Harnessing the Potentialities of Arsenic Resistant Fungal Endophytes for Improved Growth Promoting Traits under *in vitro* condition

Abstract

Endophytic fungi enhance the plant's ability to tolerate stressful conditions including heavy metal stress *via* secretion of numerous secondary metabolites. However, the role of heavy metal resistant fungal endophytes in growth promotion of plants in extreme environments is need to be understood. Therefore, eight endophytic fungal isolates having arsenic tolerance potential up to 2000 ppm explored from the arsenic stressed plants were subjected to various *in vitro* growth promoting traits *viz*., phytohormones production, phosphate solubilization, siderophore production, ammonia production, HCN production and ACC deaminase activity under normal and arsenic stress (100 ppm) condition. Indole acetic acid produced by fungal endophytes ranged between 120-610 µg/ mL, which was reduced to 50-340 µg/ mL when they were grown on arsenic induced medium. In the siderophore production test, fungal isolate S3P1S1 produced significantly higher siderophore (96 ± 0.002 µmol) compared to other isolates and reference culture. In addition, HCN production was observed in only one isolate. Therefore, present study clearly identified specific traits in the fungal endophytes, which make them good candidates as PGPR and might contribute to plant adaption to arsenic contaminated soils. These fungal endophytes, possessing metal tolerance as well growth promoting properties under *in vitro* conditions could have vital implications for the agricultural sector if used as biofertilizer.

Keywords: Endophytic fungi. PGP traits, arsenic

Introduction

Plants exhibit an intrinsic relationship with a broad range of microbial populations that colonize the rhizosphere (rhizobacteria), the phyllosphere (epiphytic bacteria) and plant tissue (endophytes) (Qin *et al*., 2011). Species of fungi that reside within living plant tissue without causing symptoms of disease in their host are known as fungal endophytes. They are the major members of endophytic population that dwell entirely within plant tissues(Ripa*et al.*, 2019). Every plant harbor at least one or more endophytic fungi in the universe. Host plants without endophyte-fungal association are devastated by the waves of extreme

temperature, drought, salinity and pathogen attack. Therefore, endophytic fungal communities can be exploited as bio-fertilizers or bio-agents to create a sustainable crop production system because fungal endophytes exhibited tolerance to abiotic stresses including heavy metal stress (Ripa*et al.*, 2019). The metal tolerance in endophytic fungi might be attributed to cell-wall binding, the extracellular precipitation, efflux of metals ions from cell, intracellular chelation, cellular compartmentalization and antioxidant systems (Jacob *et al*., 2017). Khan *et al*. (2015) assessed fungal endophyte, *Alternaria alternata* to evaluate its role in host survival and stress mitigation of *Solanum nigrum* L. and observed better growth attributes such as shoot, root length, dry biomass, chlorophyll contents and leaf area under cadmium stress as compared to controls and decreased cadmium uptake in plant. Thus, it is evident that heavy metal tolerant fungal endophytes can help in promoting plant growth and tolerance in the presence of metal stress.

This plant growth promoting characteristic of endophytic fungi is generally attributed to the production of plant growth regulators and protectants in stressful environments which include the production of plant growth promoting phyto-hormones like indole-3-acetic acid (Sukumar *et al*., 2013), gibberellins (Leitao and Enguita, 2016), auxins (Waqas *et al*., 2012) and cytokinins along with the fact that endophytes also help the plant in nutrient absorption (Shahabivand*et al*., 2012).Literature survey additionally demonstrated that plant growth promoting fungi (PGPF) maintain plant growth through the generation of a number of significant enzymes like ACCD, urease, catalase, etc. phosphate solubilization, siderophore production, antagonism to phytopathogens and take a crucial part in plant growth (Glick *et al*., 2014). Therefore, these endophytes, with their secreted plant growth regulating compounds, are of great potential interest to enhance crop yield and quality. These compounds can influence plant development as well as rescue plant growth in a stressful environment. With all these comprehensive information, it was assumed that the fungal endophytes, living in the plant host are able to influence the plant growth and fitness parameters of same and/or other plants through their promotion effects. Therefore, the present study was intended to characterize *in vitro* plant growth promoting attributes of arsenic-metal resistant fungal endophytes for improving stress tolerance and growth promoting traits under metal stress conditions.

Materials and method

Preparation of fungal inoculum

Endophytic fungal isolates *viz*., S1P1R1, S3P1S1, S3P1S3, S4P2L2, S5P1S1, S6P1S3,

S8P1L4 and S10P1L1 used in the current experiment were isolated previously from the arsenic contaminated sites of Karnataka and screened for their arsenic tolerance potential *in vitro* (Abhinandana *et al*., 2023).Reference strain, *Piriformospora indica* was collected from the Popularization of buofertilizer scheme, Department of Agricultural microbiology, University of Agricultural Sciences, Bengaluru. Inoculum of all the endophytic fungal isolates (1 mL of freshly prepared spore suspension having 10^6 -10^7 spores/mL) was prepared from ten days old pure fungal culture. Fungal endophytes were cultured on PDA supplemented with 100 µg/ mL ampicillin and grown at 30 ^oC.After 15 days of growth, mycelia and spores were harvested from plates by adding 10 mL of sterile water with 20 µL of Tween 20 and gently scaping off fungal culture using sterile hairbrush. The suspension was filtered through four layers of sterile cotton cheese cloth gauze. The spore load in the suspension was estimated (10^6 spores mL^{-1}) using haemocytometer.

