

UNVEILING THE ALLELOPATHIC POTENTIAL OF AN ENDOPHYTIC BACTERIA FROM *PERSICARIA ORIENTALIS* (L.) SPACH

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Abstract

This study aimed to identify and evaluate the allelopathic potential of a bacterial isolate from *Persicaria orientalis* (L.) Spach. *P. orientalis* (Oriental Pepper) is known for its rapid growth and tolerance to various habitats, particularly near waste wetlands. Twenty morphologically distinct bacterial colonies were initially isolated, from which isolate E/29 was selected for comprehensive analysis. Based on colony morphology, Gram staining, and biochemical profiling, E/29 was preliminarily identified as *Tatumella pyseos*. Molecular characterisation via 16S rRNA gene sequencing, however, revealed its identity as *Stenotrophomonas maltophilia*. Culture filtrates from *S. maltophilia* markedly improved seed germination. 100% seed germination observed in case of mung bean, 70% in rice, and 60% in case of field mustard compared to untreated controls. Additionally, the isolate significantly stimulated radicle and hypocotyl elongation across all three plant materials with the most pronounced effects in mung lentil (52.5 mm and 88.5 mm), followed by rice (~11 mm and 7.4 mm) and mustard (6.3 mm and 8.2 mm), demonstrating its potential as a bioinoculant for better agriculture.

Introduction

Plants can form associations with members of their ecosystem to thrive in their natural environments. Microorganisms are one of the most important organisms that can develop better relationships with plants (Santoyo *et al.* 2016). Endophytes were originally described in 1866 by de Bary, who observed and outlined possible relationships between plant pathogens (Gouda *et al.* 2016). Such microorganisms can be found from roots of aerial plant structures, surviving a portion of or all of their life cycle within their host plants without causing apparent damage or disease. Endophytic microorganisms generally invade plant tissues through the vascular system, apoplasts, and outer cell layers, where the pathways of penetration might be wounds or emergency areas of lateral roots and rootlets in germination (Liu *et al.* 2017). Furthermore, endophytes constitute an important source of a plethora of natural compounds (alkaloids, phenolic acids, quinones, steroids, tannins, and terpenoids), which play a vital role in plant resistance to phytopathogenic infections (Fadji and Babalola 2020, Morelli *et al.* 2020).

Proteobacteria, especially the γ -Proteobacteria class, are the most prevalent and varied group of endophytic bacterial communities found in a variety of plant species (Miliute *et al.* 2015, Santoyo *et al.* 2016). While Acidobacteria, Planctomycetes, and Verrucomicrobia are less frequently recorded (Reinhold-Hurek and Hurek 2011), other bacterial phyla that are commonly discovered include Actinobacteria, Bacteroidetes, and Firmicutes (Santoyo *et al.* 2016). Depending on the type of plant host, these microbial communities frequently have different compositions. The two most common bacterial genera are *Bacillus* and *Pseudomonas*, followed by *Burkholderia*, *Microbacterium*, *Micrococcus*, *Pantoea*, and *Stenotrophomonas* (Reinhold-Hurek and Hurek 2011, Chaturvedi *et al.* 2016).

Plant-associated microorganisms are known to be rich in secondary metabolites and unique phytochemicals, making them ideal biofertilizers and natural growth promoters. These microbial

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products provide sustainable alternatives to synthetic agrochemicals (Omomowo and Babalola 2019). Furthermore, allelopathy, the process by which plants or microbes release biomolecules (allelochemicals) into the environment, has the potential to influence neighbouring organisms (Mallik 1998). These substances can either impede or stimulate seed germination, plant growth and development, and the survival of other microbes, resulting in complicated ecological interactions.

Persicaria orientalis (L.) Spach (Oriental Pepper) from Polygonaceae family is a popular medicinal herb with a rich history in traditional medicine. Various plant parts—stems, leaves, roots, fruits, and flowers—have different pharmacological effects. Research on the methanol extract of *P. orientalis* leaves (Po-MeOH) found significant anti-inflammatory and anti-diarrheal activity, moderate thrombolytic effects, and low cytotoxicity, indicating its promise as a phytotherapeutic agent (Ansari *et al.* 2017). *P. orientalis* is a prevalent weed in Bangladesh, growing in damp and desolate places. Given its pharmacological versatility and capacity to thrive in contaminated soils, it is thought that its endophytes may have unique plant growth-promoting characteristics and produce bioactive metabolites. As a result, the primary goal of this research is to isolate and characterize a bacterial endophyte from *P. orientalis* (L.) Spach and to evaluate its allelopathic potential.

