PGPR-Mediated Growth Enhancement and Antioxidant Properties in Bok Choy (Brassica rapa subsp. chinensis): A New Frontier in Plant Biotechnology

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Abstract. Bok choy (*Brassica rapa* subsp. chinensis) is a nutrient-rich leafy vegetable valued for its economic and nutritional importance. However, its growth and physiological performance can be adversely affected by various biotic and abiotic stressors. This study aimed to evaluate the potential of Plant Growth-Promoting Rhizobacteria (PGPR) isolates IZ01 and IZ02 in enhancing the growth and antioxidant defense mechanisms of bok choy. The application of PGPR significantly improved several physiological parameters. Fresh weight increased by 25% in IZ01-treated plants and by 45% in those treated with IZ02 compared to the control. Chlorophyll content also showed an increase of 8.33% (IZ01) and 16.67% (IZ02), indicating enhanced photosynthetic capacity. Total sugar content was likewise elevated, with increases of 25% and 33.33% in IZ01- and IZ02-treated plants, respectively, suggesting improved carbohydrate metabolism. Antioxidant enzyme activity was markedly enhanced in response to PGPR treatment. Catalase (CAT) activity increased by 50% in IZ01 and 30% in IZ02, while guaiacol peroxidase (GPX) activity was enhanced by 175% and 75%, respectively, compared to the control. These results demonstrate that PGPR isolates IZ01 and IZ02 effectively promote plant growth and physiological resilience by enhancing photosynthesis, carbohydrate accumulation, and antioxidant enzyme activity, thereby strengthening stress tolerance in bok choy.

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1 Introduction

In recent years, sustainable agricultural practices have gained significant attention as a response to the growing challenges posed by climate change, soil degradation, and increasing global food demand [1]. One promising approach to enhancing crop productivity while minimizing the use of chemical fertilizers and pesticides is the application of plant growth-promoting rhizobacteria (PGPR) [2]. Beneficial soil bacteria play a crucial role in improving plant growth by facilitating nutrient uptake, producing phytohormones, and inducing systemic resistance against pathogens [3]. Among various leafy vegetables, bok choy (Brassica rapa subsp. chinensis) has emerged as an important horticultural crop due to its high nutritional value, rapid growth cycle, and adaptability to different growing conditions [4]. However, strategies to enhance its growth and resilience through eco-friendly biotechnological interventions remain underexplored [5]. Plant growth-promoting rhizobacteria (PGPR) have been extensively studied for their ability to enhance crop performance through mechanisms such as nitrogen fixation, phosphate solubilization, and siderophore production [6]. In addition, they are known to improve plant stress tolerance by activating antioxidant defense systems, thereby reducing oxidative damage caused by drought, salinity, and heavy metal exposure. While many studies have demonstrated the beneficial effects of PGPR in a wide range of crops, there is still limited evidence on their specific influence on the growth performance and antioxidant capacity of bok choy [7]. This research provides new insights by demonstrating that PGPR inoculation not only significantly increases bok choy biomass and nutrient uptake but also enhances its antioxidant enzyme activity, leading to improved stress resilience. These findings highlight the novelty of applying PGPR to bok choy cultivation and underline their potential to develop sustainable agricultural practices that maximize yield and nutritional quality while reducing dependence on synthetic agrochemicals.

In this study, PGPR isolates IZ01 and IZ02 were utilized to assess their influence on the growth and antioxidant properties of Bok Choy. This study aims to investigate the role of PGPR in enhancing the growth and antioxidant properties of bok choy, focusing on key physiological and biochemical responses. By assessing plant biomass, chlorophyll content, enzyme activity, and antioxidant accumulation, we seek to provide new insights into the mechanisms through which PGPR influences plant health and productivity. Furthermore, this research contributes to the broader field of plant biotechnology by exploring the potential of PGPR as a natural biofertilizer, paving the way for more sustainable and resilient cropping systems.

2 Material and Methods

2.1 Plant Material and PGPR Isolates

Bok choy (*Brassica rapa* subsp. Chinensis) seeds were used as the plant material in this study. Two bacterial isolates, IZ01 and IZ02, which exhibited plant growth-promoting traits in preliminary screening (phosphate solubilization, indole-3-acetic acid production, and siderophore activity), were selected for the experiment. Both isolates were obtained from mature vegetable-based compost and subsequently cultured in nutrient broth (NB) medium. The bacterial suspension was adjusted to a final concentration of approximately 1×10^8 CFU mL^{-1}. Treatments were applied once a week by foliar spray application.