Determination of phytohormones in fungal culture filtrate

Fungal inoculum (1 ml of freshly prepared spore suspension having 10^6-10^7 spores/ml)of each fungal culture was inoculated in each 100 mL of PDB and 100 PPM arsenic amended PDB and incubated at 30 ^{o}C for 14 days. Uninoculated medium was used as control. For IAA quantification, the broth was supplemented with 5 mg/ mL of tryptophan. After 14 days, the culture filtrate of each flask was passed through Whatman No. 42 filter paper. The pH of filtrate was adjusted to 2.5 by adding 0.1 N HCl or KOH. Extraction was done as per the method described by Rachev*et al.* (1993). The culture filtrate was extracted three times using ethyl acetate in separating funnel. The organic layer was separated and passed through anhydrous sodium sulphate. The solvent was evaporated in a rotary vacuum evaporator at 40 ^{o}C and 10 rpm (Hasan *et al.*, 2002). The residue was dissolved in HPLC-grade methanol. The HPLC analyses was carried out on a Shimadzu instrument (Prominence–I, LC-2030C) equipped with a UV detector (LC-2030 UV detector) and fitted with a C_{18} reverse phase HPLC column (Shim-pack GIST C_{18}, Dimension 250 X 4.6 mm, particle size 5 µm). The column temperature, 30^{o}C was maintained for all the samples with other specific conditions (Hasan *et al.*, 2002) described in table.

List 1 : Details of HPLC conditions

Phytohormone	**Solvent**	**Wavelength (nm)**	**Flow rate (mL/min)**

IAA	Methanol: water (80:20)	270	1.0
GA	Methanol: water (70:30)	208	0.8
SA	Acetonitrile: Acetic acid 0.5 % (90:10)	302	1.0
ABA	Acetonitrile: Acetic acid 0.5 % (80:20)	254	0.8

Phosphate solubilization activity of fungal endophytes

The phosphate solubilizing potential of fungal endophytes was evaluated *in- vitro* as described by Doilom*et al.* (2020). Fungal inoculum was inoculated on the plates of Pikovskaya medium and incubated at 30 ^{o}C for 7 days. The diameter (mm) of clear zones around fungal plugs was measured and phosphorus solubilizing index was calculated.

The complex phosphate, $Ca_3(PO_4)_2$ solubilization activity of isolates was examined. About 1 ml of freshly prepared spore suspension having 10^6–10^7 spores/ml was inoculated in 100 ml PKV broth medium in conical flasks. The flasks were inoculated at 27 ± 2 ºC in the rotatory shaker at 130 rpm for 10 days. Five milliliters of cultured aliquot was centrifuged at 10,000 rpm for 10 minutes. Then, 1 ml of the supernatant of each was transferred to 50 ml volumetric flask. This was followed by a 5 ml sodium bicarbonate solution and 10 ml distilled water. After that one drop of a p-nitrophenol indicator was added and pH of solution was adjusted 5.0 by adding 2.5 M Sulphuric acid. Then, 8 ml of Murphy–Riley reagent was added and made the volume up to 50 ml with deionized water. After incubation for 15 minutes, the intensity of blue color on UV Spectrophotometer at 730 nm was measured. The phosphate solubilization was estimated from the standard curve of KH_2PO_4 against 730 nm with UV spectrophotometer.

Potassium solubilization

The ability of potassium solubilization by fungal endophytes was determined by growing on Aleksandrow agar medium (Hu *et al.*, 2006). The discs (5 mm) of fungal mycelia were transferred on plates containing Aleksandrow medium and incubated at 30 ^{o}C for 7 days. After incubation, the clear zones formed around the colonies were recorded.

Siderophore production

The siderophore production was assessed by placing 5 mm freshly grown mycelial discs on Chrome Azurol S (CAS) solid medium (Schwyn and Neilands, 1987). The fungi-inoculated plates were incubated at 30 ^{o}C for 7 days and diameter of the colony and orange colour zone surrounding the colony was measured. Siderophore producing index (SPI) was calculated as the ratio of (colored zone + colony)/ colony diameters (Desai *et al.*, 2012).

Iron deferrated Grimm-Allen liquid media was used for growing fungi (10^6 spores/mL, ^{o}C for 15 d) and culture filtrate was used to estimate siderophore content. The filtrate (1.5 mL) added with 1.5 mL of CAS liquid solution, 10 µL of shuttle solution (0.2 M 5-sulfosalicylic acid, store in dark) was incubated at room temperature for 10 min and absorbance measured at 630 nm. The siderophore content was calculated using the following formula:

$$\% \text{ Siderophore units} = [(Ar - As)/Ar]$$

Where, Ar = Reference absorbance at 630 nm

As = Absorbance of sample at 630 nm

Qualitative analysis of ACC deaminase activity

To determine the ACC deaminase activity, spore suspension of endophyte (20 µL) was added to 10 mL synthetic medium (SM) for 48 h at culture conditions mentioned above. Mycelia that grew out of the spores were then collected, washed and incubated in 5 mL of SM containing 0.3–3 mM. After incubation of seven days, the fungal culture was re-suspended and homogenized in half volume of 0.1 M tris buffer (pH 8.5). Finally spot inoculated on DF minimal salts medium (with and without sodium arsenite as arsenic inducer) with 3 mM ACC as sole nitrogen source (Singh and Jha,2015). Fungal growth was monitored daily and those that grew in ACCsupplementedDFmediumwereconsideredputativeACCdegrading fungi.

HCN production test

The hydrogen cyanide production test was performed using a modified protocol of Miller and Higgins (1970). HCN medium was prepared using 0.3% Picric acid solution along with 1.5% sodium carbonate. To the solution, sterilized strips of Whatman filter paper No. 1 (China) were soaked and dried in a sterile environment. Fungal cultures were inoculated onto PDA plates and the treated filter paper strips were placed on the petriplateslid

simultaneously closing the lid tightly by wrapping with parafilm. The plates were incubated for 7–14 days. The rate of HCN production was determined by the color changes in the filter paper strips, from the original yellow color to brown or reddish brown. Scoring was done as weak (yellow to light red), moderate (brown) and strong (reddish brown.

Result and discussion

The global concern for the development of more sustainable agriculture has increased in recent years, and research has been performed to decipher ecology and explore the potential of endophytic interactions in plant growth. The plant endosphere is a complex micro-ecosystem where different niches can be occupied by different types of microorganisms representing rich and genuine sources of novel bioactive metabolites. To date, many studies point to the positive aspects of endophytic colonization (Miller *et al*., 2012). However, there are only a few reports that describe fungal colonization and their potential role in plant growth promotion in metal stressed condition. Therefore, in the present experiment plant growth promoting attributes of fungal endophytes were evaluated under *in vitro* arsenic stress condition.

