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Exploration of endophytic bacterial isolates from Kasni leaves (*Cichorium intybus*) for Phyto-hormone and Phyto-stimulating metabolite production

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ABSTRACT: Endophytic bacteria improve plant growth through diverse biochemical and metabolic activities that involve enhancement of nutrient availability, phytohormone production, and stress tolerance. In the present study, 5 endophytic bacteria from the leave of Kasni (*Cichorium intybus*) were isolated and evaluated for their *invitro* plant growth-promoting (PGP) potential. The isolates were qualitatively evaluated for nutrient (phosphate) solubilization, siderophore ammonia, biofilm, Indole-3-acetic acid (IAA) and antioxidants production. During qualitative analysis, only 3 isolates demonstrated positive results and were further quantified for their PGP and antioxidants activities. The maximum concentration of IAA was produced by the isolate KSBP2 in tryptic soy broth (TSB) (170.13 ± 0.7 $\mu\text{g/mL}$) followed by KSBP1 (61.15 ± 0.7) at 96 h of incubation. The isolate was also found positive for gibberellic acid, salicylic acid, siderophore production and phosphate solubilization. The antioxidant potential of isolates were evaluated by quantifying total phenolics and flavonoid contents. The isolate KSBP2 showed maximum total phenolics (2.81 ± 0.02 mg GAE/g) and flavonoids (0.18 ± 0.01 mg QE/g) contents in ethyl acetate extract. Furthermore, the isolate KSBP2 was assessed for biochemical and molecular characterization. Molecular characterization of KSBP2 based on 16S rRNA gene sequence analysis demonstrated 99.7% similarity of the isolate with *Bacillus velezensis*. Overall, the endophytic isolate KSBP2 possesses multiple plant growth promoting and antioxidant potential which might be a promising approach towards the enhancement of plant growth and development through improved nutrient availability and phytohormone production.

Keywords: Antioxidants, Endophyte, Medicinal plant, Phyto-stimulant potential

Endophytes are microorganisms that inhabit the internal tissues of plants without causing apparent harm and establish mutualistic, commensalistic, or symbiotic associations with their hosts (Makuwa and Dlamini, 2021). Among them, bacterial endophytes constitute an important component of the plant microbiome owing to their diverse functional roles in promoting plant growth, improving nutrient acquisition, and enhancing tolerance to biotic and abiotic stresses (Agrawal and Bhatt, 2023; Kumar *et al.*, 2025). These beneficial bacteria facilitate plant development through multiple mechanisms, including biological nitrogen fixation, phosphate solubilization, siderophore-mediated iron acquisition, phytohormone biosynthesis, and modulation of plant defense responses (Varga *et al.*, 2020; Saad and Badry, 2020). In addition, endophytic bacteria mitigate environmental stresses such as salinity, drought, and heat through the production of osmoprotectants, antioxidant metabolites, and 1-aminocyclopropane-1-carboxylate (ACC)

deaminase, thereby improving plant adaptation to adverse conditions (Bilal *et al.*, 2018).

Among the various plant growth-promoting mechanisms, bacterial synthesis of phytohormones, including indole-3-acetic acid (IAA), gibberellic acid (GA_3), cytokinins, and salicylic acid, has received considerable attention because these metabolites regulate root architecture, cell division, nutrient uptake, and plant development (Orozco-Mosqueda *et al.*, 2023). Besides phytohormone production, research evidence suggests that endophytic bacteria synthesize antioxidant metabolites such as phenolic compounds, flavonoids, and ascorbic acid, which contribute to scavenging of reactive oxygen species (ROS) and maintenance of cellular redox homeostasis. Their antioxidant ability is commonly assessed using total phenolic content, total flavonoid content, ascorbic acid estimation, and DPPH radical scavenging activity (Arivo *et al.*, 2023; Linh *et al.*, 2025). These multifunctional traits not only enhance

bacterial survival under stress but may also improve plant resilience under unfavorable environmental conditions. (Linh *et al.*, 2025; Arivo *et al.*, 2023). Endophytic bacteria also contribute to plant health through indirect mechanisms, including the production of antimicrobial metabolites, competition for nutrients and ecological niches, biofilm formation, and induction of systemic resistance, thereby suppressing the establishment of plant pathogens (Fadiji *et al.*, 2022). However, the expression and efficiency of these beneficial traits are largely strain-dependent and are influenced by host genotype, environmental conditions, and plant microbe interactions (Hardoim *et al.*, 2015). Therefore, the identification and characterization of efficient endophytic bacterial strains from diverse medicinal plants remain an important area of research for sustainable crop production.

