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PLANT GROWTH PROMOTING POTENTIAL OF CALIDIFONTIBACILLUS ERZURUMENSIS AS11 ISOLATED FROM GARLIC (ALLIUM SATIVUM L.) AND ITS ANTAGONISTIC ACTIVITY AGAINST ASPERGILLUS NIGER

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ABSTRACT

Garlic (*Allium sativum* L.) is an economically important spice crop affected by black mold disease caused by *Aspergillus niger*. The present study aimed to isolate and characterize endophytic bacteria from garlic and evaluate their plant growth promoting and biocontrol potential against *A. niger*. A total of 20 bacterial endophytes were isolated and screened for antagonistic activity using the dual culture assay. Among the isolates, strain AS11 exhibited the highest antifungal activity with 79.33% mycelial growth inhibition and an inhibition zone of 13.00 ± 1.73 mm. Molecular identification based on 16S rRNA gene sequencing identified isolate AS11 as *Calidifontibacillus erzurumensis*. The isolate exhibited multiple plant growth-promoting traits including indole acetic acid production, ammonia production, biological nitrogen fixation, siderophore production, and phosphate solubilization. Germination and pot experiments demonstrated significant improvement in garlic growth parameters under *Calidifontibacillus erzurumensis* AS11 treatment and partial protection against pathogen stress. Statistical analysis was performed using Student's *t*-test and one-way ANOVA followed by Tukey's HSD test at $p < 0.05$ using Python. The findings suggest that *C. erzurumensis* AS11 possesses strong plant growth promoting and biocontrol potential and may serve as an eco-friendly bioinoculant for sustainable garlic cultivation.

KEYWORD: *Allium sativum*, *Calidifontibacillus erzurumensis, Plant Growth promotion, Biocontrol, Aspergillus niger*

INTRODUCTION

Crop productivity enhancement is essential to meet the food demands of the growing population, particularly in developing countries, is often dependent on the extensive application of chemical fertilizers. However, prolonged use of these fertilizers has been reported to reduce the soil biodiversity[1,2] and adversely affect the environment through nutrient leaching, groundwater contamination and soil pollution.[3] Therefore, sustainable agricultural strategies focusing on the use of beneficial microorganisms have gained considerable attention as eco-friendly alternatives to chemical fertilizers.[4,5] Plant growth promotion promoting bacteria (PGPB), particularly those forming symbiotic associations with plants, play a significant role in improving plant growth, productivity and stress tolerance under diverse environmental conditions [6,7,8]. Among them, bacterial endophytes inhabiting the internal plant tissues without causing visible pathogenic symptoms and establishing mutually beneficial interactions with their host plants are seen in various studies. These microorganisms promote plant growth through several mechanisms, including phytohormone production, enzyme secretion, nutrient immobilization, phosphate solubilization, nitrogen fixation and ammonia production [9,10,11]. In addition, many endophytic bacteria exhibit antimicrobial activities that contribute to the suppression of phytopathogens and reduction of crop losses [12-16]. Furthermore, metabolites produced by endophytes have shown considerable potential in various biotechnological and agricultural applications [17-20].

The genus *Bacillus* comprises Gram positive, rod shaped, endospore forming bacteria belonging to the family *Bacillaceae* and are ubiquitously distributed across ecological niches, including agricultural soils, plant tissues, marine and hydrothermal sediments and extreme environmental habitats such as hot springs [21-23]. Owing to their remarkable physiological adaptability and metabolic versatility, *Bacillus* species are widely recognized for their ecological, agricultural and biotechnological significance. Several *Bacillus* species have been reported as plant growth promoting and biocontrol agents due to their ability to produce phytohormones, hydrolytic enzymes, antimicrobial compounds and nutrient solubilizing metabolites. In addition, members closely related to *Bacillus,* including *Calidifontibacillus erzurumensis*, have attracted increasing interest because of their unique physiological characteristics and potential applications in agriculture and biotechnology. Advances in phylogeny and genome based taxonomic approaches have further improved the classification and characterization of *Bacillus* and related taxa within the family *Bacillaceae,* facilitating a better understanding of their diversity, ecological roles and functional potential.

Although numerous plant growth promoting rhizobacteria and endophytic microorganisms have been isolated from diverse plant hosts, only a limited number have been reported to possess both plant growth promotion and disease suppressive properties[24,25,26]. This study primarily focuses on the isolation and characterization of endophytic bacteria from Garlic and the evaluation of their plant growth potential and disease suppressive potential against black mold disease causing agent *Aspergillus niger.* Owing to its unique biochemical composition and medicinal significance, Garlic represents a promising host for the exploration of beneficial endophytic microorganisms with potential applications in sustainable agriculture, biocontrol and plant disease management [27].