Evaluating phytohormones production by arsenic resistant fungal endophytes under arsenic stress and non stress condition

Phytohormones are essential for plant growth and development, as well as plant interactions with microorganisms. Microbes also have the ability to biosynthesize phytohormones as secondary metabolites. Indole acetic acid is the most prevalent kind of auxin that influences many aspects of plant growth and development. Indole acetic acid (IAA) produced by fungal endophytes ranged between 120-610 µg/ mL, which was reduced to 50-340 µg/ mL when they were grown in arsenic induced medium(Table 1). Isolate S1P1R1 (0.55µg/ mL, 0.34 µg/ mL) showed highest production of IAA under stress and normal condition, respectively. Isolate S3P1S1 showed highest IAA production under non stressed condition (0.61µg/ mL). However compared to control, IAA production was maximum in all the isolates.Musa*et al.* (2023) observed that IAA level was increased in *P. lilacinus*-inoculated *S. lycopersicum*plants up to 40.20 µg/mL. Similarly, Suebrasri*et al.* (2020) reported increased growth of Sunchoke when inoculated with auxin producing fungal endophytes (*Macrophominaphaseolina* BUP2/ 3 and *Diaporthephaseolorum*, *Trichoderma koningii* ST-KU1, *Trichoderma erinaceum* ST-KU2, *Macrophominaphaseolina* SS1L10 and *Macrophominaphaseolina* SS1R10). Moreover,

the use of phytohormone (IAA and GA)-producing fungi for remediation of heavy metals and enhancing plant resistance to heavy metal toxicity has also been extensively reviewed by Deng and Cao (2017); these effects were found to be exerted by the proliferation of roots for exudation of various metabolites to detoxify and chelate heavy metal contaminants to restrict their uptake by soybean.

Fungal endophytes also produced GA in significant quantity where isolates S4P2L2 (150 µg/ mL and 310 µg/ mL) showed highest GA production under normal and stress condition respectively. Exogenous production of IAA and GA both under stress and normal conditions suggested that these fungal endophytes are capable of inducing growth promotion in host plant. This finding is similar with the study conducted by Khan *et al.* (2014) who reported that secretion of phytohormones by the fungal endophytes *Paecilomycesformosus* LHL10 promoted the host plant growth and alleviated adverse effects of salt stress. Salicylic acid (SA) produced by fungal endophytes ranged between 50 µg/ mL to 250 µg/ mL under normal condition and 70 µg/ mL to 220 µg/ mL under stress condition. Salicylic acid (SA) is a stress hormone which functions critically in CO_2 assimilation, antioxidation, stomatal regulation, and photosynthesis. Fungal endophytes also produced ABA in a significant amount where reference strain showed highest production of ABA under both stress and normal conditions (30 µg/ mL and 5 µg/ mL respectively) followed by isolate S1P1R1 (7 µg/ mL and 6 µg/ mL respectively). ABA acts as a signaling mediator in plants to regulate plants response to environmental stresses (Sah *et al.*, 2016). Moreover, endophytes that produce growth-promoting hormonal contents have long been thought to be the ideal aspirants for plant growth promotion. IAA and GA3 are highly essential phytohormones in plants as it govern their developmental process and growth. While ABA and SA have been involved in abiotic and biotic stress alleviations through various cross-talks in plants (Sehar *et al.*, 2022).

Evaluating phosphate solubilization activity of fungal endophytes

All the eight fungal endophytes except S6P1S3 formed halo zone around the colony when grown on Pikovskaya's Agar medium (Plate 1). This suggested that fungal endophytes were able to solubilize tri-calcium phosphate in the medium by producing significant organic acids or inorganic acids (Khan *et al.*, 2014). Isolate S4P2L2 showed highest phosphate solubilization index (Table 2) compared to other endophytes under both arsenic stress and non stress condition (1.32 and 1.67 respectively). It was followed by isolate S1P1R1 with the solubilization index 1.27 and 0.78 under non stress and stress conditions. Results showed that P solubilization by endophytes decreases in the presence of arsenic stress. The possible

reason could be arsenic is an analog of phosphorus that enters through phosphate transporters into the biological system (Pinter *et al*., 2017). Adhikari and Pandey (2019) demonstrated the potential of endophytic fungi (*Penicillium* and *Aspergillus* spp.) isolated from roots of *Taxus wallichiana*, for their ability to solubilize insoluble phosphates through production of phosphatases, phytases and organic acids. In the present study, there was no significant difference between P solubilization by reference strain *P. indica* under arsenic stress and non stress condition. Investigation by Kushawaha*et al.* (2022) suggests that fungus can solubilize bound Pi without inhibition up to 5 mM As in the environment. While *S. indica* can tolerate arsenic toxicity up to 1 mM and a growth inhibition was observed at 2.5 mM and 5 mM As treatment.

Phosphorus solubilization efficiency also found to be higher in the isolate S4P2L2 with 113% under normal condition and 89% under arsenic stress condition. While lowest was observed with S6P1S3 which showed 0% efficiency. The results are in accordance with Kanimozhi and Panneerselvam (2010), who isolated and screened phosphate solubilizing microorganisms and showed that, the solubilization efficiency of the phosphate solubilizing fungus *Aspergillus niger*was 166.66 %. However, the phosphate solubilization efficiency of *P.indica* recorded in the present study, is lower in comparison to the previous reports. This appears to be an indicative of the influence of prevalent climatic conditions on the bioactivities performed by the microorganisms.

Endophytes were further screened for their ability to solubilize inorganic phosphate by growing them in Pikovskaya's broth containing tricalcium phosphate. The P*i* released by the fungal isolates at six days after inoculation ranged from 03.25 to 104.24 mg L^{-1} in normal condition and 3.1 to 97.34 mg L^{-1} in arsenic stress condition, whereas the reference strain showed 68.15 mg L^{-1} (without stress) and 54.51 mg L^{-1} (with arsenic stress) P*i* released in the broth. It was followed by S8P1L4 fungal isolate which recorded significantly higher release of P*iviz*., 93.65 mg L^{-1} (without stress) and 86.83 mg L^{-1} (with arsenic stress) than the reference strain. A similar method of screening was adopted by Nopparat*et al*. (2017), who screened 30 fungal strains and reported endophytic fungal strains SA07P3332, SA22P3406, SA14P2418 and SA19P2120 solubilized tricalcium phosphate and showed the highest available phosphorus in liquid medium.

