Potential application of bacteria for xenobiotic removal from agricultural soils

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Introduction

The contamination of soils by numerous pesticide residues may lead to increased environmental hazard as well as human health risk. Aniline is a toxic and recalcitrant element, which can remain in the soil for a long time. Bioremediation is based on using living organisms, particularly microbes for the removal of pollutants from different substrates. Moreover, some bacterial species besides their degradation properties can also have positive impact on plant growth. The main aim of this study was the isolation of bacterial strains with aniline-degrading ability.

Materials and methods

Aniline degradation assay

The bacterial strains ANH1, 2, 3, 4, 5, 6, 7, 8, 9, 10 and VCs1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19 - maintained in the culture collection of the Department of Microbiology, FSI, University of Szeged (Szeged Microbiological Collection; SZMC) - were inoculated into liquid minimal medium (LMM: 1 g/L KH_2PO_4, 3 g/L Na_2HPO_4, 1 g/L $MgSO_4$ in distilled water) containing 1 mg/mL aniline-HCl. The cultures were incubated at 25 °C and samples were taken after 1, 2 and 3 weeks. 5 mL from each liquid culture was centrifuged at 3000 g for 15 min. 100 µL of each sample was mixed with 100 µL aniline reagent (4-dimethylaminocinnamaldexyde in 2% citric acid, 1 mg/mL) and absorbance was measured at 520 nm.

Strain isolation

The isolation of further potential aniline-degrading bacteria was carried out on solid minimal medium (SMM: 1 g/L KH_2PO_4, 3 g/L Na_2HPO_4, 1 g/L $MgSO_4$ 20 g/L agarose in distilled water) containing 1 mg/mL aniline-HCl as sole carbon and nitrogen source, amended with 0.1 mg/L nystatin and carbendazim. Suspension was prepared from 5 g soil sample pretreated with the aniline-type herbicide Stomp in 50 ml 0.9% NaCl. SMM plates were inoculated in 3 different ways. 1) Direct inoculation: spreading 50 µL of the serially diluted (5-step, 10-fold) soil suspension. Enrichment methods: 0.5 mL of the soil suspension was inoculated into 50 mL 2) mineral salt medium (MSM: 1 g/L NH_4NO_3, 0.5 g/L KH_2PO_4, 0.2 g/L $MgSO_4$*7 H_2O, 0.5 g/L NaCl, 1.5 g/L K_2HPO_4 in distilled water) or 3) enrichment medium (ER: 1.0 g/L glucose, 2 g/L Na_2HPO_4, 1 g/L KH_2PO_4, 1 g/L $MgSO_4$*7 H_2O, 0.5 g/L NaCl in distilled water), both supplemented with 1 or 0.1 mg/mL aniline-HCl as well as 0.1 mg/L nystatin and carbendazim, then the samples were incubated on a rotary shaker (100 rpm) at 25 °C. After 1 week of incubation SMM plates were inoculated from the shaken cultures as described above. Subsequently, 1 mL MSM-culture was transferred into fresh MSM and all samples were incubated for 1 more week, then plating was repeated. The inoculated SMM plates were incubated for 7-10 days at 25 °C with daily monitoring. The bacterial strains being able to form colony on SMM containing 1 mg/mL aniline-HCl were considered as potential aniline-degraders. Individual colonies were isolated and maintained on potato dextrose agar (PDA) medium. The isolates were inoculated on SMM containing 1 mg/mL aniline-HCl and the colony diameters were measured following 7, 10 and 14 days of incubation at 25 °C.

Species identification

Strain VCs14 was grown on PDA medium for 24 hours at 25 °C. Cell suspension prepared in 50 µL double distilled water served as DNA template the PCR amplification of the RNA polymerase beta subunit (*rpoB*) gene, using primers rpob-PSF (5'-AGTTCATGGACCAGAACAACC-3') and rpoB-PAR (5'-CCTTCACGGTGAATTCGTTC-3'). Amplification was carried out using the following temperature profile: 94 °C, 3 min (1 cycle); 94 °C, 1 min; 58 °C, 1 min; 72 °C, 1 min (30 cycles); 72 °C, 10 min. Automated sequencing of the amplicon was determined by using external service and the obtained sequence was subjected to NCBI BLAST analysis (https://blast.ncbi.nlm.nih.gov/Blast.cgi).