MATERIALS AND METHODS

Collection of Sample

Fresh and healthy garlic was collected from Calicut, Kerala, India and was transported to the laboratory and processed for the isolation of endophytic bacteria

Surface Sterilization

Healthy Garlic cloves were properly washed under tap water to remove all the soil and dust particles. It was then again washed with the detergent Tween 20. The samples were then subjected to serial rinsing with tap water. Subsequently the cloves were then subjected 1% of Sodium hypochlorite for 2 minutes, followed by rinsing it with sterilized distilled water for 5 times. The cloves were then treated with 70% of ethanol for approximately 30 seconds and was followed by rinsing with sterilized distilled water for 5 times. The surface sterilized cloves were then blot dried with sterile filter paper. To confirm effective sterilization of the cloves, the distilled water from the final rinse was then plated onto Tryptic Soy Agar (TSA) and incubated at 28 ± 2° C to check microbial growth.[28]

Isolation of Endophytic Bacteria

The surface sterilized garlic cloves were aseptically transferred into sterile petri plates and cut into small segments of 1 cm in length using sterilized scalpel and forceps and were then transferred onto TSA plates under aseptic conditions. Sterile TSA plates were kept as control and maintained along side the samples. All the plates were incubated at 28 ± 2° C for 5 days and monitored periodically for bacterial growth. Bacterial growth emerging from within the garlic were selected, sub cultured and maintained at 4°C on TSA slants for further studies.[29]

Isolation of Phytopathogen from Garlic

The infected Garlic clove was surface sterilized and the excised with sterile scalpel and forceps into segments and placed on Potato Dextrose Agar (PDA) and was then incubated for 7 days at 28 ± 2° C for the growth of fungal pathogen. The fungal growth obtained from the garlic cloves with disease was subjected to macroscopic and microscopic observations to study the colony morphology, shape and arrangement of hyphae, conidia etc. [30]

Invitro Antagonism Test

The Invitro Antagonism Test was performed by Dual Culture method [31]. The isolated endophytic bacteria was tested for antagonistic activity against *Aspergillus niger* by streaking one side of the sterilized petri plate containing a combination of Nutrient agar and Potato Dextrose agar in 1:1 ratio with isolated bacterial endophyte and a fungal culture disc of 5mm diameter was placed on the adjacent side of the agar plate. Control plates were also maintained containing only the fungal colony of *Aspergillus niger*. The percentage of inhibition was calculated using the formula

$$\frac{\text{Growth in control (C)} - \text{Growth in Antagonist (T)}}{\text{Growth in control}} \times 100$$

Molecular Identification

Genomic DNA of the endophytic bacterial isolates was extracted using the HiPurA™ bacterial genomic DNA purification kit (MB505, HiMedia) according to the manufacturer's instructions. The extracted DNA was verified on 0.8% agarose gel under UV transillumination. The 16S rRNA gene was amplified by polymerase chain reaction (PCR) using Emerald AMP® GT PCR Master Mix (RR310A, Takara) with universal bacterial primers: forward primer 27F (5′-GAGTTTGATCCTGGCTCA-3′) and reverse primer 1492R (5′-ACGGCTAACTTGTTACGACT-3′). The PCR amplification was carried out in a 20 µl reaction mixture under standard thermal cycling conditions, and the amplified products were confirmed on 1,5% agarose gel. The PCR products were purified and subjected to Sanger sequencing using the ABI PRISM® BigDye™. Terminator Cycle Sequencing Kit with AmpliTaq® DNA polymerase (FS enzyme) (Applied Biosystems). The obtained sequence chromatograms were analysed using SnapGene Viewer to assess read length and sequence quality. High quality sequences were compared with reference sequences in the NCBI database using BLASTn tool for taxonomic identification based on maximum similarity score coverage. A phylogenetic tree was constructed using the Neighbor-Joining method in MEGA version 11.0.13 to determine the evolutionary relationship of the isolates.[32]

Invitro Plant Growth Promoting Traits

Phosphate Solubilization

The bacterial endophytic isolates were screened for Phosphate Solubilization on Pikovskaya medium with bromocresol green as an indicator. The medium was spot inoculated with the endophytic bacterial isolate and incubated at 28 ± 2° C for 48 - 72 hours. Pikovskaya medium without the endophytic bacteria was kept as control [33]. The formation of clear zones around the colony, due to the ultization for tricalcium phosphate, was measured to assess the ability of endophytes to solubilize phosphates. The phosphate solubilizing index was calculated using the formula below

$$PSI = \frac{\text{Colony diameter + Zone of clearance}}{\text{Colony diameter}}$$

Ammonia Production

The ability of the endophytic bacteria to produce ammonia was assessed by inoculating actively growing bacterial culture to peptone water. Peptone water was without bacterial culture was also kept as control. Following incubation at 28 ± 2° C for 72 hours, 1ml of Nessler's reagent was added into the peptone medium to assess the ammonia production. Control were also kept without the bacterial inoculation. A colour change to deep yellow or brown indicated the production of ammonia.[34]