Evaluating siderophore producing activity of fungal endophytes

The metabolically important class of substances called siderophores are organic molecules with a high affinity for Fe (III), thus participating in iron availability for plants, even under limited essential metal availability or in polluted soil (Rajkumar *et al*., 2010; Schalk *et al*., 2011). Therefore, siderophores indirectly support plant development and survival in metal-polluted soils by reducing the number of pathogens.

Significantly higher siderophore production was observed with isolate S3P1S1 (4.00- siderphore production index, 147% - siderophore production efficiency), followed by S6P1S3 (3.33- siderphore production index, 123% - siderophore production efficiency)(Table 3, Plate 2). The results obtained were directly proportional to the halo zone produced in a CAS agar test and this observation is in accordance with the findings of Calvente *et al.* (2001). Likewise, Kajula*et al.* (2010) reported the production of extracellular siderophore by the foliar endophytic fungi isolated from Scots pine (*Pinus sylvestris* L.) and labrador tea. Chowdappa*et al.* (2020) also reported per cent siderophore content among endophytic fungi ranged from a higher value of 56.6% by *P. chrysogenum*(CAL1) to a lower siderophore efficiency of 10.35% by *C. truncatum*. Besides enabling fungi to grow in an iron-starved environment, siderophore confers an added advantage of increasing resistance to high arsenic concentration as compared to the non-siderophore producers. The previous report also indicated that siderophore forms a stable complex with As; thus, the application of fungi with siderophore production activity proves beneficial for As remediation (Jeong *et al*., 2014).

On the contrary, Schwyn and Neilands (1987) reported that quantification of siderophores was possible only by CAS liquid assay as it was not accurate to access siderophore quantity on CAS solid agar. In the present study, isolate S3P1S1 produced significantly higher amount of siderophore (96 ± 0.002 µmol) compared to other isolates and reference culture. This result is supported by the findings of Das*et al.* (2020) who documented 78.7µmol siderophore production under *in vitro* assay in their metal tolerant isolate under unstressed condition. Siderophore changes the oxidation states of heavy metals including Cd, Cu, Ni, Pb, Zn and Th^{4+} andU^{4+} to make them less toxic in nature (Schalk *et al.*, 2011). Siderophores also bind different toxic metals such as Cr^{3+}, Cu^{3+}, Pb^{2+}, Cu^{2+}, V^{4+} and Al^{3+}, while the binding capability of siderophores to Fe is more as compared to toxic heavy metals (Bayssee*t al*., 2000; Braud *et al*., 2009). These siderophores bind to toxic heavy metals and thus toxic heavy metals do not hinder the efficiency of plant cells (Braud *et al*., 2009). Therefore, the toxic heavy metal detoxifying and binding capability of siderophore plays a remarkable role in plant growth under heavy metal polluted land.

Evaluation of K solubilization, HCN production, Ammonia production and ACC deaminase production by fungal endophytes under *in-vitro*

In terms of potassium solubilization, none of the fungal endophytes showed the ability to solubilize potassium in Aleksandrow agar medium (Table 4). HCN production was observed with only one isolate S4P2L2 (Plate 3). Acc deaminase activity was observed in all the eight arsenic resistant fungal endophytes in presence of ACC source i.e. all the isolates showed considerable growth in DF minimal media supplemented with 3mM ACC source. Under stress condition, endophytic bacterial/microorganisms produces ACC deaminase which break the ACC (prerequisite of ethylene production) to α-ketobutyrate and ammonia and thereby diminishes level of "stress ethylene" in the stressed host plants. In this regard, there are only a few reports have shown previously for ACC deaminase-producing fungal endophytes associated with the abiotic stress tolerance in plants. The favorable impact of the ACC deaminase generating fungal endophyte *Trichoderma asperellum* (MAP1) in plant-microbe interaction for improving waterlogging stress tolerance in wheat has recently been examined by Rauf *et al.* (2021). The presence of the ACC deaminase gene in the genomes of several species of the *Penicillium* and *Trichoderma* persuaded the control of ethylene synthesis by the fungus ACC deaminase, which might be thus associated with the plant resistance response to numerous forms of biotic and abiotic stimuli (Zhang *et al*., 2015).

These findings provide evidence that the endophytic fungi residing in the healthy tissues of plants posses the ability to enhance plant's fitness under stressful conditions. Therefore, these tested fungal strains with their endophytic character and plant growth-promotion activity, along with its high metal tolerance ability could be a novel, effective and sustainable bioremediation strategy for arsenic-polluted soils.

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Table 1: Determination of phytohormones secreted by fungal endophytes under *in-vitro* conditions

Fungal endophytes	Indole acetic acid (IAA) (mg/ mL)		Gibberellic acid (GA_3) (mg/ mL)		Salicylic acid (SA) (mg/ mL)		Abscisic acid (ABA) (mg/ mL)	
	Without stress	With stress	Without stress	With stress	Without stress	With stress	Without stress	With stress
S1P1R1	0.55^{ab}	0.34^{a}	0.09^{b}	0.063^{a}	0.21^{b}	0.13^{b}	0.007^{b}	0.006^{a}
S3P1S1	0.61^{a}	0.31^{a}	0.07^{cd}	0.032^{b}	0.25^{a}	0.21^{a}	0.005^{cd}	0.005^{b}
S3P1S3	0.41^{cd}	0.12^{cd}	0.04^{e}	0.011^{cd}	0.08^{g}	0.09^{cd}	0.002^{e}	0.002^{d}
S4P2L2	0.52^{ab}	0.27^{ab}	0.15^{a}	0.031^{b}	0.19^{c}	0.10^{c}	0.006^{bc}	0.005^{b}
S5P1S1	0.38^{d}	0.09^{d}	0.06^{d}	0.019^{bcd}	0.13^{ef}	0.04^{f}	0.004^{d}	0.003^{c}
S6P1S3	0.12^{e}	0.05^{d}	0.08^{bc}	0.025^{bc}	0.05^{h}	0.12^{b}	0.002^{e}	0.002^{d}
S8P1L4	0.49^{bc}	0.21^{b}	0.01^{f}	0.003^{d}	0.15^{d}	0.08^{de}	0.001^{e}	0.001^{e}
S10P1L1	0.50^{bc}	0.19^{bc}	0.06^{d}	0.013^{bcd}	0.12^{f}	0.09^{cd}	0.005^{cd}	0.002^{d}
Fungal reference culture	0.49^{bc}	0.26^{ab}	0.04^{e}	0.028^{bc}	0.14^{de}	0.07^{e}	0.03^{a}	0.005^{b}