Colony morphology was characterized based on size, shape, margin, elevation, surface texture, and pigmentation, following standard microbiological guidelines [10]. The diameter of the colonies was measured using a digital caliper, while color and texture were recorded

through direct visual observation. This characterization was conducted to provide preliminary information about the isolates prior to subsequent plant growth-promoting trait evaluation

2.2 Experimental Design and Growth Conditions

The experiment was conducted under controlled greenhouse conditions with a completely randomized design (CRD) [9]. Each treatment was replicated three times. Bok Choy seeds were sown in sterilized soil and allowed to grow for a designated period. PGPR treatments were applied as root inoculations, and control plants were maintained without PGPR application. Growth parameters, including fresh weight, chlorophyll content, and total sugar levels, were recorded at the end of the experiment. During the experiment, plants were grown in a greenhouse under adequate natural light conditions. Fertilization was applied according to standard horticultural practices to ensure optimal plant growth.

2.3 Determination of Chlorophyll Content

Approximately 0.1 g of fresh leaf tissue was ground with 2 mL of 95% (v/v) ethanol. The homogenized samples were placed on a shaker for 30 minutes at room temperature, followed by centrifugation at 5,000 rpm for 5 minutes. The supernatant was collected, and chlorophyll a and b concentrations were determined using a UV-visible spectrophotometer at 664 nm and 648 nm, respectively. The total chlorophyll content was calculated using Lichtenthaler's formula : $C = (5.24 \times OD664) + (22.24 \times OD648)$, where C represents chlorophyll concentration in micrograms per gram [1].

2.4 Determination of Total Sugar

Total sugar content was quantified using the anthrone reagent method as described by Kurniawan (2021) [8]. Fresh leaf samples (0.1 g) were extracted with 80% ethanol, centrifuged at 10,000 rpm for 10 min, and 200 µL of the supernatant was evaporated to dryness. The residue was dissolved in distilled water and mixed with 2 mL of 0.4% anthrone reagent in concentrated sulfuric acid. After heating in boiling water for 10 min, absorbance was measured at 600 nm, and total sugar content was determined using a standard calibration curve.

2.5 Determination of Antioxidant Enzyme

Antioxidant enzyme activities, including catalase (CAT) and guaiacol peroxidase (GPX), were analyzed based on the methods described by Aebi (1984) and Koduri & Tien (1995). Leaf samples (0.2 g) were homogenized in 1.2 mL of 0.2 M potassium phosphate buffer (pH 7.8) containing 0.1 mM EDTA and centrifuged at 12,000 rpm for 20 min at 4°C. The supernatant was used for enzyme activity assays. CAT activity was determined by adding 10 µL of the enzyme extract to a reaction mixture containing 0.1% (v/v) H_2O_2, 0.1 mM EDTA, and 100 mM potassium phosphate buffer (pH 7.0). Absorbance at 240 nm was recorded, and CAT activity was calculated based on H_2O_2 decomposition. GPX activity was measured by mixing 40 µL of enzyme extract with 1.5 mL of a reaction solution containing 1% (v/v) guaiacol, 40 mM H_2O_2, and 100 mM potassium phosphate buffer (pH 7.0). The reaction was incubated for 3 min, and absorbance at 450 nm was recorded. Enzyme activities were expressed as U/mg protein [1].

2.6 Statistical Analysis

All experimental data were analyzed using one-way analysis of variance (ANOVA) followed by Tukey's post-hoc test to determine significant differences between treatments ($p < 0.05$). Data were presented as mean ± standard deviation, and statistical analyses were performed using SAS software, and data visualization and additional processing were conducted using Phyton (version 3.12)

3 Result and Discussion

The results of this study demonstrated that PGPR isolates IZ01 and IZ02 significantly enhance the growth and antioxidant defense mechanisms in Bok Choy (*Brassica rapa* subsp. Chinensis). The application of these isolates increased fresh weight, chlorophyll content, and total sugar levels while reducing oxidative stress through enhanced antioxidant enzyme activity.

3.1 Bacterial Colony Characterization

The PGPR isolates IZ01 and IZ02 were characterized based on their colony morphology, pigmentation, and growth pattern. IZ01 formed creamy to slightly yellowish colonies with a circular appearance at a glance; however, under closer observation, the colony edges appeared irregular with a powdery or fragmented texture. The surface was slightly convex, and the colony diameter was less than 1 mm. In contrast, IZ02 produced yellowish colonies with a consistently smooth, circular shape and a more uniform, moist surface. The colony diameter was also less than 1 mm.