Indole Acetic Acid Production

The ability of bacterial endophyte to produce Indole acetic acid (IAA) was analysed in nutrient broth supplemented with 0.5% L – Tryptophan and incubated on a shaker incubator. Following incubation, the bacterial culture were centrifuged at 10000 rpm for 10 minutes to obtain cell free supernatant. To 2ml of the supernatant, 2 ml of Salkowski reagent was added and mixed properly and was kept at room temperature in dark condition for 30 minutes. Control were also kept without the bacterial inoculation. Development of pink to reddish colour indicates IAA production. [35]

Biological Nitrogen Fixation

The nitrogen fixing ability of the endophytic bacterial isolate was assessed using nitrogen free malate medium containing bromothymol blue indicator. The isolates were inoculated into sterile semi solid nitrogen free malate medium and incubated at 28 ± 2°C for 3 days. Control were also kept without the bacterial inoculation. Following incubation development of blue colouration along with pellicle formation indicates nitrogen fixation.[36]

Siderophore production

The endophytic bacteria was analysed for siderophore production. Actively grown bacterial culture was inoculated into Kings B broth and incubated at 28 ± 2° C for 3 days on an incubator shaker. Following incubation, the bacterial culture were then centrifuged at 10000 rpm for 20 minutes to obtain cell free supernatant. To the cell free supernatant 2% Ferric chloride was treated and kept for 30 minutes undisturbed. Control were also kept without the bacterial inoculation. The development of orange to brown colour indicates siderophore production. [37]

Abiotic Stress Responses

The ability of bacterial isolate to tolerate different stress conditions such as salinity, drought, temperature and pH was checked [38]

Salinity Stress : The salt tolerance of endophytic bacterial isolate was determined by observing their growth potential on nutrient media supplemented with different NaCl. Concentrations (0.5, 5, 10, 15, 20 %; w/v) the endophytic bacteria was streaked on these plates and was kept for incubation for 72 hours at 28 ± 2° C.

Drought Stress : The drought resistance of the endophytic bacteria was determined on nutrient agar medium supplemented with polyethylene glycol (6000) at various concentrations (1, 5, 10%; w/v). the endophytic bacteria was streaked on the plates and was kept for incubation at 28 ± 2° C for 72 hours.

Temperature Stress : The endophytic bacterial isolate was examined for its ability to grow at 28, 37, 40, 50°C on nutrient agar plates. Pure culture of the bacterial endophyte was streaked on the plates and were incubated for 48 hours.

pH Tolerance : the pH tolerance of the actively growing endophytic bacteria were examined on the nutrient agar media at various pH values (5, 7, 9, 11 and 12). The pure culture was streaked on the nutrient agar plate with different pH values and was incubated at 28 ± 2° C for 72 hours.

Effect of Endophytic Bacterial isolate on growth of *Allium sativum*

Seedling Germination Test

The cloves of Garlic were collected and washed thoroughly with tap water to remove all the dust and soil particles. It was then surface sterilized with 1% of sodium hypochlorite solution for 2 minutes and then washed with sterile distilled water for 5 times. The cloves were then treated with 70% ethanol followed by rinsing it with sterile distilled water for 5 times. Disinfected cloves were used for the sprouting test and for pot experiment.

10 garlic cloves were treated with actively growing endophytic bacteria for 24 hours in peptone water and was then placed on sterile filter paper in a sterile petri plate. A control was also placed with garlic cloves not treated with the bacterial isolates. The plates containing the cloves were then kept for incubation at room temperature. After 10 days of incubation sprouted seedlings were counted and the sprouting percentage and the seedling vigour index was determined using the formula [39]

$$\text{Sprouting Percentage} = \frac{\text{No. of sprouted cloves}}{\text{Total No. of Cloves}} \text{ X } 100$$

Seedling vigour index = (Mean of Shoot length + Mean of Root length) X Sprouting Percentage

Pot Experiment

The pot experiment was carried out in a completely randomized design with five treatments including the uninoculated control and each treatment was maintained in triplicate. Approximately 10 kg of loamy soil was collected from a depth of 4-5 inches from campus of SAFI Institute of Advanced Study. The collected soil was sterilized by autoclaving at 121°C and 15 psi for 20 minutes on three consecutive days before the use for the experiment, the physical and biochemical characteristics of the soil were analysed. The experiment was conducted in the greenhouse facility at SAFI Institute of Advanced Study under controlled conditions at an average temperature of 28 ± 2°C. Plastic pots measuring 18.0cm in diameter and 8.0cm in height were used for the study. The endophytic bacterial isolate *Calidifontibacillus erzurumensis* AS11 was cultured in nutrient broth and incubated at 28 ± 2°C for 24 hours on a rotary shaker at 120 rpm. Garlic cloves used for planting were surface sterilized by immersing it to 2 % of sodium hypochlorite solution for 2 minutes, followed by five rinses with sterile distilled water. The cloves subsequently treated with 70% ethanol for 1 minute and again rinsed five times with sterile distilled water to remove residual sterilizing agents.[40] Five treatment groups were prepared for the study as follows