Note:Numerical values are mean of three replicates. Treatments with the different superscripts in the same column represent a significant difference as determined by DMRT ($p \leq 0.05$)

Table 2: Phosphate solubilizing potential of arsenic resistant fungal endophytes

Isolates	Phosphate solubilization index		Phosphate solubilization (mg/L)	
	Without stress	With stress	Without stress	With stress
S1P1R1	1.27^{b}	0.78^{c}	69.87^{d}	55.45^{d}
S3P1S1	1.03^{cd}	0.89^{b}	76.54^{c}	62.72^{c}
S3P1S3	0.87^{e}	0.66^{d}	49.92^{e}	42.54^{e}
S4P2L2	1.67^{a}	1.32^{a}	104.34^{a}	97.34^{a}
S5P1S1	0.67^{f}	0.54^{e}	67.01^{d}	54.18^{d}
S6P1S3	0^{g}	0^{g}	3.25^{f}	3.1^{f}
S8P1L4	1.1^{c}	0.77^{c}	93.65^{b}	86.83^{b}
S10P1L1	1.01^{d}	0.78^{c}	89.28^{b}	83.06^{b}
Fungal reference culture	0.67^{f}	0.45^{f}	68.15^{d}	54.51^{d}

Note:Numerical values are mean of three replicates. Treatments with the different superscripts in the same column represent a significant difference as determined by DMRT ($p \leq 0.05$)

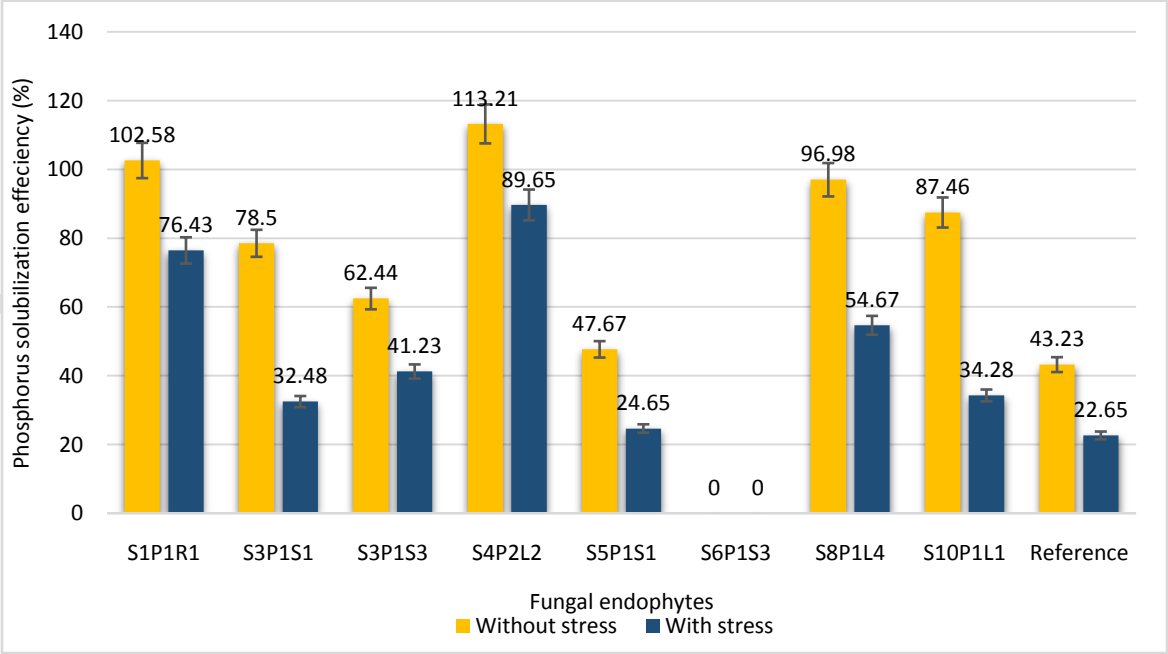


Fig 1: Phosphorus solubilization efficiency of arsenic resistant fungal endophytes

Table 3: Siderophore producing ability of selected arsenic resistant fungal endophytes

Isolates	Siderophore production index	Siderophore producing efficiency	Siderophore (psu)
S1P1R1	2.72^{d}	112.23^{c}	52^{d}
S3P1S1	4.00^{a}	147.45^{a}	96^{a}
S3P1S3	1.50^{f}	36.00^{e}	12^{g}
S4P2L2	2.00^{e}	82.00^{d}	41^{e}
S5P1S1	1.66^{f}	43.00^{e}	5^{h}
S6P1S3	3.33^{c}	123.65^{b}	73^{c}
S8P1L4	2.00^{e}	77.00^{d}	34^{f}
S10P1L1	2.00^{e}	78.00^{d}	49^{d}
Fungal reference culture	3.71^{b}	128.00^{b}	86^{b}

Note:Numerical values are mean of three replicates. Treatments with the different superscripts in the same column represent a significant difference as determined by DMRT ($p \leq 0.05$)