Medicinal plants represent unique ecological niches for endophytic microorganisms because of their rich phytochemical diversity and long-term adaptation to environmental stresses. Endophytes associated with medicinal plants frequently produce diverse secondary metabolites, including phenolics, flavonoids, alkaloids, terpenoids, and other bioactive compounds that possess agricultural and pharmaceutical significance (Pandey *et al.*, 2023; Wu *et al.*, 2023). Among these medicinal plants, *Cichorium intybus* L. (Kasni or chicory) is widely recognized for its medicinal, nutritional, and economic importance. The plant is rich in bioactive constituents such as inulin, sesquiterpene lactones, flavonoids, coumarins, terpenoids, carotenoids, tocopherols, and essential minerals, which are associated with its antioxidant, anti-inflammatory, antidiabetic, and hepatoprotective properties (Al-Snafi, 2016; Petropoulos *et al.*, 2017). Previous studies have shown that the endophytic bacterial community of *C. intybus* is dominated by members of the Proteobacteria, Firmicutes, and Actinobacteria, suggesting that this medicinal plant harbors diverse bacterial taxa with potential functional importance (Dolinski and Olek, 2013).

Despite increasing interest in plant-associated microbiomes, information on the plant growth-

promoting and antioxidant characteristics of endophytic bacteria associated with *C. intybus* remains limited. Therefore, the present study aimed to isolate and characterize endophytic bacteria from the leaves of *C. intybus* and evaluate their *in vitro* plant growth-promoting attributes, including phytohormone production, phosphate solubilization, siderophore production, ammonia production, biofilm formation, and antioxidant activities. The study further sought to identify the most promising isolate using biochemical and molecular approaches, providing a foundation for future investigations on its functional role in plant growth promotion, stress mitigation, and plant microbe interactions under greenhouse and field conditions.

MATERIALS AND METHODS

Isolation of Endophytic Bacteria

Kasni (*Cichorium intybus*) plant samples were collected from Medicinal Plants Research and Development Center (MRDC), Pantnagar. For the isolation the leave samples were taken and surface sterilized following the method given by Hallmann *et al.* (2006). The leave samples were washed under running tap water and surface sterilized with 96% ethanol (2 min), followed by 2% sodium hypochlorite (5 min) and rinsed 3-4 times with sterile distilled water. The final rinse water was plated onto nutrient agar medium to check the effectiveness of the sterilization. Sterilized tissues were aseptically macerated and plated on Luria Bertani agar (LB) and nutrient agar (NA) medium. Plates were incubated at 28±2°C for 24–72 h.

In vitro qualitative screening of plant growth promoting (PGP) potential of endophytic bacterial isolates

Siderophore, Ammonia, and biofilm production

The siderophore production was assessed on Chrome Azurol S (CAS) agar method. The formation of an orange halo zone around the colonies indicates a positive result (Schwyn and Neilands, 1987). For ammonia production the bacterial isolates were evaluated by utilizing peptone water as suggested by Dye *et al.* (1962). Bacterial cultures were inoculated in tube containing 5.0 mL of sterile

peptone water and incubated at 30°C for 24 h. Following incubation, 1.0 mL of Nessler's reagent was added to each tube. The ammonia production was indicated by a color change from yellow to brown, while lack of color change indicated negative result.

Biofilm formation was assessed by the test tube method (Raksha *et al.*, 2020) with minor modifications. Bacterial isolates were inoculated into test tubes containing 5.0 mL of tryptic soy broth and incubated at 30°C for 48 h under static conditions. After incubation the medium was decanted and the tube was gently washed with phosphate buffer saline (PBS) (pH 7.3) to eliminate non-adherent cells. The adhering biofilm was dyed with 0.1% crystal violet for 10–15 min, washed with distilled water and tubes were dried in inverted position and were observed for biofilm formation. The affirmative result indicated a blue layer lined on the wall of the test tube.

