

**Orgonochlorine pesticides negatively influenced the cellular growth, morpho-  
structure, cell viability, biofilm formation and phosphate solubilization of  
*Enterobacter cloacae* strain EAM 35**

**\*Mohammad Shahid<sup>a</sup>, Salim Manoharadas<sup>b</sup>, Mohammad Altaf<sup>b,c</sup>, Abdulwahed Fahad  
Alrefaei<sup>d</sup>**

<sup>a</sup>Department of Agricultural Microbiology, Faculty of Agricultural Sciences, Aligarh Muslim University, Aligarh-202002; Uttar Pradesh, India

<sup>b</sup>King Saud University, Department of Botany and Microbiology, Central Laboratory, College of Science, PO Box 2455, Riyadh 11451, Saudi Arabia

<sup>c</sup>King Saud University, Department of Chemistry, Central Laboratory, College of Science, P.O. Box 2455, Riyadh 11451, Saudi Arabia

<sup>d</sup>King Saud University, Department of Zoology, College of Science, P.O. Box. 2454, Riyadh 11451, Saudi Arabia

\*Correspondence to:

**E-mail: [shahidfaiz5@gmail.com](mailto:shahidfaiz5@gmail.com)**

Department of Agricultural Microbiology,  
Faculty of Agricultural Sciences,  
Aligarh Muslim University, Aligarh, India  
+91-08090939511

## **Supplementary Methods**

### **S 4.1 Identification of rhizobacterial strains using 16SrRNA gene sequencing**

The total genomic DNA was extracted by the method as previously described by Yadav et al. (2011). The forward primer pA (5'AGA GTT TGA TCC TGG CTC AG3') and reverse primer pH (5'AAG GAG GTG ATC CAG CCG CA3') (Solanki et al. 2012) were used to amplify the 16S rRNA gene from genomic DNA. The total volume of reaction mixture was 100  $\mu$ L containing 50–80 ng of template DNA, 10X reaction buffer, 2.5 mM dNTPs, 20 pM of each primer and one unit Taq DNA polymerase (Bangalore Genei, India), and reactions were performed on G-storm thermocycler (G-STORM, UK). The amplification conditions were as follows: initial denaturation at 94°C for 5 min, followed by 35 cycles of denaturation at 94°C for 40 s, annealing at 52 °C for 45 s, and elongation at 72 °C for 1min 30s. At the end of 35 cycles, the final extension step was at 72 °C for 8 min. The amplified product was resolved by electrophoresis in 1.2% agarose gel in 1X TAE buffer. Gels were stained with ethidium bromide (EtBr) (10 mg mL<sup>-1</sup>) and visualized on gel documentation system (BIO-RAD, USA). Strong and clear bands were scored for similarity and clustering analysis using the software, NTSYS-2.02e package (Numerical taxonomy analysis program package, Exeter software, USA). The purified 16S rRNA PCR products were send to Macrogen, Seol, South Korea for 16S rRNA sequencing. The 16S rRNA amplicons were sequenced from both ends and consensus sequence was generated. The partial 16S rRNA gene sequences were compared with those available in the databases (<http://www.ncbi.nlm.nih.gov/BLAST/>) and identification to the species level was determined on the basis of sequence similarity of >99% with the closest relative in the GenBank. The phylogenetic tree was constructed on the aligned datasets using the neighbour-joining method implemented in the program MEGA 4.0.2 (Tamura et al. 2007). Bootstrap analysis was performed on 1,000 random samples taken from the multiple alignments.

### **S4.2 Effect of OCPs on PGP traits of *E. cloacae* EAM 35**

#### **S4.2.1 Indole-3-acetic acid (IAA) activity**

Indole-3-acetic acid (IAA) produced by *E. cloacae* strain EAM 35 was quantitatively assayed by the modified method of Brick et al. (1991). Here, strain EAM 35 was grown in Luria Bertani (LB) broth (gL<sup>-1</sup>: tryptone 10; yeast extract 5; NaCl 10 and pH 7.5). A- 100 mL LB broth containing fixed concentration of tryptophan (100 mg mL<sup>-1</sup>) was treated with 0, 25, 50, 75, 100,

150 and 200  $\mu\text{M}$  concentrations of test OCPs. The pesticide containing LB medium was then inoculated with 100  $\mu\text{L}$  culture ( $10^8$  cells  $\text{mL}^{-1}$ ) of EAM 35 strain and incubated at  $28\pm 2$   $^{\circ}\text{C}$  for four days with shaking at 120 r/min. Following complete incubation, culture (5mL) was centrifuged (8000 r/min) for 10 min. and two mL supernatant was added with 100  $\mu\text{L}$  orthophosphoric acid and four mL Salkowsky reagent (2% 0.5 M  $\text{FeCl}_3$  prepared in 35% perchloric acid) and incubated for one hour at  $28\pm 2^{\circ}\text{C}$  in dark for color development. The absorbance of pink color developed during reaction was measured at 530 nm. The quantity of indole-3-acetic acid was calibrated using pure IAA as a standard.

### **S4.3 Surface morphology assessment of OCPs treated bacteria**

Pesticide induced morphological distortion in bacterial cells was observed under scanning electron microscope following the method of Shahid and Khan (2018) by growing bacterial strain in NB medium treated with 100  $\mu\text{M}$  concentrations each of benzene hexachloride (BHC), chlorpyrifos (CP), dieldrin (DE) and endosulfan (ES) at  $28\pm 2$   $^{\circ}\text{C}$  for 24 h. For this, cells were grown in 10 mL NB medium at  $28\pm 2$   $^{\circ}\text{C}$  overnight. The culture was centrifuged (at 12,000 rpm) for 10 min. and pellet was suspended in 1X PBS. Untreated (control) set of experiment was run in parallel. The suspension was then centrifuged, and the cell pellet was washed three times with 1X PBS and pre-fixed with 2.5% glutaraldehyde for 1 h at  $4^{\circ}\text{C}$ . The cells were recovered by centrifugation at 10,000 rpm for 5 min. and the pellet was again washed three times with PBS. After three successive washes with 1X PBS, the fixed specimens were dehydrated (in 30, 50, 70, 90 and 100 % ethanol) for 5 min. each and dehydrated specimens were embedded in white resin overnight. The sections were mounted on carbon coated copper grids and finally the changes in