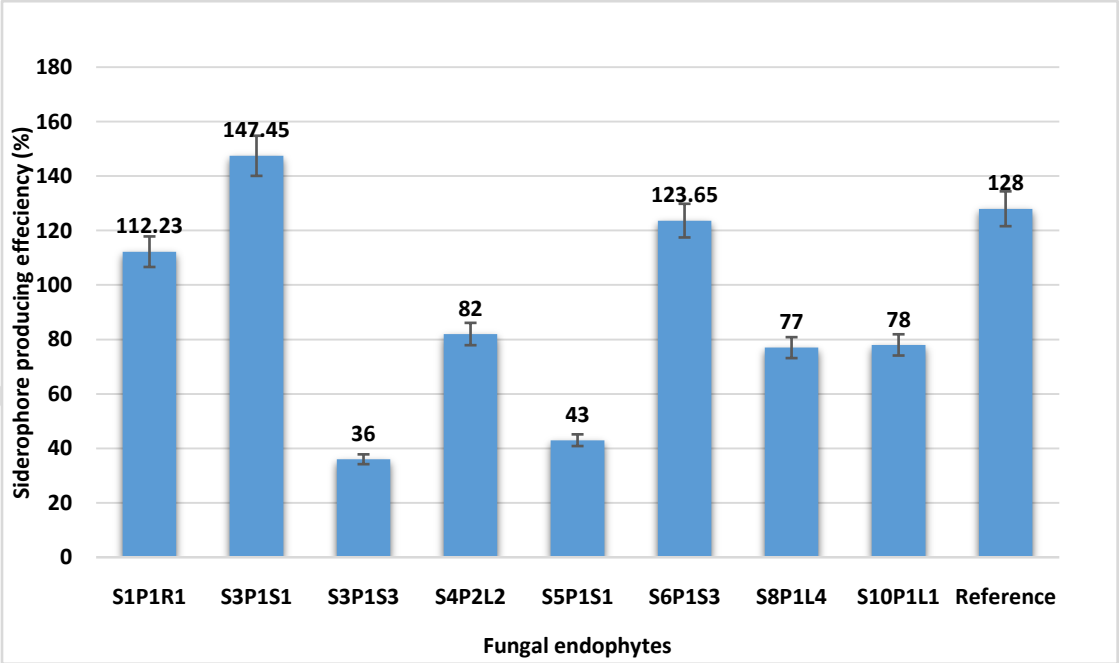
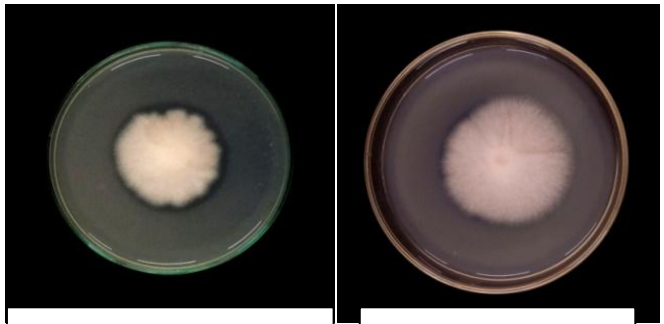


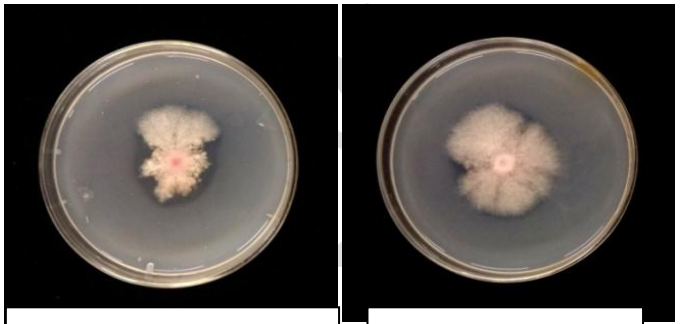
Fig 2: Siderophore producing efficiency of arsenic resistant fungal endophytes

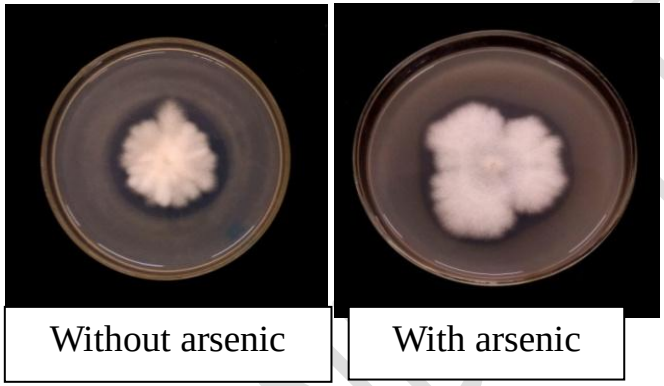
Table 4: Determination of K solubilization, HCN production and ACC deaminase production by fungal endophytes under *in-vitro*

Isolates	K solubilization		HCN production		ACC deaminase production	
	Without stress	With stress	Without stress	With stress	Without stress	With stress
S1P1R1	-	-	-	-	+	+
S3P1S1	-	-	-	-	+	+
S3P1S3	-	-	-	-	+	+
S4P2L2	-	-	+	+	+	+
S5P1S1	-	-	-	-	+	+
S6P1S3	-	-	-	-	+	+
S8P1L4	-	-	-	-	+	+
S10P1L1	-	-	-	-	+	+
Fungal reference culture	-	-	-	-	+	+

Note: HCN- Hydrogen cyanide, ACC- aminocyclopropane-1-carboxylate, (+)- Positive, (-)- Negative







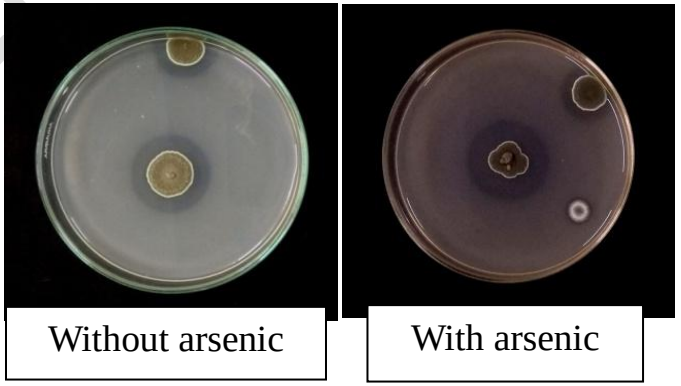


Plate 1a: Phosphate solubilization by arsenic resistant fungal endophytes under *in vitro* normal and arsenic (100 ppm) condition