T1 - Garlic cloves treated with *C. erzurumensis* AS11 alone
T2 – Garlic cloves inoculated with *Aspergillus niger*
T3 – Garlic cloves treated with both AS11 and *Aspergillus niger*
T4 – Garlic cloves treated with Fungicide (Carbendazim) and *Aspergillus niger*
T5 – Uninoculated Garlic cloves (Control)

The treated garlic cloves were planted in the sterilized soil filled pots and maintained under greenhouse conditions. All pots were irrigated regularly with tap water throughout the experimental period. After 45 days of growth, the plants were carefully uprooted and growth parameters, shoot length and root length were recorded for each experiment.

Statistical Analysis

Statistical data were expressed as Mean ± SE for Germination test and Mean ± SD for Pot experiment. An independent Student's *t*-test was used for Germination analysis, while one-way ANOVA followed by Tukey's HSD test was applied for Pot experiment comparisons. Statistical significance was considered at $p < 0.05$. All analyses were performed using Python.

RESULTS AND DISCUSSIONS

Plant endophyte interactions contribute significantly to plant growth and development through multiple direct and indirect mechanisms. In recent years, the excessive use of chemical fertilizers to meet the increasing global food demand has raised serious concerns regarding environmental pollution, soil degradation, and public health hazards[41]. Garlic (Allium sativum L.) is an important spice crop frequently affected by black mold disease caused by the fungal pathogen *Aspergillus niger* during preharvest or post-harvest. The use of plant growth promoting endophytic bacteria has gained attention as an ecofriendly and sustainable approach for the management of the black mold disease. The beneficial microorganisms suppress the pathogen growth through production of antifungal metabolites, siderophores, extracellular enzymes and competition for nutrient and space, while simultaneously enhancing plant growth and health. In this context, these microorganisms enhance plant growth through biological nitrogen fixation[42], indole acetic acid production, siderophore production, phosphate utilization thereby improving nutrient availability and plant health[43,44]. Therefore, the present study focuses on the isolation and characterization of endophytic bacteria and the evaluation of their growth promoting and biocontrol potential.

A total of 20 bacterial endophytes were isolated from garlic (*Allium sativum*) and screened for its antagonistic activity against black mold pathogen *Aspergillus niger* using the dual culture method. Among the isolates AS11 exhibited the highest antagonistic activity, showing 79.33% inhibition of mycelial growth with a mean inhibition of 13.00 ± 1.73 mm (Mean ± SD). The strong antifungal activity demonstrated by AS11 indicates its potential as an effective biocontrol agent against *A. niger* induced black mold disease in garlic (Figure 1).

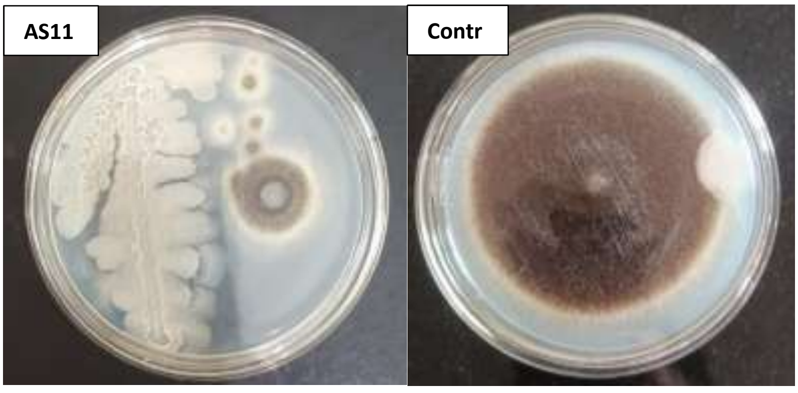


Figure 1 : Antagonistic activity of AS11 against *Aspergillus niger*

Among the screened isolates, the highly potent strain AS11 was selected for further evaluation of its biocontrol potential against *Aspergillus niger*. Molecular identification of isolate AS11 was carried out through 16S rRNA gene sequencing (Figure 2). Genomic DNA was extracted using the HiPurA™ Bacterial Genomic DNA Purification Kit (HiMedia) and successfully amplified using universal bacterial primers 27F and 1492R. The amplified PCR products were confirmed on agarose gel, purified, and subjected to Sanger sequencing using the ABI PRISM® BigDye™ Terminator Cycle Sequencing Kit (Applied Biosystems). Sequence quality was analyzed using SnapGene Viewer, and the obtained sequence was compared with reference sequences in the NCBI database using the BLASTn tool (Figure 3). The analysis revealed a high degree of similarity with *Calidifontibacillus erzurumensis*, confirming the taxonomic identity of isolate AS11. Phylogenetic analysis using the Neighbor-Joining method in MEGA version 11.0.13 further supported the identification by clustering AS11 closely with reference strains of *C. erzurumensis strain P2* (Figure 4).