Materials and Methods

Roots of *P. orientalis* plants were collected from the Hazaribagh area in Dhaka, Bangladesh (Fig. 1). A total of 20 samples were collected from five distinct locations. At each site, samples were aseptically collected using sterile tools and placed into sterile polythene bags. All samples were labeled with relevant details such as sampling location, date, and sample type. After collection, the samples were promptly transported to the laboratory to prevent contamination or degradation.

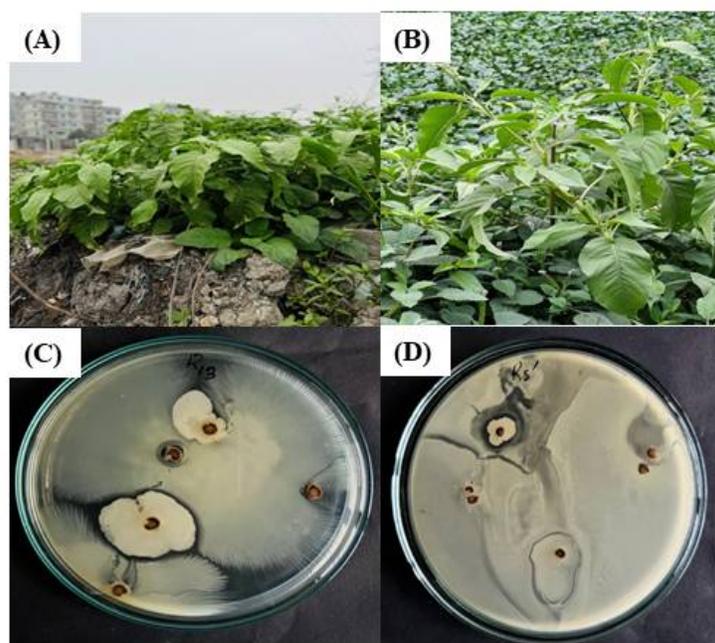


Fig. 1(A-B). Sample plant *Persicaria orientalis* (L.) Spach (C-D) Isolation from roots.

For the enumeration and isolation of aerobic heterotrophic bacteria from root samples, Nutrient Agar (NA) and Luria-Bertani Agar (LBA), each supplemented with 1% glucose, were used as growth media. The pH of both media was adjusted to 7.0 ± 0.2 prior to the addition of agar and sterilization to optimize bacterial growth. Bacterial enumeration and isolation were carried out using the technique as described by Greenberg *et al.* (1985).

Morphological, cultural, and biochemical characterization of the bacterial isolates was conducted following standard microbiological protocols outlined in laboratory manuals (Sneath 1986, Krieg and Holt 1984, Collins and Lyne 1976, APHA 1998).

Out of the 20 bacterial isolates obtained, one isolate, E/29, was selected for evaluating its allelopathic potential based on its consistent growth characteristics and pronounced activity observed during preliminary screening. Seeds of rice (*Oryza sativa* L.), mustard (*Brassica rapa* L.), and mung bean (*Vigna radiata* L.) were used as plant materials for the bioassay. Broth culture of the selected bacterial isolate was prepared using the nutrient broth medium according to the method described by Mallik (1998) and incubated at 37°C for three days. Following incubation, the cultures were vacuum filtered through 0.45 µm membrane filters to obtain cell-free filtrates. These filtrates were used to moisten sterile filter paper placed inside Petri dishes, onto which seeds of the test plants were evenly scattered. Control treatments were prepared with sterile nutrient broth medium without any bacterial inoculation. All plates were incubated in a POL-EKO APARATURA incubator at 27-30°C in the dark and humid conditions for four days. The incubation temperature of 27-30°C was selected as it falls within the optimal germination range for rice, mustard, and mung bean, allowing uniform seedling growth and reliable assessment of allelopathic effects. After the incubation period, the lengths of radicles and hypocotyls were measured, and seed germination percentages were calculated to assess the allelopathic effects of the bacterial isolates.

