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Promotion of Growth and Modulation of As-Induced Stress Ethylene in Maize by As-Tolerant ACC Deaminase Producing Bacteria

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Abstract**—**One of the major pollutants in the environment is arsenic (As). Due to the toxic effects of As to all organisms, its remediation is necessary. Conventional technologies used in the remediation of As contaminated soils are expensive and may even compromise the structure of the soil. An attractive alternative is phytoremediation, which is the use of plants which can take up the contaminant in their tissues. Plant growth promoting bacteria (PGPB) has been known to enhance growth of plants through several mechanisms such as phytohormone production, phosphate solubilization, siderophore production and 1-aminocyclopropane-1-carboxylate (ACC) deaminase production, which is an essential trait that aids plants especially under stress conditions such as As stress. Twenty one bacteria were isolated from As-contaminated soils in the vicinity of the Janghang Smelter in Chungnam Province, South Korea. These exhibited high tolerance to either arsenite (As III) or arsenate (As V) or both. Most of these isolates possess several plant growth promoting traits which can be potentially exploited to increase phytoremediation efficiency. Among the identified isolates is *Pseudomonas* sp. JS1215, which produces ACC deaminase, indole acetic acid (IAA), and siderophore. It also has the ability to solubilize phosphate. Inoculation of JS1215 significantly enhanced root and shoot length and biomass accumulation of maize under normal conditions. In the presence of As, particularly in lower As level, inoculation of JS1215 slightly increased root length and biomass. Ethylene increased with increasing As concentration, but was reduced by JS1215 inoculation. JS1215 can be a potential bioinoculant for increasing phytoremediation efficiency.

Keywords**—**As-tolerant bacteria, plant growth promoting bacteria, As stress, phytoremediation.

I. Introduction

ARSENIC is one of the most hazardous substances in the environment [1]. Prolonged exposure to low concentrations or immediate exposure to sufficiently high concentrations of inorganic As in the natural environment such as in the soil and water are toxic to organisms. The attention to As has increased in the past two decades due to the number of people who were exposed to the contaminant. In addition to naturally occurring As, it is estimated that over 80% of all the As ever produced by man has dissipated to the environment [2]. The main activities that contributed to As contamination are mining, smelting and ore processing, use of arsenic-containing fertilizers and pesticides, and wood preservation using Chromated Copper Arsenate (CCA) [3]. Inorganic forms are considered more toxic than organic forms and the most common species found in As-contaminated soil are arsenate and arsenite. These are also the main phytoavailable forms of arsenic in the soil solution [4]. Microorganisms have an important role in the cycling of As in the environment as these can biotransform arsenite to arsenate (arsenite oxidation), arsenate to arsenite (arsenate reduction).

Due to the hazardous effects of As exposure, efforts to clean up contaminated soil and waters are necessary. Compared to organic pollutants, As cannot be degraded and thus requires appropriate methods for its removal [5]. Remediation technologies such as physical and chemical techniques that are being employed are not only costly but may also compromise soil physical, biological and chemical properties [6]. An alternative technology that has advanced in recent years is phytoremediation. The discovery of plants that can take up heavy metals in large amounts created optimism for the remediation of polluted lands. However, even tolerant plants can have limited growth when exposed to high levels of heavy metals. The biotechnological use of microorganisms in association with plants offers more advantages for metal(loid) uptake or removal [7], [8] Heavy metal contaminated soils are known to harbor heavy metal tolerant microorganisms. Some of the isolated bacteria from such soils were characterized as having plant growth promoting (PGP) traits [9], [10], [11], [12]. Known PGP traits of bacteria such as IAA production, P solubilization, siderophore production, nitrogen fixation, and ACC deaminase production are among the most studied traits. It was the aim of this study to isolate and characterize As-tolerant bacteria possessing various plant growth promoting traits which can be potentially used in enhancing phytoremediation of As.

II. Methodology

A. Characterization and Identification of As-tolerant Isolate

The isolate was characterized by its Gram reaction, oxidase, and catalase activity following standard methods. Tolerance of the bacteria to As III and As V was determined by its minimum inhibitory concentration (MIC) of that particular As species. MIC is defined as the lowest concentration that causes no visible growth. Strains were grown in 5 ml TLP broth media without As for 48-72 h at 28±2°C on a rotary shaker

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(150 rpm). Each well of a 96 well microtiter plate was filled with 130 µl sterile TLP medium and supplemented with different concentrations of As III as $NaAsO_2$ (0 to 15 mM) and As V as $Na_2HAsO_4.7H_2O$ (0 to 250 mM). Twenty microliters of bacterial inoculum was placed in each respective well. Cell density was initially measured using EZ Read 400 Microplate Reader (Biochrom) at 590 nm before incubation at 28±2°C. Bacterial growth was determined every after 24 h for 72 h. The strain was identified by16S rRNA gene sequence.