The observed morphological characteristics of isolates IZ01 and IZ02, such as small colony diameter (<1 mm), yellowish pigmentation, and distinct surface textures, are commonly associated with members of the *Bacillus* genus. Specifically, the dry, fragmented surface of IZ01 and the smooth, moist appearance of IZ02 resemble traits often reported in various *Bacillus* species [10]. However, such colony morphology is not exclusive to *Bacillus*, as certain species of *Pseudomonas*, *Paenibacillus*, or even *Micrococcus* may exhibit overlapping traits under specific culture conditions [11,12]. Although the colony morphology of IZ01 and IZ02 suggests possible affiliation with Bacillus spp., further analysis such as Gram staining and 16S rRNA gene sequencing is required to confirm their taxonomic identity. Xu explained that the differences in colony characteristics suggested variability in extracellular polysaccharide production, which may contribute to their ability to enhance plant growth. These variations also indicated possible differences in their mechanisms of action, such as phosphate solubilization, nitrogen fixation, or phytohormone production [13].

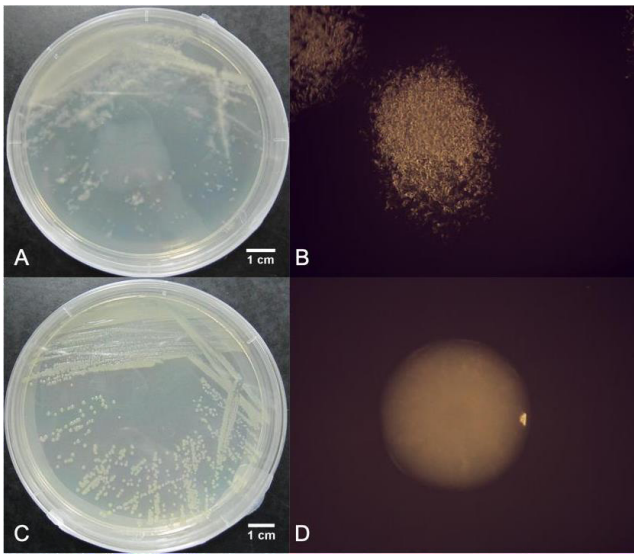

Fig. 1. Morphological and microscopic characteristics of PGPR isolates IZ01 and IZ02. (A) Colony morphology of IZ01 isolate on NA medium. (B) Microscopic observation of IZ01 isolate showing cellular characteristics. (C) Colony morphology of IZ02 isolate on NA medium. (D) Microscopic observation of IZ02 isolate highlighting structural features.

3.2 Plant Growth and Physiological Responses

Our results demonstrated a significant increase in fresh weight in plants treated with PGPR isolates compared to untreated plants. Notably, treatment with isolate IZ01 led to a 25% increase in fresh weight, while IZ02 resulted in a 45% increase (**Fig. 2A**). These findings indicated that both isolates may enhance nutrient uptake and metabolic processes, thereby promoting greater biomass accumulation. The potential of PGPR to improve plant growth and yield has been widely reported in various crops, including bok choy [14], rice [15], and chili [16], highlighting their promising role in sustainable agriculture. Photographic evidence and a visual comparison of plant condition between control and treatments can be observed in **Fig 2B and 2C**.

In addition, our treatment had a positive effect on increasing chlorophyll content in plants. Chlorophyll levels in IZ01 and IZ02-treated plants were 45.6 mg/mL and 48.5 mg/mL, respectively, compared to 41.9 mg/mL in the control. This correspond to an increase of 8.33% for IZ01 and 16.67% for IZ02. The observed enhancement suggested that the PGPR isolates may stimulate nitrogen assimilation, which plays a central role in chlorophyll biosynthesis [17]. Since chlorophyll is essential for light absorption and energy conversion during photosynthesis, its increased content serves as a key indicator of improved photosynthetic efficiency and overall plant health [18].

Our treatment also resulted in a significant increase in total sugar content in PGPR-treated plants. Plants treated with IZ01 and IZ02 had total sugar levels of 4.5 mg/mL and 4.8 mg/mL, respectively, compared to 3.6 mg/mL in the untreated plants. This correspond to an increase of 25% for IZ01 and 33.33% for IZ02 compared to control. The higher sugar accumulation suggests improved carbohydrate metabolism, providing a more efficient energy supply to support plant growth and defense responses [19]. These findings imply that PGPR enhances photosynthetic carbon fixation, enabling plants to synthesize and store more carbohydrates for sustained development and physiological resilience.