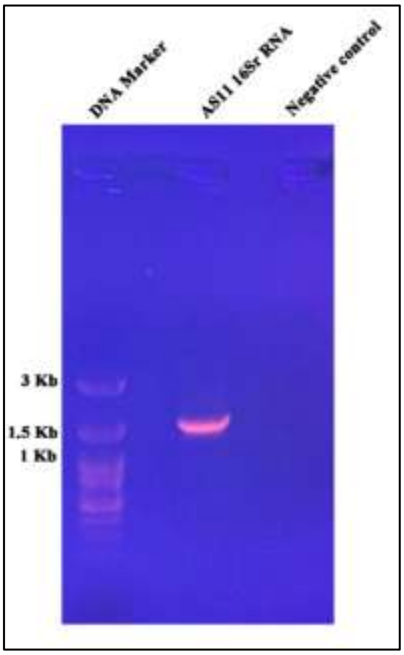
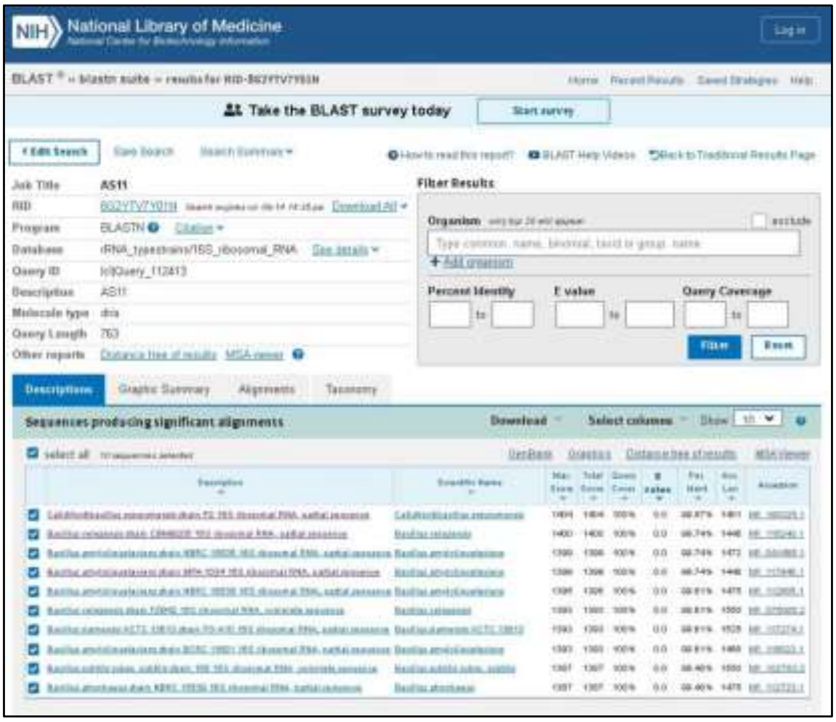


Figure 2 : PCR amplification of 16S rRNA of AS11



Job Title AS11
Program BLASTN
Database rRNA_typestrains/16S_ribosomal_RNA
Query ID lcl|Query_112413
Description AS11
Molecule type dna
Query Length 763

