Electronic Supporting Information

Bacillus mojavensis, A Metal-tolerant Plant Growth Promoting Bacterium improves Growth, Photosynthetic Attributes, Gas-exchange Parameters and Alkalo-polyphenol contents in Silver Nanoparticles (AgNPs)-treated *Withania somnifera* L. (Ashwagandha)

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Supplementary Methods

Molecular identification and Construction of Phylogenetic tree

Primarily, the bacterial cultures were identified to genus level by microbiological and biochemical techniques and further to species level by 16S rRNA sequence-based method. The identification of the isolated bacterial strains to species level was done by 16S rRNA partial gene sequencing service, Macrogen, Seoul, South Korea using universal primers, 785F (5′-GGATTAGATACCCTGGTA-3′) and 907R (5′-CCGTCAATTCMTTTRAGTTT-3′). The nucleotide sequences so obtained were trimmed using BioEdit program version 7.2.4. The similarity search was performed using the BLASTn online program of the National Center for Biotechnology Information (NCBI) with a highly similar sequence search limited to only type material. The processed nucleotide sequence was then submitted to GenBank sequence database through sequin software to obtain accession number. Later, for tracing the evolutionary relatedness of selected bacterial strains with other bacteria, the sequences displaying maximum similarity (percent identity) with the sequence of the test organism were selected and aligned in FASTA format. For phylogenetic tree construction, pair wise and multiple sequence alignment was done by the CLUSTAL W sequence alignment tool of MEGA7.0. This alignment was then exported in dot Meg format. This format was then used to prepare phylogenetic trees using neighbor-joining algorithm with the help of MEGA 7.0 software.

S2.5 ***Biofilm development assessment***

Biofilm development on microtiter plate in the presence and absence of NaCl was assayed adopting the standard procedure described by (O'Toole 2011) using 1% crystal violet (CV) to stain biofilms formed in microtiter plate (96 well plate). Biofilm bonded dye was extracted in 75% ethanol, and absorbance was measured at 595 nm in microtiter plate reader (Thermo Scientific Multiskan EX, UK) to examine the concentration of dye retains by the biofilm and compared with control (without salt). Each treatment was replicated in five independent wells for biofilm quantification, and experiment was repeated three times.

S2.5.1 assessment of alginate production

The alginate produced by PGPR was quantified. For the assay, cells were grown in liquid medium added with different concentrations of NaCl. Supernatant obtained after centrifugation and filtration was subjected to deacetylated alginate isolation by adding the isopropanol in equal

volume and stored at room temperature for 24 h. Precipitate was collected by centrifugation for 10 min at 10,000 rpm followed by washing with graded concentrations of ethanol (70%). The washed and dried pellet was suspended in 1 mL of DW. Further, 100 µL of suspension was kept in fresh tubes and made the volume up to 1 mL by adding Milli-Q water for the quantification. Freshly prepared 1 mL of borate sulfuric acid solution (10 mM) was supplemented after the addition of fresh carbazole reagent (30 µL) and then mixed properly. The solution mixture was left at room temperature for 15 min and read the absorbance at 500 nm against reagent blank. The quantitative extraction of alginate was expressed in terms of µg/mg wet biomass (Wozniak et al. 2003).

Determination of total flavonoid content

The total flavonoids concentration in *B. mojavensis* inoculated and AgNPs treated roots of ashwagandha plants was determined using the aluminum chloride colorimetric test, as described by Kim et al. (2003). For the estimation, a-100 µl of the plant extract was diluted with 900 µl in a test tube. The volume was made to 4 ml using distilled water. In this 0.3ml of 5% Na2NO3 was added and kept for 5 minutes. After few minute 0.3 ml of AlCl3 and 2.0 mL of 1.0 M NaOH was added. The reaction mixture was allowed to stand for few minutes and the absorbance was measured at 510 nm against a blank solution. The amount of flavonoids in the extracts was expressed as mg equivalents of quercetin equivalents (QE)/gram extract, using a calibration curve of quercetin (20-100 µg).