3.3 Antioxidant Enzyme Activity and Gene Network Regulation

To evaluate the plant's enzymatic antioxidant response, the activities of catalase (CAT) and guaiacol peroxidase (GPX) were measured. CAT activity increased by 50% in IZ01-treated plants (0.15 U/mg protein) and by 30% in IZ02-treated plants (0.13 U/mg protein), compared to the control (0.10 U/mg protein). Similarly, GPX activity showed a substantial rise, with an increase of 175% in IZ01 (1.1 U/mg protein) and 75% in IZ02 (0.7 U/mg protein), relative to the control (0.4 U/mg protein). These findings indicated that PGPR treatment significantly enhances the plant's antioxidant defense system, thereby potentially reducing oxidative damage and improving resilience under stress conditions.

It is well documented that PGPR can improve plant tolerance to both biotic and abiotic stresses by enhancing the antioxidant activity [20]. Under stress conditions such as drought, salinity, pathogen attack, or heavy metal exposure, reactive oxygen species (ROS) tend to accumulate and cause cellular damage [21]. PGPR-mediated induction of antioxidant enzymes such as CAT, GPX, SOD, and APX helps to detoxify ROS, maintain cellular redox balance, and protect vital cellular components [22]. This mechanism plays a crucial role in strengthening the plant's defense system and promoting survival and productivity under adverse environmental conditions.

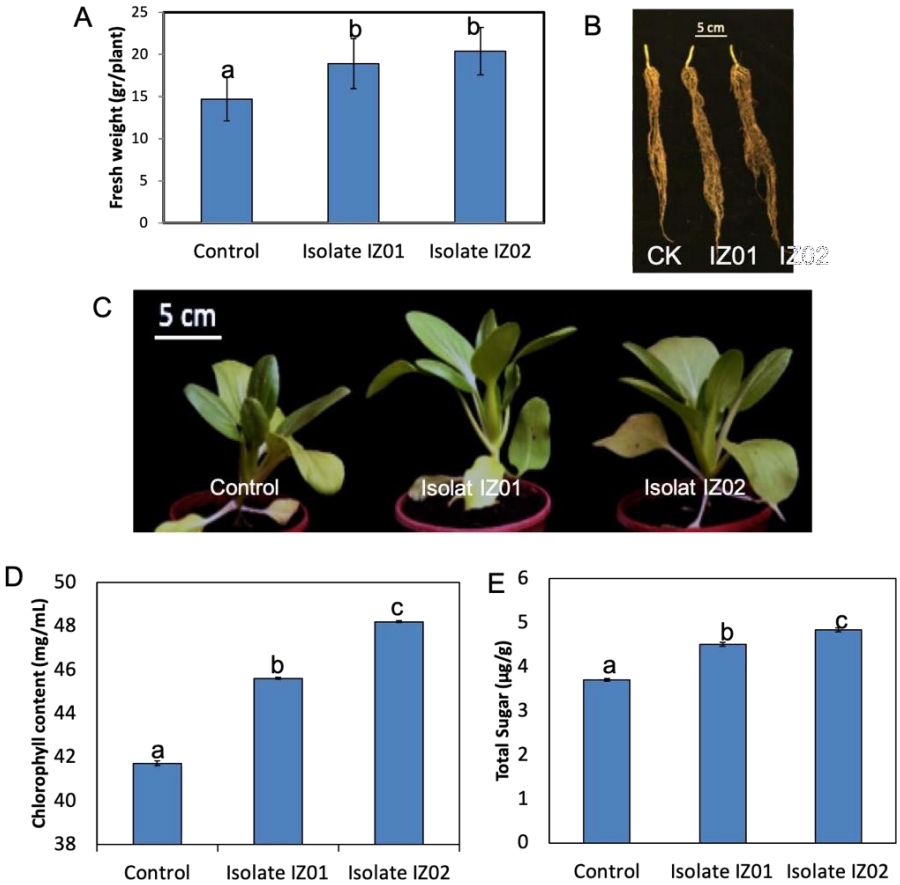

Fig. 2. Effects of PGPR treatment on growth and physiological parameters of bok choy. (A) Fresh weight of bok choy plants measured at 4 weeks after planting. (B) In vitro plant growth of bok choy on agar medium: from left to right—control, IZ01-treated, and IZ02-treated plants. (C) Visual

condition of bok choy plants grown in soil, showing control and PGPR-treated plants (IZ01 and IZ02) at 4 weeks after planting. (D) Chlorophyll content (mg/mL) in leaf tissues of bok choy under different treatments. (E) Total sugar content (µg/g fresh weight) in bok choy plants treated with PGPR isolates.