Sequences producing significant alignments

Figure 3 : Blast Analysis

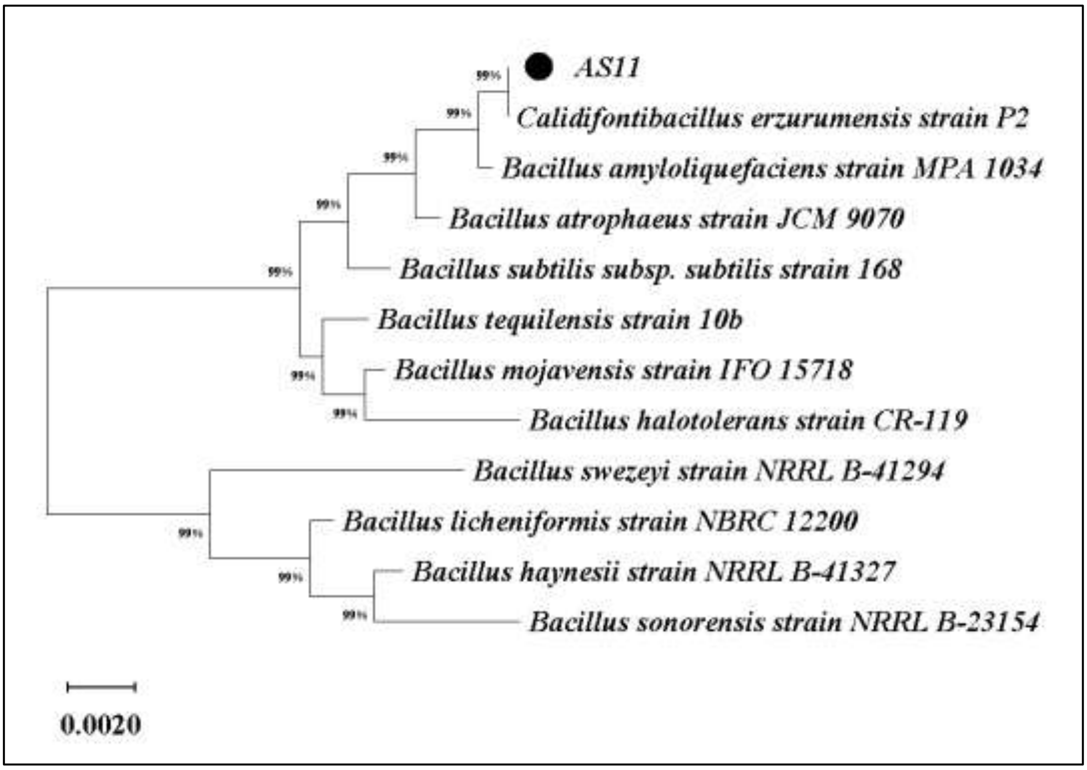


Figure 4 : Phylogenetic placement of 16S rRNA sequences of AS11

The primary screening for plant growth promoting parameters were evaluated for *Calidifontibacillus erzurumensis* AS11, which includes phytostimulant production, nutrient solubilization and plant protectants. The results revealed that *Calidifontibacillus erzurumensis* possesses a variety of plant growth promoting capabilities such as production of IAA, biological nitrogen fixation, ammonia production, siderophore production and phosphate solubilization as shown in Table 1, were +++ shows strong activity, ++ shows moderate activity. Phosphate solubilization of *C. eruzurumensis* was observed in the form of yellow zone formed around the bacterial colony on the Pikovskaya agar plate and showed phosphate solubilization index (PSI) as 2.3 after 72 hours of incubation. Plant growth promoting bacteria show numerous plant growth promoting traits under different environmental and soil circumstances [45]. Various *Bacillus* sp. have been reported to be effective plant growth promoters in solubilizing phosphate, production of indole acetic acid, siderophore production, ammonia production and biological nitrogen fixation [46,47]. As in our study *C. erzurumensis* have multiple plant growth promotion traits, it can be applied in the field to supplement nutrients and augment plant growth.

Endophytic Bacterial Isolate	**IAA production**	**Ammonia Production**	**Nitrogen Fixation**	**Siderophore Production**	**Phosphate Solubilization**
C. erzurumensis	++	+++	+++	++	+++

Table 1 : Screening of Plant Growth promotion traits of *Calidifontibacillus erzurumensis*

Abiotic stress is responsible for significant yield losses on crops, worldwide. *Calidifontibacillus erzurumensis* responded well to the abiotic stresses like osmotic resistance, salt tolerance, temperature tolerance and pH stress and results are noted in Table 2. The growth of *C. erzurumensis* was examined on nutrient agar plates at temperatures ranging from 28 to 50°C and results were seen good over the range of temperature. Plants and bacteria are found to produce various proteins that increase their tolerance to temperature and specifically *Bacillus* sp. are more tolerant due to the production of heat shock proteins and the presence of resistant and dormant endospores [48]. *C. erzurumensis* showed growth in nutrient agar plate with 0.5 to 10% (w/v) salt. No growth was observed in 15 to 20 % (w/v) salt. The growth was observed on nutrient agar plates having different pH values from 5.0 to 13.0. all the range of pH showed growth which may be observed that *C. erzurumensis* can thrive in a range of pH environments, which includes acidic, neutral and alkaline. The osmotic resistance was evaluated with the PEG (6000) at 5, 10 and 20% (w/v) concentration which is equivalent to osmotic potential levels -0.27, 0.54 and 1.09 MPa, respectively. *C. erzurumensis* was found to tolerate polyethylene glycol concentration upto 20% and stress upto -1.09 MPa and maintain high water activity level. The study shows that *C. erzurumensis* showed multiple abiotic stress tolerance, hence could be useful for field application.

Parameters/ Growth	**Parameters range**				
Temperature (°C)	**28**	**RT**	**37**	**40**	**50**
Growth	+++	+++	+++	+++	+++
Salt tolerance (% w/v)	**0.5**	**5.0**	**10.0**	**15.0**	**20.0**
Growth	+++	+++	+	-	-

pH tolerance	**5.0**	**7.0**	**9.0**	**11.0**	**13.0**
Growth	+++	+++	+++	+++	+++
Osmotic Resistance PEG (6000) % w/v	**1.0**	**5.0**	**10.0**		
Growth	+++	+++	+++		

Table 2 : Abiotic responses of *Calidifontibacillus erzurumensis*

Germination is one of the crucial phases of growth cycle in plant development. The application of *C. erzurumensis* strain AS11 resulted in measurable improvements in early growth parameters of garlic seedlings (Table 3).