Phosphate solubilization and Nitrogen fixation

The bacterial isolates were inoculated on Pikovskaya's agar to evaluate the phosphate solubilizing ability. Clear halo zones around the colonies indicate the ability of phosphate solubilization by the cultures (Yasmin *et al.*, 2011). The ability of nitrogen fixation was assessed in Jensen's nitrogen free medium, growth on the medium indicated the ability of the bacterial isolates to fix atmospheric nitrogen (Jensen, 1955). Ammonia production of bacterial isolate was evaluated by utilizing peptone water as suggested by Dye *et al.* (1992). Bacterial cultures were inoculated in tube with 5.0 mL of sterile peptone water and incubated at 30°C for 24 h. Following incubation, 1.0 mL of Nessler's reagent was added to each tube. The ammonia production was indicated by a color change from yellow to brown, while a lack of color change indicated a negative result.

***In- vitro* Quantitative estimation of plant growth promoting potential of selected endophytic bacterial isolates**

Phosphate solubilization

P-solubilizing efficiency of selected bacterial

isolates (KSBP1, KSBP2, KSBP5) was carried out in MSM broth medium supplemented with tricalcium phosphate (TCP) as a bound phosphate source. Samples were collected every 24 h and centrifuged for 10 min at 5000 rpm at 4 °C. Uninoculated broth was used as control. The supernatant obtained was further employed for estimation of released phosphate as outlined by Kapri and Tewari (2010). Absorbance was read at 405 nm using a UV-Vis spectrophotometer (Biomate 3S, Thermo Scientific). The concentration of liberated phosphate was determined from standard curve prepared from KH_2PO_4 .

Siderophore production

Quantitative estimation of siderophore production was performed in bacterial isolates by Chrome Azurol S (CAS) assay as described by Schwyn and Neilands (1987). Bacterial cultures were cultivated in L.B. broth (pH 6.8) at 30°C for 24 h time interval and centrifuged for 10 min at 10,000 rpm to obtain cell free supernatant. The supernatant (500µL) was combined with an equal volume of CAS reagent (500µL), and was incubated at room temperature for 20 min. The decrease in the intensity of the blue color was evaluated spectrophotometrically at 630 nm against a reference (CAS reagent with uninoculated broth). The siderophore production was reported as percent siderophore units (SU%) using the following formula:

$$\text{SU (\%)} = (\text{Ar} - \text{As}) / \text{Ar} * 100,$$

where Ar- represents the absorbance of the reference (uninoculated broth); As- represents the absorbance of the sample.

Indole acetic acid (IAA) production

Indole-3-acetic acid (IAA) production by bacterial isolates was determined using the Salkowski colorimetric method as described by Gordon and Weber (1951) with minor modifications. Isolates were grown in Tryptic Soy Broth (TSB) and Luria-Bertani (LB) broth separately, each supplemented with L-tryptophan (0.5%, w/v), and incubated at 30°C in dark for 24 h intervals. Cell-free supernatants were prepared by centrifugation of cultures for 10 min at 10,000 rpm. 2.0 mL of

Salkowski reagent (0.5 M FeCl_3 in 35% perchloric acid) was added to 1.0 mL of supernatant and incubated for 30 min in dark. Development of pink color indicates positive IAA production. Absorbance was measured at 530 nm against a reagent blank, with uninoculated tryptophan-amended broth as a negative control. IAA concentration was determined from a standard curve prepared with pure IAA and expressed as $\mu\text{g/mL}$ of culture filtrate.

Production of Gibberellic Acid (GA_3)

The extraction of gibberellic acid (GA_3) by the bacterial isolates was estimated using the method described by Borrow *et al.* (1955) with slight modifications. Bacterial cultures were cultivated in L.B. broth and incubated for 5 days at 30°C . The culture was centrifuged at 10,000 rpm for 10 min at 4°C and the cell free supernatant was collected and acidified to pH 2.0 with 5.0 N HCl. By treating the acidified culture filtrate with equal volume of ethyl acetate (twice / thrice), ethyl acetate phase was evaporated at 32°C and the remaining residue was dissolved in 2.0 mL of distilled water containing 0.05% tween. The estimation of gibberellins was performed by following the method given by Mahadevan and Sridhar (1982), the dissolved residue was treated with 2.0 mL zinc acetate and incubated for 2 min. 2.0 mL of potassium ferrocyanide was added to precipitate interfering substances. The supernatant obtained after centrifugation was combined with 5.0 N HCl and incubated for 70 min at 20°C . Absorbance was recorded at 254 nm using spectrophotometer.

