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Valorisation Of Chicken Feather By Exiguobacterium Sp. For Enhanced Seed Germination And Plant Growth In Rice.

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Abstract

The transformation of industrial poultry waste into value-added products through microbial processes has attracted significant interest for reducing pollution and environmental health. This study aims to investigate the potential of feather hydrolysate (FH) for seed germination and plant growth studies of Oryza sativa (L.) The feather hydrolysate obtained from the lab isolate Exiguobacterium sp. was tested to check plant growth promoting activity. The Plant growth parameters measured were fresh weight (FW), dry weight (DW), shoot length (SL) root length (RL), seed vigour index were monitored in the experiment where bacterial pellet and feather hydrolysate were compared to uninoculated in terms of dry and fresh weight however feather hydrolysate significantly improved plant biomass (5fold increase) when compared to control similarly, the levels of chlorophyll a, chlorophyll b and carotenoids were found to be high. qualitative studies of feather hydrolysate for amino acids exhibits better growth parameters compared to the control which was not supplemented with feather hydrolysate. Qualitative estimation of amino acids indicated the presence of α – amino acids, phenyl alanine, tyrosine, tryptophan and sulphur containing amino acids.

Keywords: Feather hydrolysate, Oryza sativa, plant growth, amino acids

Introduction`

The development of organic products from natural waste materials has become a key focus of modern microbiological research. Over the past few decades, numerous studies have explored ways to enhance the agronomic use of organic waste. Various waste materials of both animal origin (such as blood meal, hooves, horns, feathers, bones, nails, hair, wool, and scales) and plant origin (including wheat straw, paddy straw, rice husk, and leaves) have been investigated as potential organic fertilizers [1–2]. Previous study, report

degradation of chicken feather hydrolysate by keratinolytic bacteria which then slowly release amino acids that support N – fertiliser as slow-release nitrogen (N) act as an organic fertiliser [3].

Keratin protein is a natural source of essential amino acids, peptides, and minerals that can be used in animal feed supplements, and also as biofertilizer [4]. Feathers represent 5–7% of the total weight of mature chickens thereby producing substantial amounts of poultry wastes [5]. Feathers contain about 80–90% keratin on dry mass basis and contains about 15% N [6-7]. Feather hydrolysate produced by keratinolytic bacteria presents a superior alternative to synthetic fertilizers for developing slow-release nitrogen fertilizers. The breakdown of feathers releases essential amino acids, which act as precursors for plant growth-promoting compounds.[8] Feathers primarily consist of fibrous β-keratin (90% w/w), a mechanically strong, unreactive protein with high resistance to degradation. Various keratinase-producing soil microorganisms, such as bacteria and fungi, can break down feathers, making them valuable source in cost-effective and eco-friendly manner [9-11].

This study aimed to investigate the impact of feather hydrolysate using Exiguobacterium sp. as natural biofertilizer used for seed germination, and plant growth promotion. Thus, providing a sustainable and eco-friendly alternative to conventional fertilizers.

Materials and methods

Feather hydrolysate

Chicken feather hydrolysate from Exiguobacterium sp. was acquired from P2BL laboratory, Department of microbiology, Osmania university, Hyderabad, India.

Preparation of bacterial culture and feather hydrolysate

Bacterial culture i.e., Exiguobacterium sp. obtained from P2BL laboratory was grown in mineral salt medium (50ml vol in 100ml flask) incubated at 37 ^{o}C for 24 hrs. After incubation the MSM media was centrifuged at 5000 rpm and pellet was used for seed priming.

For preparation of feather hydrolysate, one percent feather taken in MSM medium along with 4 percent inoculum incubated at 37 ^{o}C for 48 hrs then the medium was centrifuged at 10000rpm for 20minutes and the supernatant was used for seed priming studies.