The bacterial strains were screened for plant growth promoting traits. Quantitative estimation of the amount of Indole-3-acetic acid (IAA) produced was done after [13]. ACC deaminase activity was determined by growing the bacterial isolates on nitrogen-free medium amended with 3 mM ACC as nitrogen source [14] and the amount of α-ketobutyrate produced by the enzymatic hydrolysis of ACC was estimated following [15]. Quantitative estimation of phosphate solubilization was done using NBRIP liquid medium [16] and phosphate determination was done by the method of [17]. Siderophore production was determined using modified CAS agar plates according to [18] with the production of orange zones around the growing bacteria as indication of production of siderophore.

B. Inoculation Effect JS1215 on Growth and Ethylene Emission of Maize under Gnotobiotic Condition

Surface-sterilized maize seeds (30% H_20_2 for 5 min) were inoculated with JS1215 bacterial solution (OD_{600}=1.0) or sterile distilled water, after which, the seeds were incubated in the dark for 24h. Two maize seeds were placed in each sterilized growth pouch (MEGA) filled with sterile distilled water. Ten milliliter As V solution ($Na_2HAsO_4.7H_2O$) was added to each pouch after 72h. The maize plants were grown in the growth chamber (25ºC, 70%RH, 40 µmol m^{-2} s^{-1}) for another 7 days. Root and shoot lengths, plant weight were gathered after 7 days. Ethylene emission of maize seedlings was measured in 120 ml glass bottles covered with rubber septum stoppers. One milliliter headspace gas from each bottle after 4 h incubation was injected to a Gas Chromatograph (DASOL) and sample readings were compared to an ethylene standard curve. Analysis of variance and comparison of means were done using SAS 9.1 Software (SAS Institute Inc., Cary, NC, USA).

III. Results and Discussion

A. Characterization of As-tolerant Bacteria Isolated from As-contaminated Soil

Soils are known to harbor beneficial microorganisms which can be utilized for their beneficial effects on plant growth and development. JS1215 is one of the isolated As-tolerant bacteria from As-contaminated soil (144.87 mg kg^{-1} total As content) from Janghang, Chungnam, South Korea. It was identified through 16S rRNA sequencing as *Pseudomonas* sp. The nucleotide sequence of 16S rRNA genes was deposited to the GenBank® database under the accession number JQ014191. It is a Gram-negative rod and oxidase positive. It has moderately high tolerance to As III (7 mM) and very high tolerance to As V (310 mM). It has ACC deaminase activity, produces the phytohormone IAA, solubilizes P, and produces siderophore which are essential PGP traits (Table I). Aside from their recognized roles in plant pathogen suppression or induction of resistance to diseases, several species of *Pseudomonas* isolated from heavy metal contaminated soils have been identified for possible bioremediation of contaminated water and soil systems [19].

TABLE I
PLANT GROWTH PROMOTING TRAITS OF *PSEUDOMONAS* SP. JS1215

Strain	PGPR traits			
	ACCD activity (µmol α-KB mg-1 h-1)	IAA production (µg ml-1)	P solubilization (g l-1)	Siderophore production
JS1215	1.82±0.31	5.71±0.11	9.35±0.76	+

+ able to produce siderophores

B. Effect of Pseudomonas sp. Inoculation on Growth of Maize in the Presence of As under Gnotobiotic Condition

Root and shoot growth of maize seedlings were reduced in the presence of As. Arsenic has no known function as a nutrient [20] and may be toxic even at low concentration. Several studies show that plant species that are not resistant to As suffer considerable stress upon exposure. The symptoms of As toxicity in plants frequently include poor seed germination and very marked reductions in root growth. If plants survive, they may show reduced growth, nutrient deficiencies and chlorosis resulting to reduced chlorophyll biosynthesis [21] as well as reduced photosynthetic oxygen evolution [22].