Supplementary Table

Table S1: Physicochemical properties of test soil

Physicochemical properties	Value
pH	7.4
Organic carbon (g kg^{-1})	7.39
Kjeldahl nitrogen (g kg^{-1})	0.84
Olsen phosphorus (mg kg^{-1})	13.1
Potassium (mg kg^{-1})	16.03
Magnesium (mg kg^{-1})	12.6
Water holding capacity (mLg^{-1})	0.462
Calcium ($mgkg^{-1}$)	11.65
Sodium ($mgkg^{-1}$)	8.01
Carbonate ($mgkg^{-1}$)	23.4
Bicarbonate	11.8
Cation exchange capacity (cmol kg^{-1})	13.0
Anion exchange capacity (cmol kg−1)	6.1

Each value is a mean of three replicates

Supplementary Figures

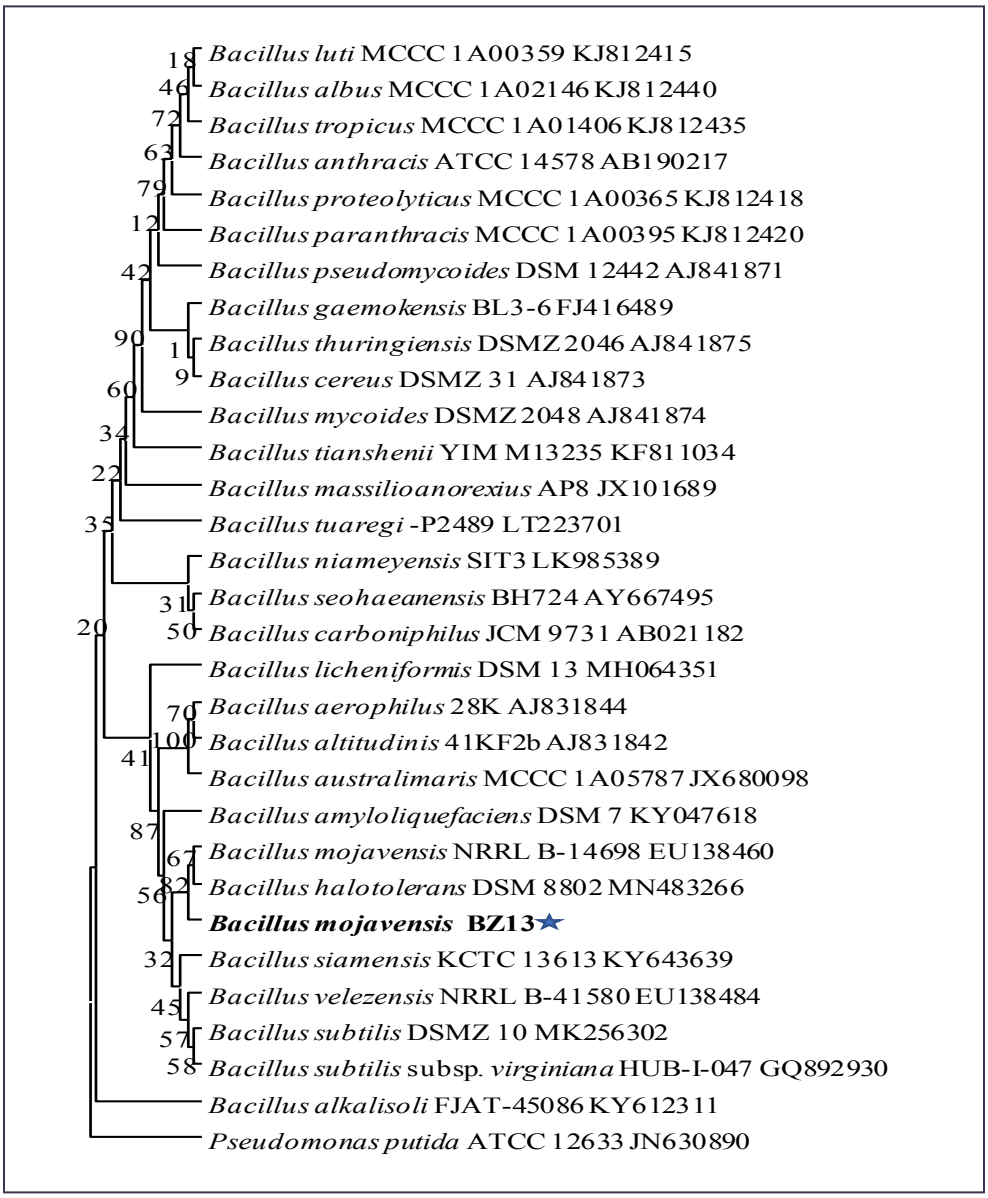

Figure S1: Neighbour-joined phylogenetic tree of *Bacillus mojavensis* BZ13. The tree was constructed based on 16S rRNA partial gene sequence of selected bacteria (marked with red) and closely related phylogenetic species derived using NCBI BLAST search tool. Sequences were aligned using Clustal W sequence alignment tool in MEGA 7.0 software.