate, which is then oxidized to IAA. Since this pathway is the same as the pathway for rice to synthesize IAA itself, it is more beneficial for rice to absorb and utilize it [27]. Many studies have shown that IAA plays different roles in different stages of rice growth. Jia et al., found that a deficiency of IAA at the tillering stage will significantly increase the number of tillers in rice [28].

In contrast, Liu Yang et al., found that endogenous IAA content is significantly or extremely significantly positively correlated with grain filling rate. The dynamic change of IAA content during grain filling is consistent with the changing trend of H+-ATPase activity in grains. Grain filling rate and H+-ATPase activity are critical indicators of sink capacity [29]. Research also shows that PGPR-producing IAA is beneficial for alleviating heavy metal stress in rice. Wang Dong’an et al., screened out a strain of arsenic methylation functional Bacillus subtilis LH14 from the rhizosphere soil of arsenic-contaminated rice fields. The strain significantly increased seed germination rate, root and shoot length, and biomass under high arsenic conditions [30].

Cell division hormone (CTK) promotes cell division in rice, enhancing rice stress resistance and delaying cell aging. In bacteria, CTK synthesis is initiated by the expression product of the ipt gene, isopentenyl transferase, which catalyzes the transfer of isopentenyl groups from DMAPP (dimethylallyl diposphate) to AMP to start CTK production or transfers isopentenyl groups from HMBDP (1-hydroxy-2-methyl-2-(E)-butenyl-4-diposphate) to AMP [31]. When inoculated with the short beard Bacillus pumilus strain TUAT1, it promotes crown root formation in rice, which may be due to B. pumilus TUAT1 upregulating the expression of crl5 and downregulating the expression of wox11 to regulate the expression of osrr [32].

In addition to producing IAA and CTK, PGPR can also produce other hormones such as gibberellin (GA), abscisic acid (ABA), and ethylene (ETH). Liu et al., isolated plant growth-promoting bacteria from rice rhizosphere soil dominated by Bacillus and Staphylococcus with the ability to solubilize phosphorus, dissolve phosphorus, and hydrolyze potassium [33]. Strains with strong nutrient transformation ability can also secrete rice auxins and gibberellins. Wu et al., inoculated tobacco rhizosphere with Paenibacillus polymyxa and found that it induced the expression of plant hormone-related genes such as auxin, cytokinin, and gibberellin in tobacco, promoting tobacco biomass and plant height [34]. In summary, PGPR can promote rice growth by secreting relevant hormones or indirectly promoting the secretion of rice hormones.

2.2 INDIRECT EFFECTS THROUGH INHIBITION OF PATHOGEN GROWTH

2.2.1 COMPETITION

Competition mainly includes competition for nutrients and living space. During growth and reproduction, biocontrol bacteria compete with pathogenic bacteria for living space, nutrients, and water, thereby hindering the growth and reproduction of pathogenic bacteria. For example, Bacillus subtilis colonizes rice roots and can hinder the colonization of pathogenic bacteria on rice through competition with the pathogenic bacteria for the attachment sites, thereby achieving the biocontrol effect [35]; it can also produce antibacterial substances such as antimicrobial peptides to compete with the pathogenic bacteria for the attachment sites, protecting rice from the invasion of the pathogenic bacteria [36]. In addition, in some extreme environments, such as low iron environments, PGPR that produce siderophores can prioritize the utilization of limited iron in soil for rice and itself, effectively inhibiting the growth of pathogenic microorganisms [37].

2.2.2 EPIZOOTIOLOGY

Epizootiology is a particular type of parasitism that takes pathogenic bacteria as the host, which involves multiple processes such as adsorption, entanglement, invasion, and penetration. Research has shown that Trichoderma hyphae can parasitize and adhere to the hyphae of the host by secreting extracellular enzymes such as glucanase, xylanase, and chitinase, then penetrate the host hyphae after lysing the cell wall to absorb nutrients [38].

2.2.3 SECRETION OF ANTIMICROBIAL METABOLITES

Antimicrobial metabolites can directly inhibit the growth of pathogenic bacteria, thereby reducing rice damage. Antimicrobial metabolites mainly include two parts: one is lipopeptide antimicrobial peptides such as fengycins, surfactins, viscosinotoxins, and mycosubtilins that are synthesized by non-ribosomal pathways; the other is protein antimicrobial substances mainly including small molecular weight bacterial proteins such as protease inhibitors and some unidentified inhibitory peptides that are synthesized by ribosomal pathways [39]. These substances act through different mechanisms such as Wu et al.,’s study found that Bacillus velezensis FZB42 can secrete clindamycin and lincomycin, leading to the downregulation of genes related to virulence, cell division, protein synthesis, and cell wall synthesis in
**Xanthomonas oryzae**. Oryza, thereby inhibiting rice sheath blight [40]. Jayaraj *et al.*, added *Bacillus subtilis* to rice. They significantly increased phenylalanine ammonia-lyase and polyphenol oxidase levels, detecting systemic resistance against *Rhizoctonia solani* (the causal agent of sheath blight) in rice [51].

### 3. FUTURE PERSPECTIVE

Numerous studies have confirmed PGPR’s ability to promote rice growth on rice plants and its biosticide activity against rice pathogenic bacteria. With increasing awareness of PGPR applications in agricultural production, there are higher requirements for their isolation and identification, especially in rice plant protection. Their application in the form of biosticides may improve sustainable rice production. Using PGPR in correct biological formulations provides a significant solution for sustainable agricultural futures.

However, transferring research results into industrial development takes work. Lack of multi-year field trial results often cannot support the development of PGPB in the commercial sector. This limitation hinders the commercial development of PGPB biosticides. In addition, due to the strict dependence of PGPB’s antibacterial metabolites on their culture substrate and surrounding abiotic and biotic stresses, their biosticide activity is hindered [42]. In addition, the efficacy of PGPB against newly emerged rice bacterial or fungal diseases remains unknown. Further extensive research on rice-microbial interactions, especially on biosticide activities and rice growth promotion, has yet to be fully explain. Revealing the symbiotic interaction between PGPR and rice may help develop efficient biosticides suitable for different soil types and environmental conditions.

Overall, more in-depth research on PGPB is still needed to meet industrial requirements and produce effective biosticides with one or more active ingredients using different carrier materials and additives, employing different methods of field inoculation to facilitate the development of PGPB.

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