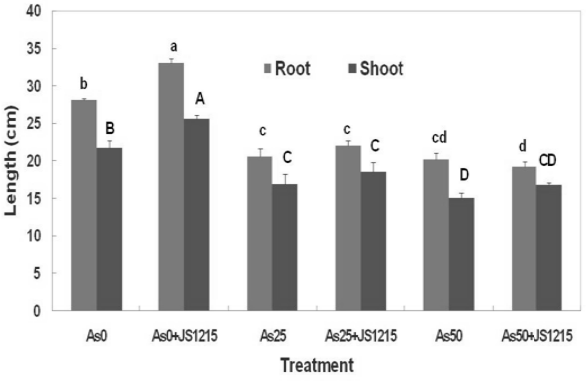

Fig. 1 Effect of bacterial inoculation on root and shoot length of maize under As stress in gnotobiotic condition
Values are means ± SE. Means per group with common letters are not significantly different at P ≤0.05 according to DMRT

Inoculation of JS1215 on maize under gnotobiotic condition stimulated growth of the crop. This is particularly more evident under normal conditions with 17% increment in root length and 18% in shoot length with inoculation compared to control (Fig. 1). Inoculation increased root length by 7% in 25µM As V treatment, and root and shoot lengths by 10% and 12%, respectively in 50 µM As V treatment. A similar trend was observed in plant dry weight of maize after 10 days of growth with 26% increment over control under normal

condition. However, only 3% and 4% increment were observed in the dry weight of inoculated plants over uninoculated plants under 25 µM and 50 µM As V conditions, respectively (Fig. 2).

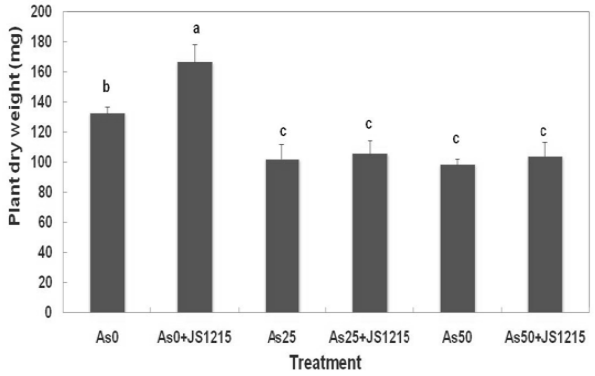

Fig. 2 Effect of bacterial inoculation on total dry weight of maize under As stress in gnotobiotic condition.
Values are means ± SE. Means per group with common letters are not significantly different at P ≤0.05 according to DMRT

C. Effect of Pseudomonas sp. Inoculation on Ethylene Emission of Maize under As Stress

Metal stress is known to cause ethylene production in plants. High production of ethylene can be detrimental to plants by inhibiting growth especially root growth. Inoculation of JS1215 was able to decrease ethylene emission due to As stress in maize plants (Fig. 3).

The synergistic use of plants and As-tolerant PGPR can be a promising approach for the clean-up of As-contaminated soils. The potential of JS1215 for increasing the phytoremediation efficiency of maize or other crops can be further explored under larger scale study.

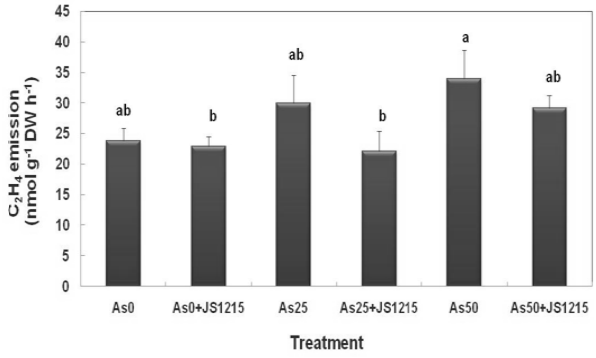

Fig. 3 Effect of bacterial inoculation on ethylene emission of maize under As stress in gnotobiotic condition.
Values are means ± SE. Means per group with common letters are not significantly different at P ≤0.05 according to DMRT

REFERENCES

[1] Agency for Toxic Substances and Disease Registry (ATSDR). Detailed data for 2011 priority list of hazardous substances. 2011. http://www.atsdr.cdc.gov/SPL/resources/ATSDR_2011_SPL_Detailed_Data_Table.pdf.

[2] Nriagu, J.O., P. Bhattacharya, A.B. Mukherjee, J. Bundschuh, R. Zevenhoven and R.H. Loppert. Arsenic in soil and groundwater: an overview. In Arsenic in Soil and Groundwater Environment. P. Bhattcharya et al. (eds). Trace Metals and Other Contaminants in the Environment. 2007, Vol. 9, pp. 3-60.