Salicylic Acid Production

The production of salicylic acid by the selected bacterial isolates was assessed by the method of Meyer *et al.* (1992) with minor modifications. Bacterial cultures were cultivated in succinate medium at 30°C for 72 h in shaking incubator at 120 rpm. Cultured broth was centrifuged at 8,000 rpm for 5 min to obtain cell-free supernatant. The supernatant was resuspended in 1.0 mL of 0.1M phosphate buffer (pH. 7.0). After that 4.0 mL cell free culture supernatant was acidified to 2.0 pH with 1.0 N HCl and extracted with chloroform. The organic phase was collected and 4.0 mL distilled

water, 5 μL of FeCl_2 was added. Then the formation of purple colored iron complex was observed and absorbance was measured at 527 nm using spectrophotometer. Salicylic acid content was estimated by using a standard curve of pure salicylic acid and expressed as $\mu\text{g/mL}$ of culture filtrate.

Antioxidant potential by selected endophytic bacterial isolates

Total phenolic content (TPC) and total flavonoid contents (TFC)

Preparation of Bacterial Extract

The selected bacterial isolates (KSBP1, KSBP2 and KSBP5) were inoculated in LB broth and cultured at 30°C for 72 h under shaking condition. Metabolite extraction was performed by adding equal volumes of ethyl acetate to bacterial culture broth after 72h of incubation. The ethyl acetate organic phase was separated by separating funnel and evaporated to dryness. The dried extract was re-dissolved in ethanol and kept in a vial for the measurement of total phenolic and flavonoid contents.

Determination of Total Phenolic Content (TPC)

Total phenolic content (TPC) was calculated using Folin Ciocalteu spectrophotometric method (Ismail *et al.*, 2019) with minor adjustments. Briefly, 200 μL of ethanolic extract of bacterial endophytes was mixed with 200 μL of 50% Folin-Ciocalteu reagent and vortexed for 1 min. After that, of 2% sodium carbonate (4.0 mL) solution was added to the reaction mixture and incubated in the dark at room temperature for 30 min. Absorbance was measured at 750 nm by using UV-Vis spectrophotometer. Total phenolic content was expressed as gallic acid equivalent (mg GAE/mL) by preparing a standard curve using known concentrations of gallic acid.

Determination of Total Flavonoid Content (TFC)

Total flavonoid content was assessed using aluminum chloride based on the method of Zou *et al.* (2004) with minor modification. In brief, 500 μL of ethanolic bacterial extract was combined with 2.0 mL of distilled water, 0.15 mL of 5% sodium nitrite (NaNO_2) solution and incubated for 6 min at room temperature. Then 10% aluminum chloride (0.15 mL) solution was added and the mixture was

vortexed and reincubated for 6 min. 2.0 mL of 1.0 N sodium hydroxide (NaOH) was added, distilled water was added to adjust total volume to 5.0 mL. The mixture was incubated at room temperature for 15 min and the absorbance was read at 510 nm in UV-Vis spectrophotometer. Total flavonoid content was expressed in mg quercetin equivalent (mg QE)/mL of extract by using known concentration of quercetin.

Ascorbic acid production

Ascorbic acid production was determined in a medium proposed by Ghosh *et al.* (2008) with the following composition: glucose (8.0 g/L), K₂HPO₄ (0.5 g/L), MgSO₄·7H₂O (0.2 g/L), L-glutamic acid (2.0 g/L), thiamine hydrochloride (200 µg/L), biotin (200 µg/L) and NH₄Cl (2.0 g/L). The bacterial cultures were inoculated and incubated at 30 °C for 72 h. The cultural broth was centrifuged at 5000 rpm for 8 min to obtain cell free supernatant. The supernatant was treated with 10 ml of 6% trichloroacetic acid and centrifuged for 2 min at 5000 rpm. The volume of the filtrate was readjusted to 50 mL with distilled water and transferred to another tube. 5.0 mL of reaction mixture was taken in another tube and combined with 2.5% solution of 2,4-dinitrophenyl hydrazine (9N H₂SO₄ with 2 drops of thiourea solution in 70% ethanol) to stop the oxidation of ascorbic acid. The mixture was incubated in a water bath for 25 min and cooled. Then 80% H₂SO₄ (5.0 mL) was added and the mixture was incubated at room temperature for 30 min. The absorbance was recorded by a UV-Vis spectrophotometer at 530 nm. Standard curve was prepared by using different known concentration of ascorbic acid.