Parameter	**AS11 (Mean ± SE)**	**Control (Mean ± SE)**	**% Change**	**Significance**
Root Length (cm)	1.70 ± 0.11	1.32 ± 0.60	+28.8%	NS
Shoot Length (cm)	4.42 ± 0.20	2.69 ± 0.53	+64.3%	*
Sprouting (%)	86.7	83.3	+4.1%	—
SVI	530.60	334.03	+58.9%	—

Table 3 : Effect of *C. erzurumensis* on early growth parameters of garlic

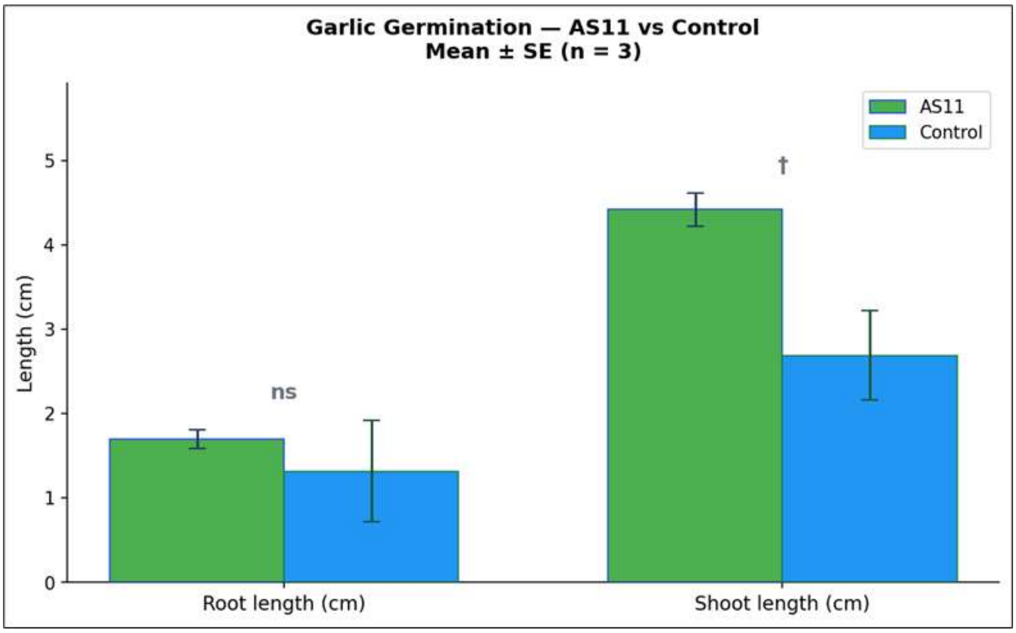


Figure 5 : Effect of *C. erzurumensis* AS11 on root and shoot growth during garlic germination (Mean ± SE, n = 3)

Shoot length increased significantly from 2.69 ± 0.53 cm (control) to 4.42 ± 0.20 cm (AS11), representing a 64.3% increase ($p < 0.05$). Root length increased from 1.32 ± 0.60 cm to 1.70 ± 0.11 cm, corresponding to a 28.8% increase, although this difference was not statistically significant ($p \geq 0.05$). Sprouting percentage showed a slight increase from 83.3% to 86.7%. The Seedling Vigour Index (SVI) increased substantially from 334.03 (control) to 530.60 (AS11), indicating an overall improvement of 58.9%. The significant increase in shoot length suggests that *C. erzurumensis* AS11 enhances early vegetative growth, likely through improved physiological activity during germination [49]. The relatively low standard error observed in treated samples indicates consistent performance across replicates. Although root length showed a positive increase, statistical significance was not achieved. This may be attributed to the high variability observed in the control group (SE = 0.60) and the limited sample size (n = 3), which reduces the statistical power of the test. The marked improvement in Seedling Vigour Index reflects the combined effect of enhanced shoot growth and stable germination percentage. Since SVI integrates both growth and germination parameters, it provides a more comprehensive indicator of early plant performance. The observed increase confirms the positive role of *C. erzurumensis* in improving early seedling establishment (Figure 5).

In the pot experiment on garlic cloves showed significant differences were seen between treatments for both shoot and root growth (Table 4). The AS11 treatment (T1) showed the highest shoot length (17.01 ± 0.69 cm) and was in group "a", similar to the control (13.28 ± 0.50 cm). The treatment with *A. niger* (T2) showed the lowest shoot length (5.60 ± 2.99 cm) and was in group "c". This confirms strong growth reduction due to the pathogen. The co-inoculation group with AS11 and *A. niger* (T3) showed moderate growth (11.11 ± 4.86 cm) and was placed in group "bc". This indicates partial recovery from pathogen stress. The fungicide treatment with *A. niger* (T4) also improved shoot length (10.31 ± 0.14 cm) and was in group "ab", showing effective recovery. And considering the root length of the treatments, AS11 alone showed the highest root length (2.92 ± 0.17 cm) and was in group "a", higher than all other treatments. The control group (T5) showed normal root growth (2.67 ± 0.06 cm) and was in group “ab”. The co-inoculation group (AS11 + *A. niger*) showed moderate root growth (2.31 ± 0.06 cm) and was in group “bc”, indicating some protection. The *A. niger* group (2.23 ± 0.04 cm) and fungicide group (2.18 ± 0.1 cm) had the lowest root values and were both in group “c”, showing strong root growth reduction (Figure 6).