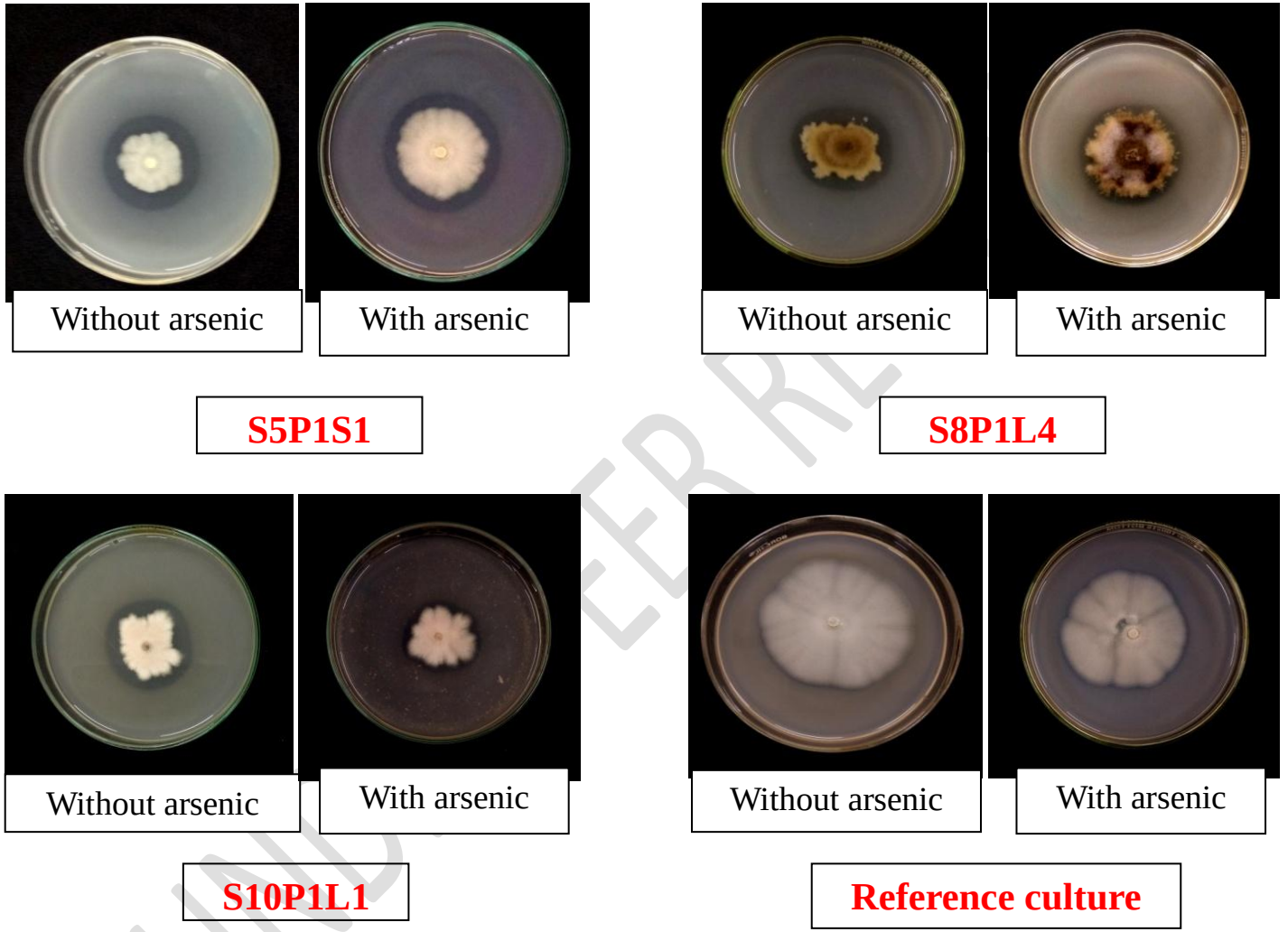


Plate 1b: Phosphate solubilization by arsenic resistant fungal endophytes under *in vitro* normal and arsenic (100 ppm)condition