DPPH activity of bacterial culture

The bacterial isolates were assessed for the ability to scavenge DPPH free radical using the method given by Kim *et al.* (2022). The bacterial isolates were inoculated in LB broth medium and incubated for 24 h at 30°C. The culture broth was centrifuged at 4000 rpm for 5 min. Cells were washed twice with phosphate-buffered saline (PBS) and finally the pellet was re-suspended in PBS and optical density was measured at 600nm to check the bacterial

growth. The suspended pellet and 0.2 mM DPPH were combined in the ratio of 1:2 (v/v) for the reaction and incubated at room temperature for 30 min and absorbance was recorded at 517 nm. The calculation of free radical scavenging activity was done by using the following formula:

$$\text{Radical scavenging activity (\%)} = (1 - A_{\text{sample}}/A_{\text{control}}) * 100,$$

where A_{sample} = absorbance of sample; A_{control} = absorbance of control.

Biochemical and molecular characterization of selected endophytic bacterial isolate KSBP2

The bacterial isolate exhibiting the maximum plant growth-promoting and antioxidant potential, was selected for biochemical and molecular characterization. For biochemical characterization, Gram staining, catalase, oxidase, starch hydrolysis, protease, gelatinase and peroxidase production tests were performed. Bacterial DNA was isolated by CTAB method, 16S rRNA was amplified and for molecular identification, Sanger sequencing by capillary electrophoresis was performed (Kumar *et al.*, 2013). BLAST was performed against the NCBI database and a phylogenetic tree was generated by using the neighborhood joining approach in MEGA 12.0 software.

Statistical analysis

All studies were carried out in triplicate and findings are expressed as mean±standard deviation. Statistical analysis was carried out using one way ANOVA and the differences were considered significant at p < 0.05.

RESULTS AND DISCUSSION

Selection of endophytic bacteria for plant growth promoting traits

Five endophytic bacterial cultures were isolated from the healthy leaf tissue of *Cichorium intybus*. The isolation confirmed the presence of cultivable endophytic bacteria present in *Cichorium intybus*. Studies have shown that endophytic colonization patterns are mostly determined by plant genotype, environmental conditions, and habitat (Hardoim *et al.*, 2015). The isolation of endophytic bacteria from

chicory leaves indicates its ecological adaptability to the internal plant environment. Qualitative screening revealed the presence of several plant growth promoting activities of endophytic bacterial isolates. The 3 isolates (KSBP1, KSBP2, KSBP5) demonstrated positive response for phosphate solubilization, siderophore and ammonia production, biofilm formation and N_2 -fixation, indicating its multifunctional potential in plant growth promotion (Table 1). The halo zone formation by all the three isolates on Pikovskaya's agar indicated positive phosphate solubilizing ability which may lead to improved plant nutrition. Similarly, the formation of an orange halo on Chrome Azurol S (CAS) agar by all the three isolates showed the positive siderophore production, which help in iron acquisition under iron-limited conditions. In addition, the production of ammonia by all the three isolates indicates the contribution to the nitrogen availability in the rhizosphere. Ammonia production is an important plant growth-promoting trait that contributes to nitrogen availability and supports plant growth by enhancing nutrient acquisition (Glick, 2012; Compant *et al.*, 2010; Prasgi, and Cahyani, 2025).

***In vitro* Phytostimulant potential of selected bacterial endophytes**

The bacterial isolates (KSBP1, KSBP2 and KSBP5) found positive in qualitative assays were further

quantified for phytohormones, siderophore production and phosphate solubilization and antioxidants potential.

Indole-3-Acetic Acid (IAA) Production

The selected bacterial isolates exhibited the ability to produce IAA in both TSB and LB medium. In TSB medium the bacterial isolate KSBP2 showed maximum IAA production ($170.13 \pm 0.7 \mu\text{g/mL}$) followed by KSBP1 (61.12 ± 0.7) at 96 h of incubation, while in LB medium maximum production was observed by KSBP2 ($60 \pm 0.9 \mu\text{g/mL}$) after 72 h of incubation (Fig.1B). The significantly higher production of IAA in TSB medium implies that the nutrient rich nature of medium is favorable for

Table 3: Microscopic and biochemical characteristics of the selected endophytic bacterial isolate KSBP2

Biochemical characterization	Isolate KSBP2
Catalase	+
Peroxidase	+
Starch hydrolysis	+
Urease	-
Casein hydrolysis	+
Gelatinase	-
Oxidase	+
Gram's reaction	+
Cell shape	Rod shaped
Cell arrangement	chain