[3] Pacyna J.M. and E.G. Pacyna. An assessment of global and regional emissions of trace metals in the atmosphere from anthropogenic sources world. *Environ. Rev*. 2001, 9:269-298.

[4] Meharg AA, Hartley-Whitaker J. Arsenic uptake and metabolism in arsenic resistant and non-resistant plant species. *New Phytologist* . 2002, 154: 29–43.

[5] Rajkumar, M, Prasad, M.N.V., Freitas, H., Ae, N. Biotechnological applications of serpentine soil bacteria for phytoremediation of trace metals. Crit Rev Biotechnol. 2009, 29:120-130.

[6] Pulford, I.D. and C. Watson. Phytoremediation of heavy metal-contaminated land by trees- A review. *Environ. Int.* 2003, 29:529-540.

[7] Glick, B.R. Using soil bacteria to facilitate phytoremediation, *Biotech Adv*. 2010, 28 (3):367-74.

[8] Zhuang, X., J. Chen, H. Shim and Z. Bai. New advances in plant growth promoting rhizobacteria for bioremediation. *Environ. Int.* 2007, 33: 406-413.

[9] Jiang, C.Y, Sheng, X.F., Qian, M., Wang, Q.Y. Isolation and characterization of a heavy metal-resistant *Burkholderia* sp. from heavy metal-contaminated paddy field soil and its potential in promoting plant growth and heavy metal accumulation in metal-polluted soil. *J. Appl. Microbiol.* 2011, 5:1065-1074.

[10] Sheng, X.F., Xia, J.J. Improvement of rape (*Brassica napus*) plant growth and cadmium uptake by cadmium-resistant bacteria. *Chemosphere*. 2006, 64: 1036-1042.

[11] Zaidi, S., Usmani, S., Singh, B.R., Musarrat, J. Significance of *Bacillus subtilis* strain SJ-101 as a bioinoculant for concurrent plant growth promotion and nickel accumulation in *Brassica juncea. Chemosphere*. 2006, 64: 991-997.

[12] Burd, G.I., Dixon D.G., Glick, B.R. A plant growth-promoting bacterium that decreases nickel toxicity in seedlings. Appl. *Environ. Microbiol*. 1998, 64: 3663-3668.

[13] Bano, N., J. Musarrat. Characterization of a new *Pseudomonas aerunginosa* strain NJ-15 as a potential biocontrol agent. *Curr. Microbiol*. 2003, 46: 324-328.

[14] Penrose, D.M., Glick, B.R. Methods for isolating and characterizing ACC deaminase-containing plant growth-promoting rhizobacteria. *Physiol. Plant*. 2003, 118:10-15.

[15] Honma, M., Shimomura, T. Metabolism of 1- aminocyclopropane-1-carboxylic acid. *Agri. Biol. Chem*. 1978, 42: 1825-1831.

[16] Mehta, S., Nautiyal, C.S. () An efficient method for qualitative screening of phosphate-solubilizing bacteria. Curr. Microbiol. 2001, 43: 51–56.

[17] Murphy, J. and J.P. Riley. 1962. A modified single solution method for the determination of phosphate in natural waters. Anal. Chim. Acta. 27: 31-36.

[18] Alexander, B., Zuberer, D.A. Use of chrome azurol S reagents to evaluate siderophore production by rhizosphere bacteria. *Biol. Fertil. Soils.* 1991, 12:39-45.

[19] Chen X, Shi J, Chen Y, Xu X, Xu S, Wang Y.2006.Tolerance and biosorption of copper and zinc by Pseudomonas putida CZ1 isolated from metal-polluted soil. *Can J Microbiol*.52 (4):308-16.

[20] Nies DH. 1999. Microbial heavy-metal resistance.Appl Microbiol Biotechnol. 51(6):730-50.

[21] Singh N., Ma L.Q., Srivastava M., Rathinasabapathi, B.2006. Metabolic adaptations to arsenic-induced oxidative stress in Pteris vittata L. and Pteris ensiformis L. Plant Sci 170:274–282

[22] Ullrich-Eberius C.I., Sanz A., Novacky A.J. 1989. Evaluation of arsenate- and vanadate-associated changes of electrical membrane potential and phosphate transport in Lemna gibba G1. Journal of Experimental Botany 40: 119–128.