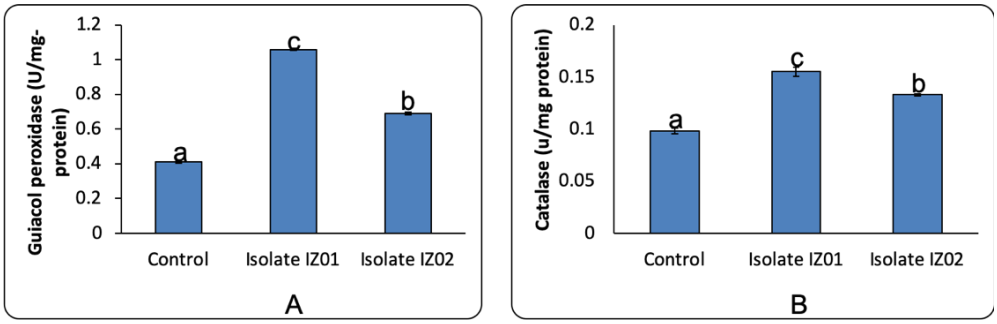

Fig. 3. Antioxidant enzyme activity in bok choy plants treated with PGPR isolates after 4 weeks planting. (A) Catalase (CAT) activity (U/mg protein) in bok choy leaves. (B) Guaiacol peroxidase (GPX) activity (U/mg protein) in bok choy leaves.

Further analysis using Gene Ontology (GO) enrichment revealed that the gene set under study is predominantly associated with molecular functions linked to antioxidant defense. The term 'antioxidant activity' emerged as the most significantly enriched category, supported by a high enrichment score, a substantial number of associated genes, and the lowest false discovery rate (FDR). This overarching antioxidant theme is further reinforced by the enrichment of specific enzymatic functions, particularly peroxidase activity and catalase activity (**Fig 4**). The presence of catalase activity underscores the role of this enzyme in mitigating oxidative stress by catalyzing the decomposition of hydrogen peroxide (H_2O_2) into water and oxygen, thereby preventing cellular damage. Collectively, these findings not only emphasize the central role of antioxidant mechanisms within the gene set but also shed light on the specific biochemical pathways—such as hydrogen peroxide detoxification—through which oxidative protection is achieved.

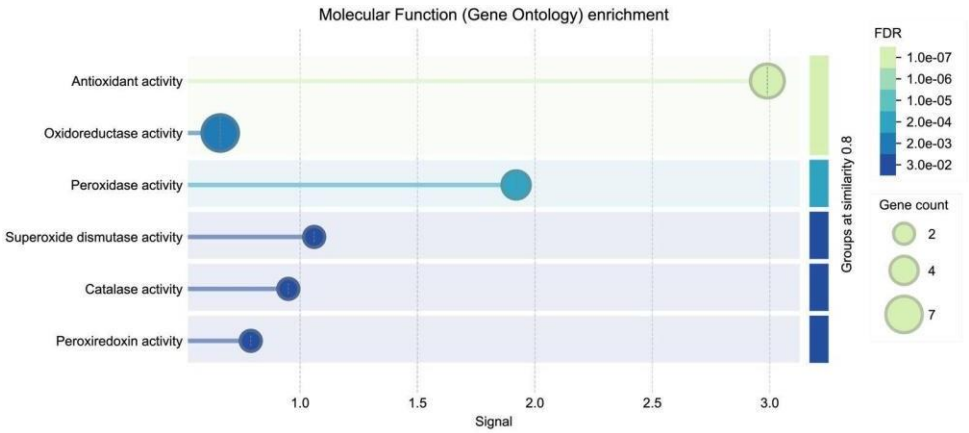

Fig. 4 Gene Ontology enrichment highlights antioxidant activity, including catalase and peroxidase functions

To further elucidate the genetic basis of antioxidant activity in *Bacillus* sp., we performed a protein–protein interaction analysis using the STRING database and visualized the results through a GeneMANIA network (**Fig. 5**). This network illustrated the molecular architecture

of the antioxidant defense system in *Bacillus* sp., with a focus on catalase-related antioxidant activity. Key catalase genes such as katA and katX appear as central nodes within a tightly interconnected functional module. These catalase genes are functionally associated with other critical antioxidant enzymes, including superoxide dismutase (*sodA*) and peroxiredoxin (*ahpC*), forming a robust core response to oxidative stress. The dense network of interactions—dominated by 'co-expression' relationships—indicated that these genes are likely co-regulated and activated in a coordinated manner during oxidative challenge. Moreover, the network connects this core antioxidant machinery to essential supporting genes such as *hemH-2*, which is involved in the biosynthesis of the heme cofactor required for catalase activity, and hfq, a global regulator of gene expression. Together, this network provides a comprehensive overview of the genetic basis of catalase-mediated antioxidant defense in *Bacillus* sp., emphasizing its integrated and systemic nature.