(+) shows presence while (-) shows absence

Table1: Qualitative screening of endophytic bacterial isolates from Kasni (*Cichorium intybus*) for *in vitro* phytostimulating potential

S. No.	PGP activities	KSBP1	KSBP2	KSBP3	KSBP4	KSBP5
1	Siderophore	+	+	-	-	+
2	P-solubilizing	+	+	-	-	+
3	Ammonia production	+	+	-	-	+
4	N_2 -Fixation	+	+	-	-	+
5	Biofilm formation	+	+	-	-	+

(+) shows presence while (-) shows absence

Table 2: *In vitro* production of antioxidant metabolites (total phenolics, total flavonoids, and ascorbic acid) and DPPH radical scavenging activity of selected endophytic bacterial isolates

S. No.	Isolates	TPC (mg GAE/g)	TFC (mg QE/g)	%DPPH	Ascorbic acid ($\mu\text{g/mL}$)
1.	KSBP1	0.180 ± 0.02^a	0.074 ± 0.03^a	7.33 ± 0.6^a	119.86 ± 1.0^a
2.	KSBP2	2.81 ± 0.02^c	0.18 ± 0.01^c	16.72 ± 0.8^c	326.78 ± 0.6^c
3.	KSBP5	0.523 ± 0.06^b	0.111 ± 0.06^b	8.52 ± 0.7^b	141.21 ± 1.0^b

Where GAE= Gallic acid equivalent; QE= quercetin equivalent

bacterial growth and enhances IAA production. (Sharma *et al.*, 2025; Vimal *et al.*, 2025). Similar results have been observed for other PGP bacteria (*Bacillus*, *Pseudomonas*, *Enterobacter*, and *Achromobacter*), where the maximum IAA production occurs between 72 and 96 h followed by a decline during prolonged incubation and nutritional content, incubation period and the concentration of precursors had a substantial effect on IAA synthesis (Wagi and Ahmed, 2019; Castillo Alfonso *et al.*, 2021). Bacterial IAA is important for root elongation, cell division, and improvement of nutrient absorption, resulting in plant growth and development.

Production of gibberellic acid (GA_3) and salicylic acid

The selected endophytic bacterial isolates were evaluated for their ability to produce phytohormones, gibberellic acid (GA_3) and salicylic acid (SA) under

in vitro conditions. The isolate KSBP2 produced maximum GA_3 ($169.47 \pm 0.8 \mu\text{g/mL}$) followed by KSBP1 ($95.49 \pm 0.4 \mu\text{g/mL}$) and the maximum concentration of SA ($30.0 \pm 0.97 \mu\text{g/mL}$) was also observed in isolate KSBP2 (Fig.1A).

The high production of gibberellic acid (GA_3) indicates the strong phyto-stimulatory potential of the isolate KSBP2, as GA_3 regulates stem elongation, seed germination, and leaf expansion. In contrast, lower salicylic acid (SA) production as compared to gibberellic acid suggests a secondary role in plant defense, as low SA levels are sufficient to trigger defense signaling (Orozco-Mosqueda *et al.*, 2023; Benjamin *et al.*, 2022; Elsis *et al.*, 2024).