Seed priming

Healthy, undamaged rice seeds ((Dhan 54 variety from the ICAR-IIRR).) were surface sterilized using 1.5% sodium hypochlorite for 5 minutes, followed by three rinses with sterile distilled water and air-drying under a laminar flow hood on sterilized blotting paper [12]. The seeds were then bio primed by mixing with 1% CMC containing bacterial pellet, feather hydrolysate as prepared above in a beaker and allowed to stand at room temperature for 12 hours. After biopriming, the seeds were shade-dried to restore their original moisture content [13]. For control, sterilized distilled water was used to soak seeds for similar time interval. Seeds were then placed in the Petri dishes with soft agar (0.8% agar) and incubated at room temperature for 2 days and germination percentage was calculated.

Quantification of plant growth parameters

The plants were observed and monitored for a period of 10 days to assess their growth and development. After this period, they were carefully harvested, and key growth parameters, including root length, shoot length, dry weight, wet weight, seed vigour index were measured [14]. Following these measurements, the plant samples were subjected to a drying process at a constant temperature of 70ºC for four days to ensure the complete removal of moisture. After the drying period, the final dry weight of the samples was recorded to evaluate biomass.

Analysis of carotenoid and chlorophyll contents

About 0.5 g of fresh leaf tissue was carefully weighed and thoroughly crushed to facilitate the extraction of pigments. The crushed tissue was then treated with 80% chilled acetone and allowed to incubate for 24 hours to ensure efficient pigment extraction [15]. After the incubation period, the mixture was subjected to centrifugation at 5000 rpm for 10 minutes at a temperature of 4ºC to separate the solid debris from the liquid extract. The resulting supernatant, which contained the extracted pigments, was carefully collected and used for spectrophotometric analysis.

To determine the concentration of different pigments, the absorbance of the supernatant was measured at specific wavelengths: 479 nm for carotenoids, 663 nm for chlorophyll a, and 645 nm for chlorophyll b. The optical density (OD) values obtained from the spectrophotometer readings were then substituted into Arnon's equation [16] to calculate the total chlorophyll and carotenoid content [17]. The final pigment concentrations were expressed in milligrams per gram (mg/g) of leaf tissue, providing quantitative data on the chlorophyll and carotenoid levels in the plant samples.

Chlorophyll Content (Arnon's Method)

1. **Chlorophyll a Content:**

$$\text{Chlorophyll a (mg/g)} = \frac{(12.7 \times \text{A663}) - (2.69 \times \text{A645})}{1000 \times \text{W}} \times \text{V}$$

2. **Chlorophyll b Content:**

$$\text{Chlorophyll b (mg/g)} = \frac{(22.9 \times \text{A645}) - (4.68 \times \text{A663})}{1000 \times \text{W}} \times \text{V}$$

3. **Carotenoid Content:**

$$\text{Carotenoid (mg/g)} = \frac{(1000 \times \text{A470}) - (1.82 \times \text{Chlorophyll a}) - (85.02 \times \text{Chlorophyll b})}{198}$$

A663 - Absorbance at 663 nm (typically used for chlorophyll a).

A645 - Absorbance at 645 nm (typically used for chlorophyll b).

A470 - Absorbance at 470 nm (typically used for carotenoids).

V - Volume of the leaf extract in millilitres (mL).

W - Weight of the fresh leaf sample in grams (g).

Qualitative analysis of amino acids

The hydrolysate was centrifuged at 5000 rpm for 10 minutes, and the resulting supernatant was utilized for the qualitative analysis of amino acids using

The qualitative estimation of amino acids was performed using standard procedures. For qualitative analysis, various established tests were employed, including the Ninhydrin test, Xanthoproteic test, Sulphur test, (Hopkins-Cole test), and Sakaguchi test.[18]

Results

Plant growth studies in rice

The plant growth parameters include germination percentage, shoot length, root length, fresh weight, dry weight, seed vigour index presented in table 2.