Treatment	**Shoot Length (cm)**	**Group**	**Root Length (cm)**	**Group**
AS11 (T1)	17.01 ± 0.69	a	2.92 ± 0.17	a
A. niger (T2)	5.60 ± 2.99	c	2.23 ± 0.04	c
AS11 + *A. niger* (T3)	11.09± 0.86	bc	2.31 ± 0.06	bc
A. niger + Fungicide (T4)	10.31 ± 0.14	ab	2.18 ± 0.1	c
Control (T5)	13.28 ± 0.50	a	2.67 ± 0.06	ab

Table 4 : Growth performance under different treatments

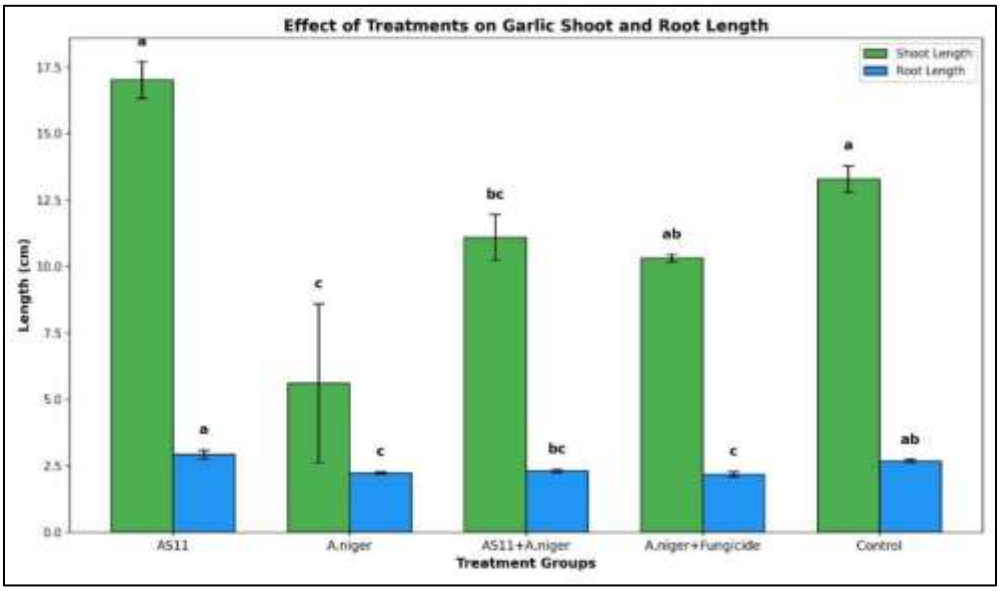


Figure 6 : Growth response of garlic under different treatments in pot experiment. Bars sharing the same letter are not significantly different (Tukey HSD, p < 0.05)

The *Aspergillus niger* treatment caused a strong reduction in plant growth. This shows that the fungus has a harmful effect on garlic. The large variation in this group means that plants responded differently under stress. The *Calidifontibacillus erzurumensis* AS11 treatment alone gave the highest shoot and root growth. This confirms that *Calidifontibacillus erzurumensis* AS11 is a strong plant growth-promoting bacterium under normal conditions. In the co-inoculation treatment (AS11 + *A. niger*), plant growth improved compared to the pathogen-only group. This shows that *Calidifontibacillus erzurumensis* can protect the plant from the fungus. However, the growth did not reach the same level as *Calidifontibacillus erzurumensis* alone, so the protection is partial. The fungicide treatment also improved plant growth compared to the pathogen-only group. However, *Calidifontibacillus erzurumensis* alone showed better root growth than the fungicide. The combined treatment showed similar shoot recovery.

CONCLUSION

The present study demonstrated that the endophytic bacterial isolate AS11, identified as *Calidifontibacillus erzurumensis*, possesses significant plant growth-promoting and biocontrol potential against Aspergillus niger causing black mold disease in garlic. The isolate exhibited strong antagonistic activity against the pathogen along with multiple plant growth-promoting traits including IAA production, nitrogen fixation, phosphate solubilization, siderophore production, and abiotic stress tolerance. Seed germination and pot experiment studies further confirmed its positive effect on garlic growth and its ability to reduce pathogen-induced growth suppression. These findings suggest that *C. erzurumensis* AS11 could serve as a promising eco-friendly bioinoculant for sustainable garlic cultivation and biological management of black mold disease.

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