Siderophore Production and Phosphate solubilization

The selected bacterial isolates were evaluated for

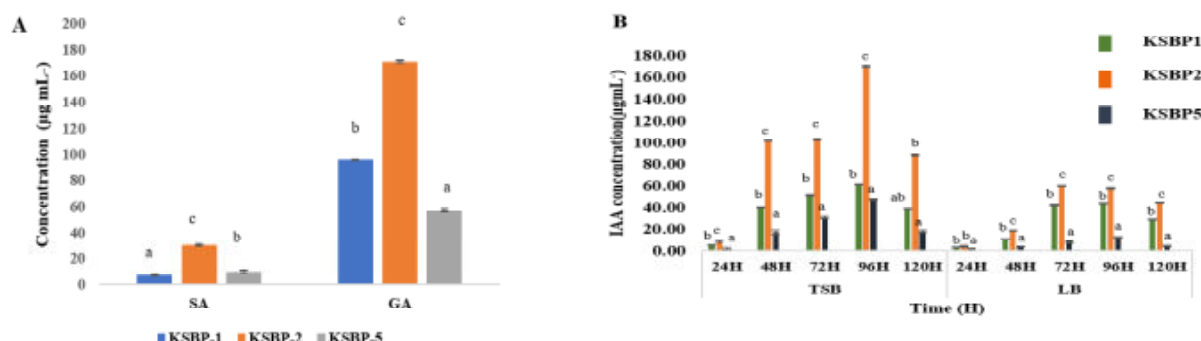


Fig 1: Quantitative estimation of phytohormone production by the selected endophytic bacterial isolates (A) Gibberellic acid (GA_3) and salicylic acid (SA); (B) Indole-3-acetic acid (IAA) production in Tryptic Soy Broth (TSB) and Luria-Bertani (LB) media supplemented with 0.5% L-tryptophan at different incubation periods (24–120 h).

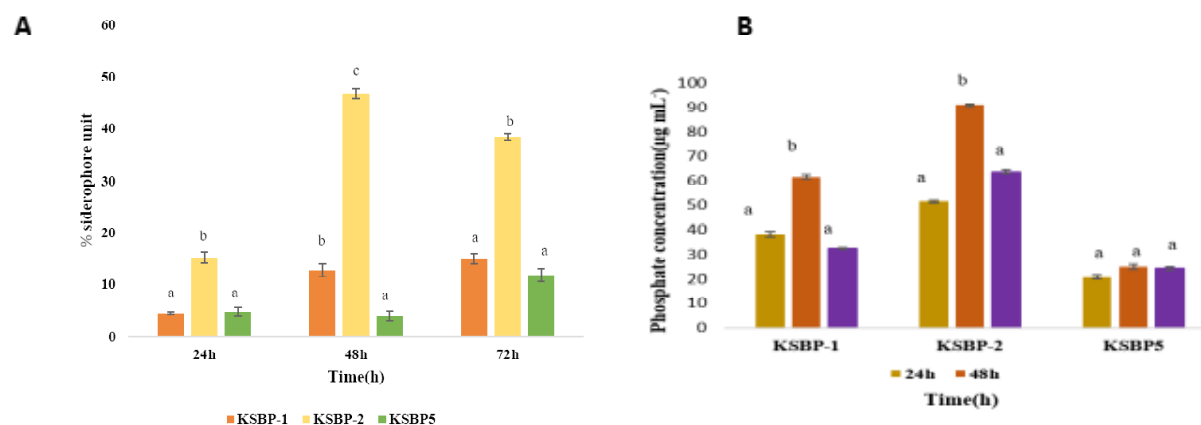


Fig.2: Quantitative estimation of phosphate solubilization and siderophore production by selected endophytic bacterial isolates at different time intervals

the siderophore production and phosphate solubilization at different incubation periods (24, 48 and 72 h). The maximum siderophore production was observed in KSBP2 (45%) after 48 h of incubation (Fig.2 A). Similarly, the phosphate solubilizing activity varied among the isolates and isolate KSBP2 recorded the highest soluble phosphate concentration ($90.95 \pm 0.6 \mu\text{g/mL}$) followed by KSBP1 ($61.61 \pm 0.8 \mu\text{g/mL}$) at 48h of incubation (Fig.2 B). Maximum siderophore production during the exponential growth phase reflects active metabolism and enhanced iron-chelating activity under iron-limited conditions. The decline after 72 h may result from reduced metabolic activity, nutrient depletion, or feedback regulation following increased intracellular iron availability (Kloepper *et al.*, 1980; Li *et al.*, 2023). The enhanced phosphate solubilization may be due to active microbial metabolism and secretion of organic acids which facilitates the conversion of insoluble phosphate into soluble forms through acidification and chelation mechanisms (Kapri and Tewari, 2010; Pant *et al.*, 2026).

Antioxidant activity

The antioxidant potential of the selected endophytic bacterial isolates was assessed by determining total phenolics (TPC), total flavonoids content (TFC), ascorbic acid production and DPPH radical scavenging activity (Table 2). Among the selected isolate KSBP2 showed maximum total phenolic content ($2.81 \pm 0.02 \text{ mg GAE/g}$), followed by KSBP5 ($0.523 \pm 0.06 \text{ mg GAE/g}$). Similarly, the maximum total flavonoid content was recorded in KSBP2