To identify the selected bacterial isolates through sequence comparison, partial amplification of the 16S rRNA gene was performed using the primer pairs 27F (AGAGTTTGAT CCTGGCTCAG) and 1492R (GGTTACCTTGTTACGACTT), as described by de Lillo *et al.* (2006). PCR was carried out in an oil-free thermal cycler (Applied Biosystems Veriti 96-well) under the following cycling conditions: initial denaturation at 95°C for 5 min; 30 cycles of denaturation at 94°C for 1 min, annealing at 55°C for 30 secs, and extension at 72°C for 1 min; followed by a final extension at 72°C for 5 min. After amplification, the PCR products were held at 4°C. Electrophoretic separation of amplified products was carried out on 1% agarose gels prepared with 1× TAE buffer (1.0 g agarose in 100 ml). Electrophoresis was performed at 100 volts for 20 min, using a 1 kb DNA ladder as a molecular weight marker. DNA bands were visualized under a UV transilluminator and photographed using a gel documentation system (MS Major Science UVDI, Taiwan).

Results and Discussion

Allelopathy is a widespread biological phenomenon where organisms, particularly plants, release biochemicals (allelochemicals) that influence the development, growth, survival, or reproduction of other species, with effects ranging from beneficial to harmful (Cheng and Cheng 2015). This process is key in plant-plant interactions and has important applications in weed control, crop protection and production, and re-establishment (Bhowmick *et al.* 2024). Plants also host diverse microbial communities, notably endophytes—microorganisms that reside within plant tissues without causing harm (Kobayashi and Palumbo 2000). These endophytes are adapted to the host's chemical environment and can contribute to specific biosynthetic pathways, making them an untapped source of natural bioactive compounds (Strobel 2003, Rosenblueth and Martinez-Romero 2006). They are known to promote plant growth via phytohormone production, nutrient

cycling, disease resistance, and phytoremediation (Cho 2007, Lodewyckx *et al.* 2002, Chi *et al.* 2005), and they may exert allelopathic effects on surrounding plant species (Rosenblueth and Martinez-Romero 2006, Cipollini *et al.* 2012).

In this study, endophytic bacteria were isolated from *Persicaria orientalis* (L.) Spach to assess their potential allelopathic and biostimulant activities. Among 20 morphologically distinct isolates, E/29 was selected for detailed characterization. The bacterial isolate E29 was initially classified as *Tatumella pyseos* based on colony morphology and conventional biochemical tests. Biochemical characterization revealed that the isolate fermented D-glucose with acid production but no gas, tested negative for MR, indole, urease, motility, citrate utilization, and propionate hydrolysis and was positive for VP, catalase, oxidase and casein hydrolysis (Table 1). Genomic DNA from the isolate was subjected to PCR amplification, and the products were separated by electrophoresis on a 1.0% agarose gel. An amplified DNA band of approximately 600 bp was observed, confirming successful amplification of the target region (Fig. 2). Molecular identification using 16S rRNA gene sequencing confirmed the isolate as *Stenotrophomonas maltophilia* strain DRLB3 (GenBank accession KU550150.1), showing 100% sequence identity, 98% query coverage, a maximum score of 1851, and an E-value of 0.0 (Table 2). *Stenotrophomonas maltophilia*—a gram-negative, motile, non-fermentative bacterium found in soil, water, and plant hosts, has garnered attention for its biofertilizer and biocontrol properties in various crop systems (Kumar *et al.* 2023).

Table 1. Selected biochemical test results of the isolate *Stenotrophomonas maltophilia* (E/29).

D-Glucose		MIU		MR	VP	Hydrolysis		Oxidase	Catalase	Citrate	Propionate
Acid	Gas	Motility	Indole	Urease		Casein	Starch				
+	-	-	-	-	-	+	+	-	+	+	-

“+” sign indicates positive activity, “-” sign indicates negative activity, MR=methyl red, VP=, Voges-Proskauer.

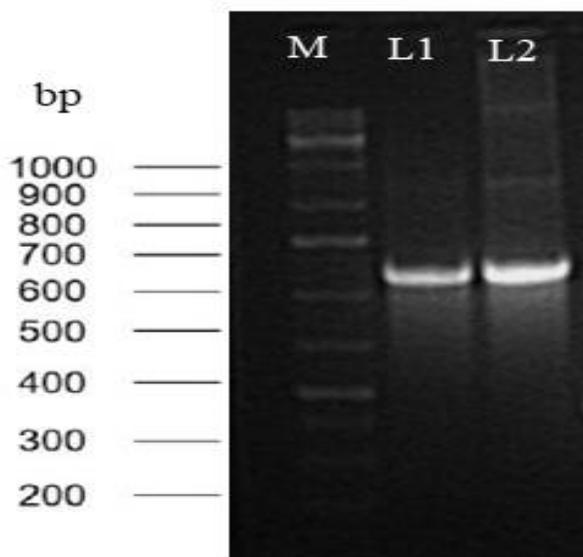


Fig. 2. PCR amplification of the 16S rRNA gene (partial sequence). Lane M: 100 bp DNA ladder (OriGene Technologies); lanes 1-2: PCR products from isolate E29; lanes 3-4. The amplified DNA fragments are approximately 600 bp in size.