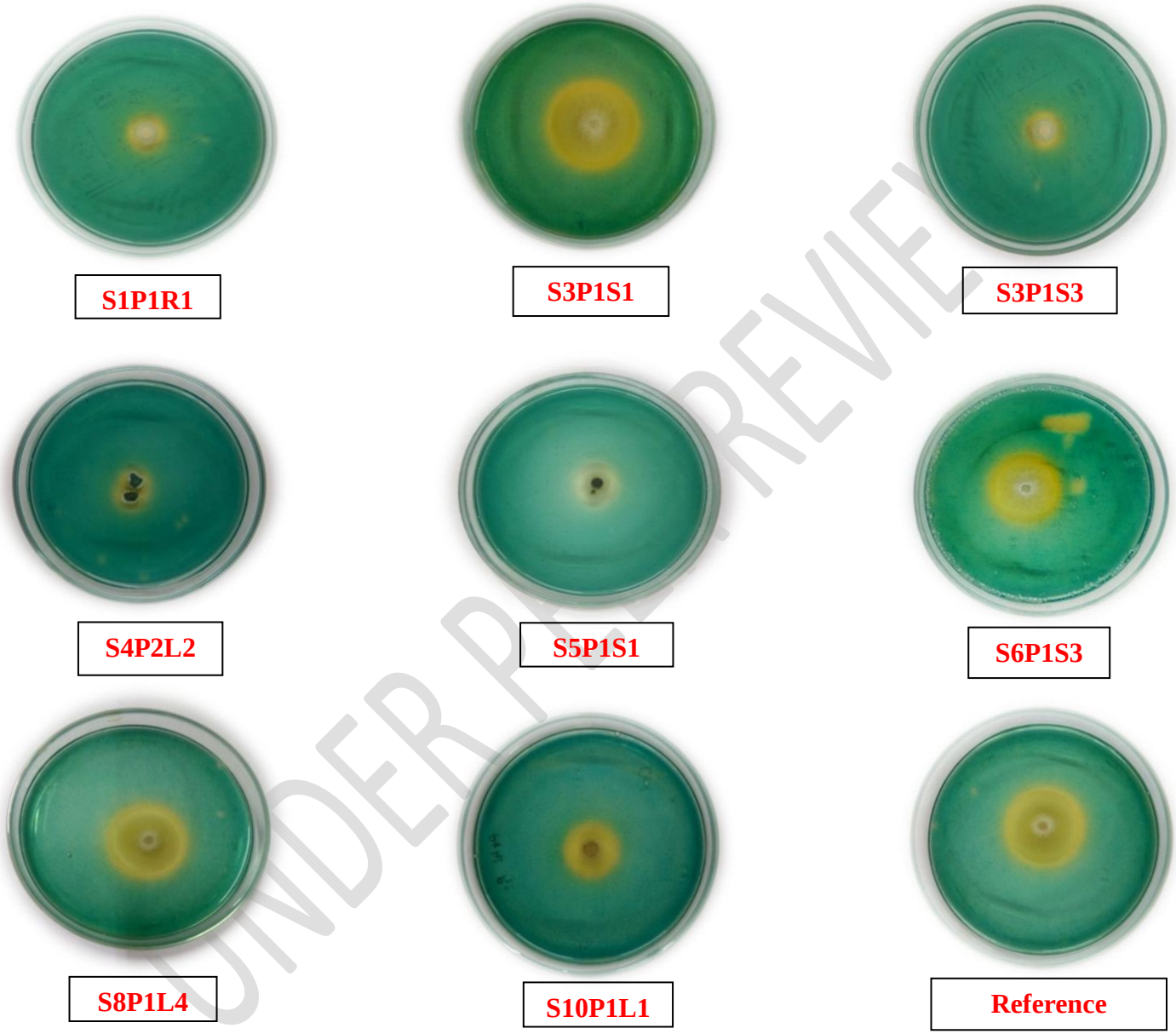


Plate 2: Siderophore production by arsenic resistant fungal endophytes under *in vitro* condition

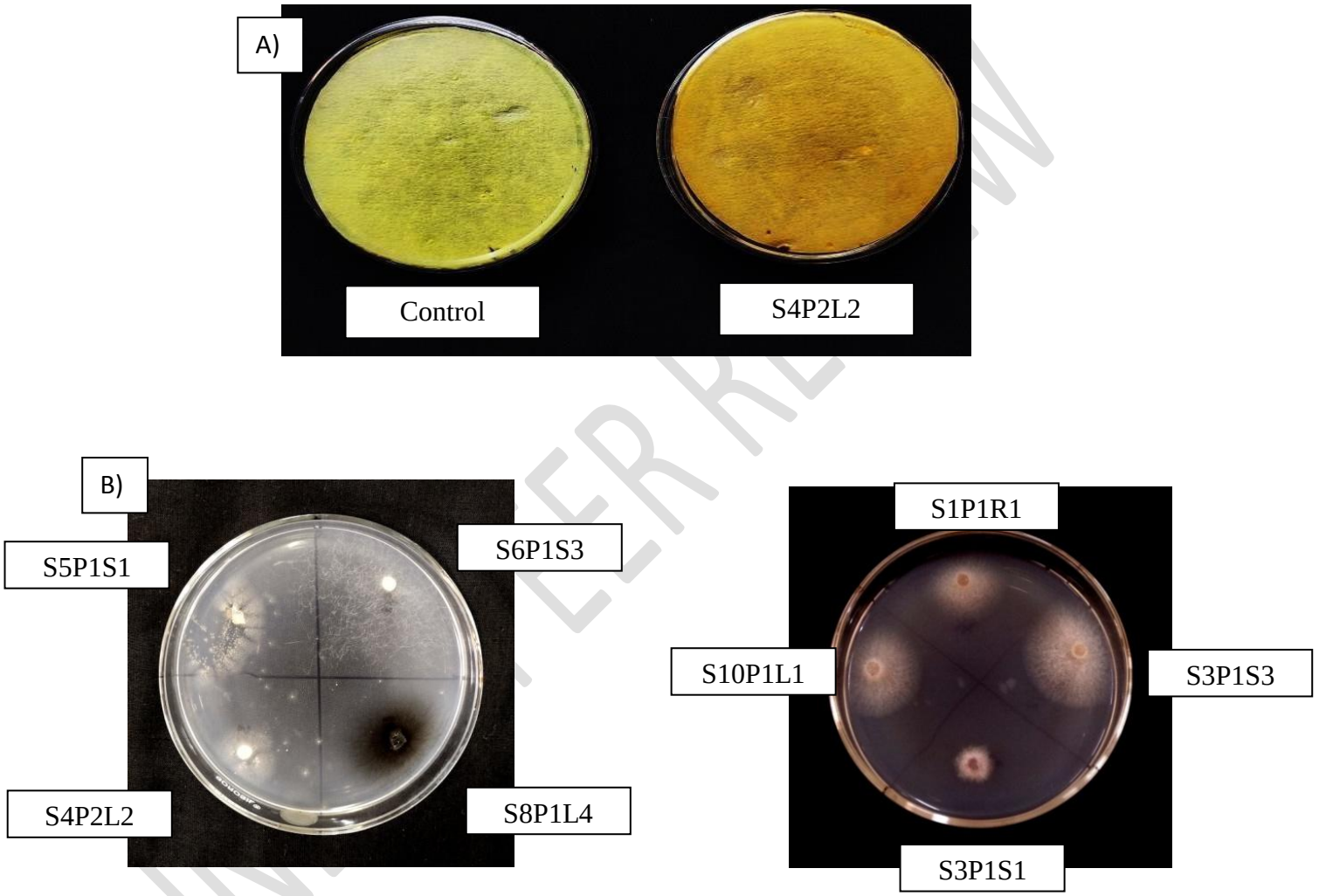


Plate 3: A) HCN and B) ACC deaminase production by arsenic resistant fungal endophytes under *in vitro* condition