($0.18 \pm 0.01 \text{ mg / QE/g}$) followed by KSBP5 ($0.111 \pm 0.06 \text{ mg QE/g}$). Studies show that phenolic and flavonoid contents are strong reducing agents, hydrogen donors and free radical scavengers and play a vital role in oxidative stress tolerance in plants and micro-organisms (Kour *et al.*, 2023; Elsharkawy *et al.*, 2025). Likewise, KSBP2 produced the highest ascorbic acid content ($326.78 \pm 0.59 \mu\text{g/mL}$) and exhibited the maximum DPPH radical scavenging activity ($16.72 \pm 0.8 \mu\text{g/mL}$) indicating strong antioxidant potential. Ascorbic acid is a key non-enzymatic antioxidant, that scavenge reactive oxygen species (ROS) maintains cellular redox balance and protects cells from oxidative damage under stress conditions (Ghosh *et al.*, 2015).

Although the DPPH radical scavenging activity of the isolates was relatively low, the higher activity exhibited by KSBP2 may be attributed to its greater accumulation of phenolics and flavonoid compounds (AlKahtani *et al.*, 2024). The presence of maximum phenolic and flavonoid content, ascorbic acid and radical scavenging activity demonstrated the antioxidative potential of KSBP2 which might help in alleviating stress in plants and hence promote growth.

Biochemical and molecular characterization of selected bacterial isolate KSBP2

Bacterial isolate KSBP2 was capable of secreting catalase, oxidase, peroxidase and casein hydrolase while urease and gelatinase activities were absent in the selected isolate (Table 3). The presence of positive catalase and peroxidase activities shows that the isolate is able to detoxify reactive oxygen species, which indicates its resilience to oxidative and environmental stress conditions. The oxidase activity also suggests that the isolate has a functional electron transport chain, confirming its aerobic metabolic nature. The ability to hydrolyze casein reflects extracellular proteolytic activity, suggesting that the isolate is capable of degrading complex protein substrates and contributing to nutrient recycling. On the other hand, there was no urease and the gelatinase activity, indicating that the isolate does not hydrolyze urea and does not degrade gelatin. These distinct biochemical characteristics are

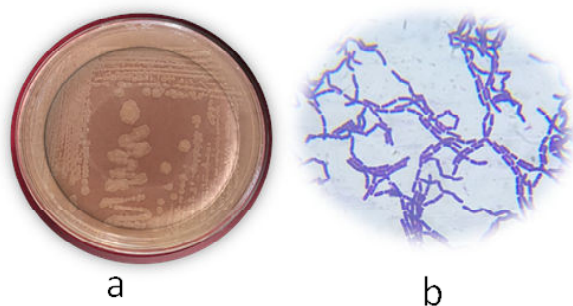


Fig.3: Morphological and microscopic characterization of selected bacterial isolate KSBP2. Plate photograph of bacterial isolate(a); microscopic photograph (b)



Fig. 4: Phylogenetic tree showing the molecular identification of endophytic bacteria KSBP2, based on 16S rDNA sequencing

significant for functional characterization of bacterial isolates. (Singh *et al.*, 2019; Rani *et al.* 2020). When observed under compound microscope, the bacterial isolate KSBP2 was found to be Gram-positive, rod shaped and grouped in chains with slender elongated cell morphology (fig.3). These features are generally seen in plant growth-promoting bacteria, especially belonging to genus *Bacillus* (Kandel *et al.*, 2017; Kaur *et al.*, 2023). The 16S rRNA gene sequencing identified the isolate KSBP2 belonging to genera *Bacillus*, with 99.7% similarity with *Bacillus velezensis* (fig.4).

CONCLUSION

The present study demonstrates Kasni (*Cichorium intybus*) as a rich source harboring a number of plant growth promoting bacterial endophytes having high antioxidant potential. Among the selected isolates, the isolate KSBP2, showing maximum *in vitro* phytohormones (IAA, GA₃, salicylic acid), siderophore, phosphate solubilization, ammonia and biofilm production was found superior. In addition, the isolate also demonstrated the maximum potential to produce total phenolic and total flavonoids contents, DPPH, and ascorbic acid in comparison to other tested isolates. Biochemical and molecular characterization identified the endophytic bacterial isolate as *Bacillus velezensis*, which is well reported for its plant growth-promoting potential. The

combination of these *in vitro* traits suggests that KSBP2 may contribute to improved nutrient acquisition, plant growth, and enhanced tolerance to environmental stresses. However, these findings require validation under greenhouse and field conditions to determine the effectiveness of the isolate in plant microbe interactions.

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