Table 2. Conventional and molecular identification of the isolates *Stenotrophomonas maltophilia* (E/29).

Isolate Name	Conventional identification	Molecular identification						
		Scientific name	Strain	Identity match (%)	Max. coverage score	E-value	Query Cover	Accession
E29	<i>Tatumella ptyseos</i>	<i>Stenotrophomonas maltophilia</i>	DRLB3	100	1851	0.0	98%	KU550150.1

Culture filtrates of E/29 significantly enhanced seed germination and early seedling development in mung lentil, rice, and mustard (Fig. 3). In mung bean, germination rates rose from 60% in the control to 100%, with rice showing an approximate 70% increase, and mustard displaying a modest but consistent improvement (Fig. 4). These stimulatory effects suggest the production of bioactive metabolites by E/29 that enhance seed viability across multiple species.

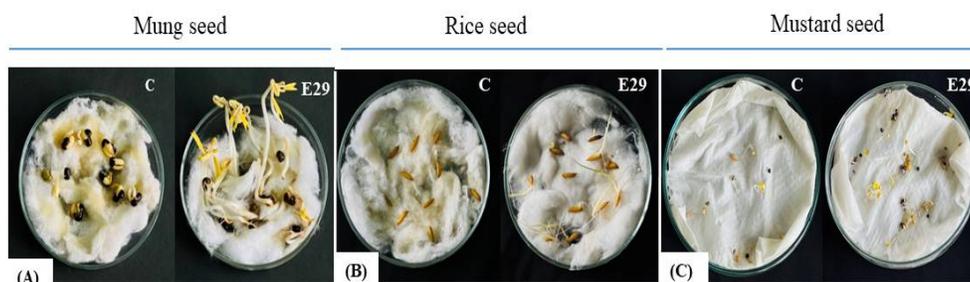


Fig. 3. Impact of culture filtrates from selected isolates on mung (A), rice (B), and mustard (C) seed germination.

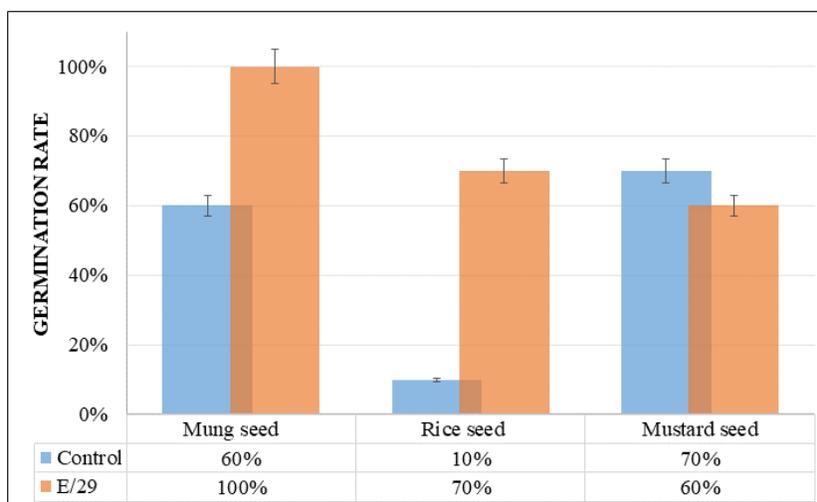


Fig. 4. Impact of culture filtrates from selected isolate on germination rate of mung, rice and mustard seeds.

Radicle growth was particularly pronounced in mung lentil, reaching 52.5 mm compared to 0.5 mm in the control, indicating strong root-promoting activity. Rice and mustard also showed significant increases in radicle length—~11 mm and 6.3 mm, respectively (Figs 5A-C and 6A). Hypocotyl development followed a similar trend: mung lentil seedlings treated with E/29 reached

88.5 mm (vs. 6 mm in controls), while rice and mustard recorded moderate increases of 7.4 mm and 8.2 mm, respectively (Figs 5A–C and 6B). These results confirm the broad-spectrum allelopathic and biostimulant properties of *S. maltophilia* E/29, especially in mung beans.