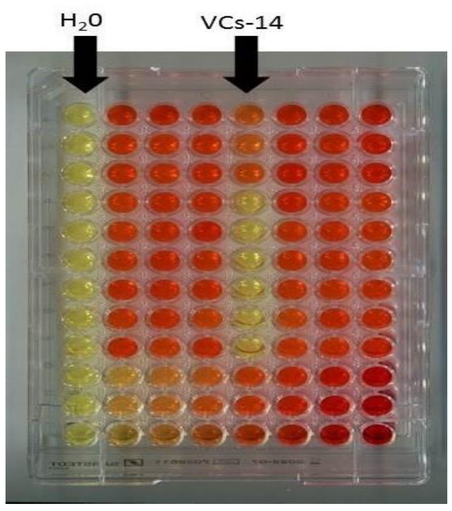
Biotest

The effect of strain VCs14 on plant growth was tested using yellow mustard (*Sinapis alba*). The strain was cultivated in 50 mL potato dextrose broth (PDB) medium for 24 hours. Seeds were surface-sterilized in 96% ethanol for 10 min, then washed with sterile distilled water 5 times. Seeds were treated in two different ways: 1) soaking in bacterial suspension 2) direct surface inoculation.1) Seeds were inoculated directly on the surface with 100 µL of the liquid culture of VCs14. Control seeds were treated with the same amount of sterile PDB medium (A) or sterile distilled water (B). 2) seeds were soaked in the liquid culture of VCs14, PDB medium or water 2 hours. The experiments were carried out on sterile filter paper discs in Petri dishes (10 seeds in each) at 25 °C. Each Petri dish was supplied with 1 mL sterile distilled water 1 and 3 days after inoculation. The rate of seed germination was counted daily; length of seedlings, length of roots and fresh mass were measured after 7 days of incubation.

Results I: Aniline degradation assay

Spectrophotometric analysis revealed no traces of aniline-HCl in the 2 and 3-week culture supernatant of the bacterial strain VCs14. Furthermore, strain VCs12 also showed significant aniline-HCl degrading potential, (Fig. 1).

Twenty seven new, potential aniline degrader bacterial strains (A1, A2, A4, A5, A6, A7, A8, A9, A10, A11, A12, A13, A14, A15, A16, A17, A19, A20, A21, A23, A24, A25, A26, A27, A28, A29, A30) have been isolated from a soil sample pretreated with the aniline-type herbicide Stomp. After 2 weeks of incubation the colony diameters of the 10 strains showing the most intensive growth on SMM plates containing 1 mg/mL aniline-HCl varied between 28 and 52 mm (Fig. 2). The degradation efficacy will be determined by spectrohotometry and HPLC analysis.