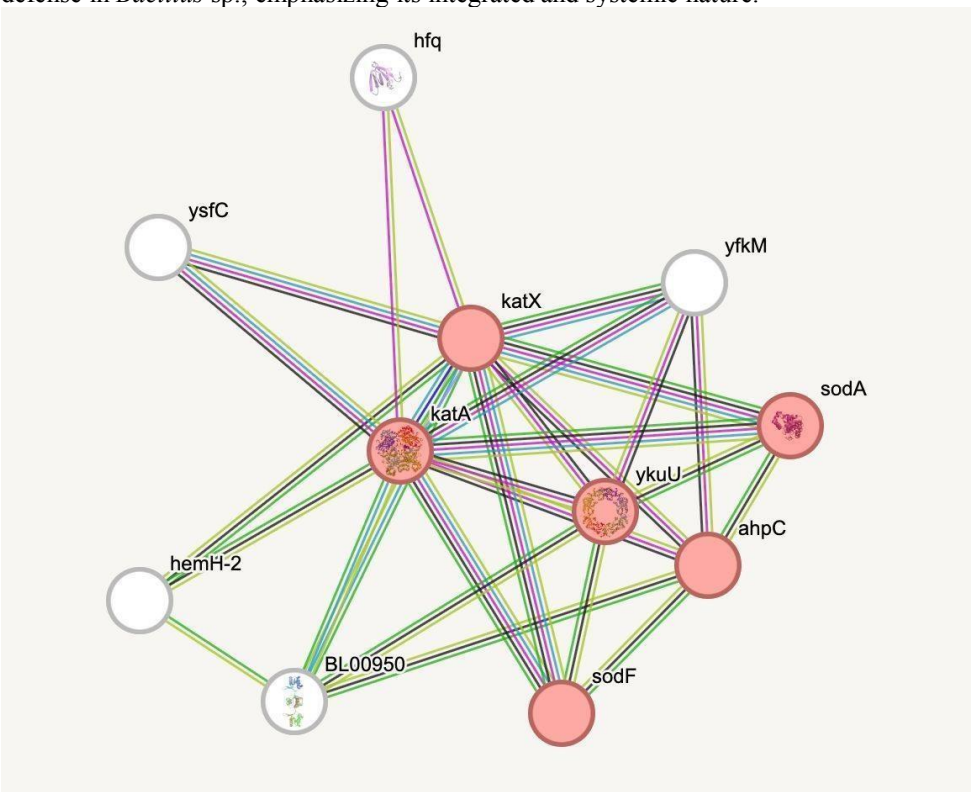

Fig. 5. Gene Interaction network of catalase-mediated antioxidant activity in *Bacillus* sp.

3.4 Implications for Sustainable Agriculture

The findings of this study emphasize the potential of IZ01 and IZ02 as biofertilizers for enhancing plant growth and stress tolerance. The improved growth parameters and increased antioxidant enzyme activity suggested that PGPR application can be an effective strategy for promoting plant health while reducing the need for chemical fertilizers and synthetic growth stimulants. By reducing oxidative stress and improving physiological responses, PGPR contributed to sustainable agricultural practices that support higher crop yields and resilience under suboptimal environmental conditions. The ability of IZ01 and IZ02 to improve Bok Choy growth and antioxidant defense mechanisms positions them as promising candidates for future biotechnological applications in plant production.

4 Conclusion

The application of PGPR isolates IZ01 and IZ02 significantly enhances Bok Choy growth by increasing fresh weight, chlorophyll content, and total sugar levels while reducing oxidative stress. The elevated activities of catalase and guaiacol peroxidase enzymes further confirm the role of PGPR in strengthening plant defense mechanisms. These results highlight the potential of IZ01 and IZ02 as sustainable biofertilizers, providing an eco-friendly approach to improving crop productivity and stress resilience. Future studies should explore the underlying molecular mechanisms and field-scale applications of these PGPR isolates to maximize their agricultural benefits.

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