Germination Percentage

The application of keratin hydrolysate resulted in the highest germination rate (100%), indicating its efficacy in enhancing seed germination fig 2. In contrast, both the control and Exiguobacterium-treated seeds exhibited a germination rate of 90%, suggesting that Exiguobacterium did not significantly influence germination

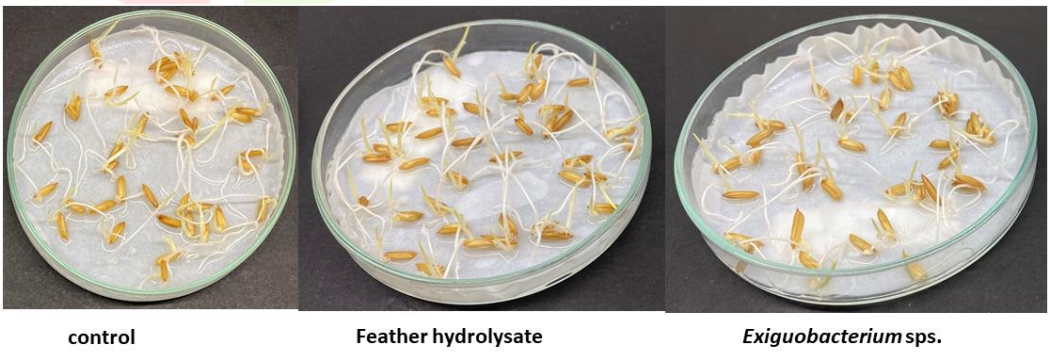

Figure 2: Germination of oryza sativa seed

Shoot and Root Growth:

The application of keratin hydrolysate resulted in the longest shoot (12 cm) and root (17 cm) lengths, indicating enhanced seedling growth. This aligns with previous studies demonstrating that keratin hydrolysates can stimulate seed germination and promote both root and shoot development [19].Exiguobacterium resulted in longer root growth (15 cm) than the control (12 cm), but its shoot length

(7.5 cm) was shorter than both Control (10 cm) and Keratin Hydrolysate (12 cm) tretment. The shorter shoot length in Exiguobacterium-treated plants may indicate different nutrient uptake patterns or stress responses compared to the other treatments fig.1

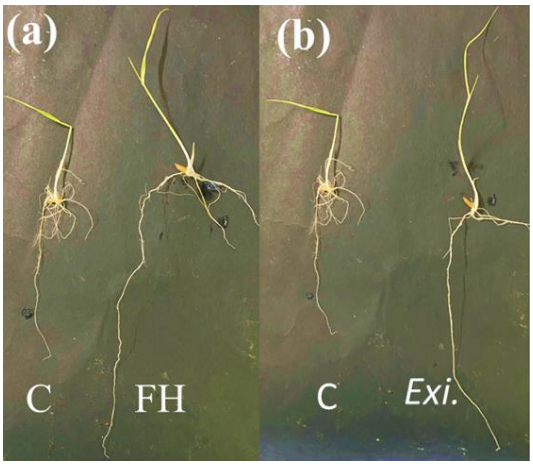

Figure1. shoot length and root length (C-control, FH-feather hydrolysate,Exi.- Exiguobacterium sp.)

Plant Biomass (Fresh and Dry Weight):

Exiguobacterium-treated plants had the highest fresh (0.3 g) and dry weight (0.15 g), suggesting it significantly improved biomass . Keratin Hydrolysate also promoted higher fresh (0.21 g) and dry weight (0.1 g) compared to the control (0.06 g and 0.02 g, respectively), indicating its potential role in enhancing plant growth

Seed Vigour Index (SVI) :

The Seed Vigour Index (SVI) is a crucial metric that reflects seedling strength and the potential for successful field establishment. A higher SVI indicates robust seedling growth, which is essential for optimal crop performance.The highest SVI (2,900) was observed with Keratin Hydrolysate, followed by Exiguobacterium (2,025) and the lowest was in the Control (1,980).