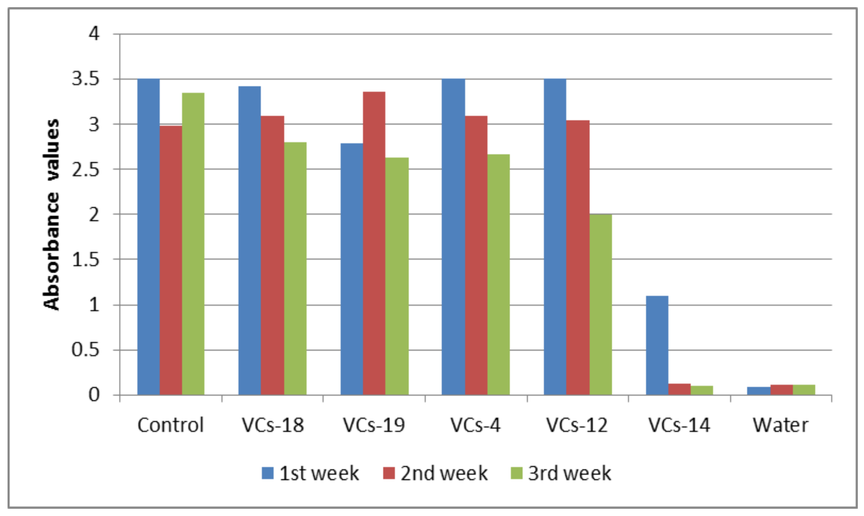


Figure 1 (A, B): Spectrophotometric visualization of aniline-HCl degradation

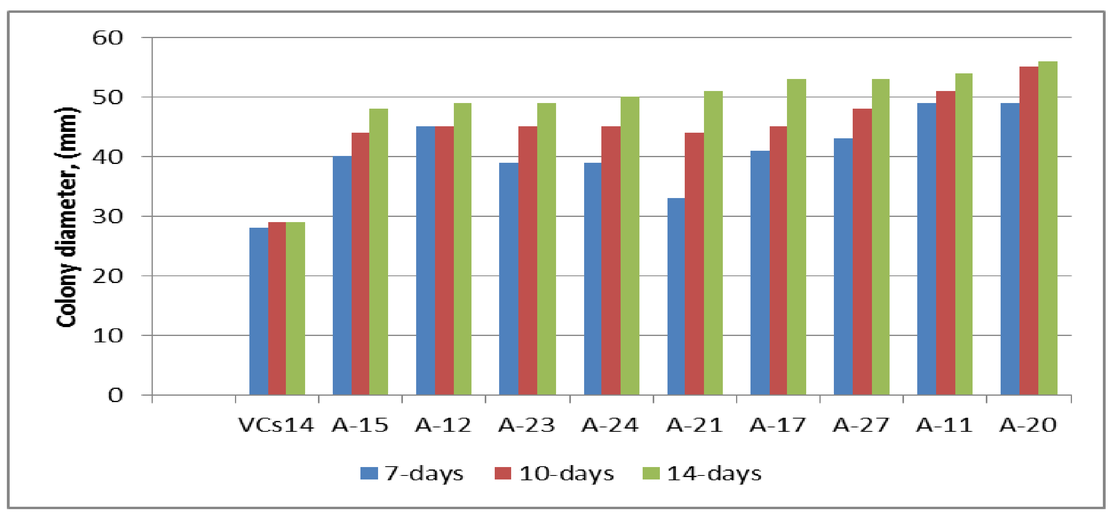


Figure 2: Growth of bacteria in the presence of 1 mg/mL aniline-HCl

Results II: Species identification

Based on the NCBI BLAST analysis of its *rpoB* gene sequence, the bacterial strain VCs14, which showed complete degradation of 1 mg/mL aniline-HCl, has been identified as *Pseudomonas fluorescens.*

Results III: Biotest

Soaking seeds in the liquid culture of *P. fluorescens* VCs14 for 2 hours resulted in the highest length of roots and seedlings of yellow mustard. Compared to the controls, 10.0 and 28.6% increase was observed in the length of roots and seedlings, respectively (Fig. 3).

Conclusions

The *P. fluorescens* VCs14 strain was found to degrade aniline with high efficacy, furthermore, it had plant growth promoting effect as well. Based on our findings it may be a potential candidate for soil bioremediation purposes.

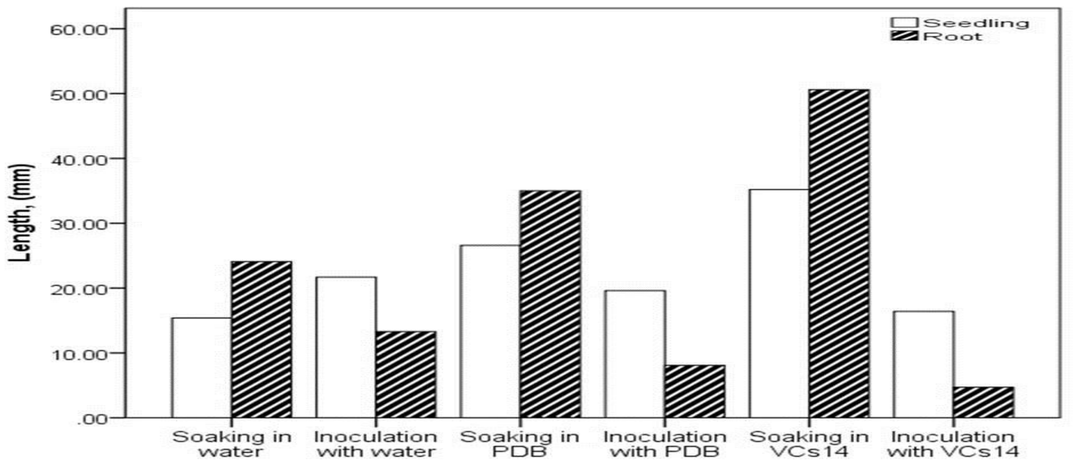


Figure 3: Growth of roots and seedlings of yellow mustard

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