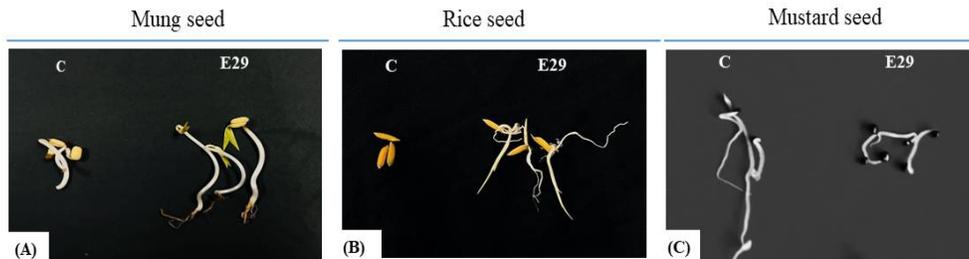


Fig. 5. Impact of culture filtrates from selected isolates on (A) mung, (B) rice, (C) mustard seed radicle and hypocotyl growth.

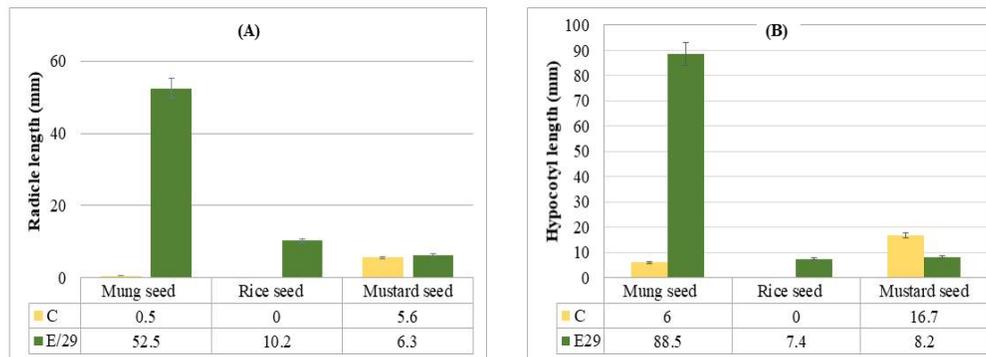


Fig. 6. The effect of different culture filtration of selected isolates on the growth and development of mung, rice, and mustard seeds (A) Impact on Radicle length (mm) and (B) Hypocotyl length (mm).

The observed effects of E/29 align closely with previous reports on *S. maltophilia* strains. For example, strains SBP-9 and BJ01 improved wheat and peanut growth under saline and nitrogen-deficient conditions via mechanisms such as phosphate solubilisation, ACC-deaminase activity, and indole-3-acetic acid (IAA) production (Singh and Jha 2017, Alexander *et al.* 2019, 2020). Similarly, endophytic *S. maltophilia* R551-3 promoted root colonisation and pathogen defence in *Brassica napus* through DSF-mediated quorum sensing (Alavi *et al.* 2013).

Under biotic stress, *S. maltophilia* strains BCM and BCM_F significantly restored wheat seed germination rates suppressed by *Rhizoctonia solani* and *Fusarium oxysporum*—from <10% in untreated stressed seeds to >45% in BCM-treated ones, also enhancing alpha-amylase activity (Sharma *et al.* 2025). This supports the hypothesis that *S. maltophilia* mediates plant growth and defence both directly and indirectly. Additionally, *S. maltophilia* strains have shown antagonistic activity against multiple fungal pathogens, including *F. oxysporum*, *Epicoccum nigrum*, *Beauveria bassiana*, *Aspergillus flavus*, and *A. candidus* (Romanenko *et al.* 2008).

Genomic analyses of *S. maltophilia* strain BCM_F revealed functional genes encoding hydrolytic enzymes (proteases, chitinases), IAA biosynthesis pathways, phosphate solubilisation mechanisms (PhoR, PhoB, PstABCD, alkaline phosphatase), and phenazine biosynthesis. Genes

like ExbB and SbnDEF support siderophore production, while nifC, NarK, and NarL aid nitrogen fixation (Sharma *et al.* 2025). These genetic traits likely underpin the strong allelopathic and growth-promoting traits observed in isolate E/29.

The isolate E/29 (*S. maltophilia*) exhibited significant allelopathic and biostimulant activity, enhancing seed germination and early seedling growth in multiple crops, particularly mung lentils. Its effects are comparable to or stronger than those reported in previous *S. maltophilia* strains, and are likely mediated by a combination of phytohormone production, nutrient mobilisation, and stress mitigation. These findings highlight the potential of endophytic bacteria like E/29 as eco-friendly bioinoculant and natural allelopathic agents, contributing to better crop production and early plant establishment.

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