Table 1. plant growth parametres of rice.

Treatment	**Germination percentage (%)**	**Shoot Length (cm)**	**Root length (cm)**	**Fresh weight (g)**	**Dry weight (g)**	**Seed vigour index (SVI)**
Control	90%	10	12	0.06	0.02	1,980
Exiguobacterium sp.	90%	7.5	15	0.3	0.15	2,025
Feather hydrolysate	`100%	12	17	0.21	0.1	2,900

Chlorophll and cartenoid contents

The treated plants have more than double chlorophyll a (0.00212mg/g) content than the control (0.0005mg/g). This suggests enhanced photosynthetic efficiency, as chlorophyll a is the primary pigment involved in light absorption and energy conversion

The chlorophyll b 0.003mg/g) content in treated plants is 3 times higher than in the control 0.0008mg/g. Chlorophyll b plays a crucial role in light absorption and energy transfer, particularly in adapting to low-light conditions. The higher chlorophyll b content indicates improved light-harvesting capacity, possibly due to better nitrogen assimilation or reduced oxidative stress.

An increase in carotenoid (0.82mg/g) content compared to control (0.41mg/g) as photoprotective pigments, by preventing oxidative damage

Amino acid qualitative estimation

The qualitative analysis of amino acids present in FH shown in Table 2 indicated the presence of a diverse range of amino acids, including phenylalanine, tryptophan, arginine, and tyrosine, along with α-amino acids and sulphur-containing amino acids essential for plant growth promotion.

Table 2. Qualitative estimation of amino acids in feather hydrolysate.

Ninhydrin test (α-amino acids)	Xanthoproteic test (phenylalanine,tyrosine,tryptophan	Suphur test Methionine,cysteine
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Discussion

In the current study application of feather hydrolysate showed increased root length and shoot length has demonstrated significant enhancement in plant growth. The highest germination rate and seed vigor index [19] were observed with keratin hydrolysate treatment, indicating its efficacy in promoting it as a good biofertiliser and early seedling development. Also feather hydrolysate promoted seed vigor index reflects enhanced seedling strength and a better potential for field establishment. Keratin hydrolysate treatment resulted in the longest shoot and root lengths, compared to treatment with Exiguobacterium sp. and control suggesting improved nutrient uptake[20] and robust seedling growth. However Previous study of alkali treated feather hydrolysate showed improvement in the growth of nursery tea plants [25]. In terms of biomass, Exiguobacterium sp. treated plants recorded the highest fresh and dry weights[21], followed by keratin hydrolysate treatment[22] both out performing the control. Chlorophyll a (0.00212mg/g) content in the treated plants was twice that of control (0.0005mg/g). This suggests enhanced photosynthetic efficiency, as chlorophyll a is the primary pigment involved in light absorption and energy conversion. In a similar study of feather hydrolysate by Bacillus sp. there was significant increase in leaf chlorophyll a and b content [23]. The chlorophyll b 0.003mg/g content in treated plants is 3 times higher than in the control 0.0008mg/g. [24]. Amino acids are essential organic compounds that contain both an amino (-NH_2) and a carboxyl (-COOH)

functional group, serving as the fundamental building blocks of proteins. Additionally, amino acids contribute significantly to the formation and development of vegetative tissues, thereby supporting plant growth and development [25]. When applied as soil fertilizers or foliar sprays, amino acids present in the keratin hydrolysate influence key physiological processes, including nutrient uptake, stress tolerance, and overall plant health.

Conclusion

Chicken feather hydrolysate which are usually left out as waste material could be valorised using efficient keratinolytic microrganisms. Feather degradation results in production of different plant growth promoting factors like amino acids, oligopeptides and dipeptides which can serve as a nutrient supplement for the growth of plants. Feather Hydrolysate prepared using Exiguobacterium sp. resulted in the best overall growth of rice plant improving germination, shoot lenght and root length, and seed vigour index.

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