

Rhizosphere biology of aquatic microbes in order to access their bioremediation potential along with different aquatic macrophytes

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Abstract

Background: Contamination of the aquatic environment by toxic metal ions is a serious pollution problem.

Aim: This study reviews the role of different Rhizospheric bacteria in bioremediation along with aquatic macrophytes.

Methods and Materials: Literature searches were done to identify relevant studies in the concerned area.

Results: Natural resources including plants and microorganisms are extensively explored to combat metal ion pollution. Certain compounds produced by bacteria have been shown to promote plant growth. Rhizospheric micro-organisms are well known for their coexistence with plants and for providing nutrition to plants. It was recently reported that these organisms facilitate the uptake of essential elements, such as iron, copper and zinc. *Eichhornia crassipes* showed increased removal efficiency of heavy metals through the activity of its Rhizospheric bacteria. The highly versatile metabolic capabilities of fungi and bacteria can be applied to reclaim polluted ecosystems and minimize the potential adverse effects of hazardous chemicals released to the environment. However, sufficient consortia of microorganisms, capable of degrading the contaminant(s), must be present, and environmental conditions conducive to degradation must be maintained.

Conclusion: Moreover, the information presented, herein illustrates the potential for Rhizosphere microbial communities to remediate systems through biotransformation of hazardous organic compounds in the root zone. Future research in this area should include investigations of the possible role and characterization of the microorganisms associated with different plant species and different histories of toxicant exposure.

Keywords: Rhizospheric, Heavy metals and Biotransformation.

INTRODUCTION

Contamination of the aquatic environment by toxic metal ions is a serious pollution problem. [1-6]. To remediate the aquatic environment, the toxic metal ions should be concentrated in a form that can be extracted conveniently, possible for reuse or at least for proper disposal. Natural resources including plants and microorganisms are extensively explored to combat metal ion pollution (Christensen *et al.*, 1994) [7]. Recently, there are a number of new studies related to rhizospheres of aquatic plants and specifically their increased potential for remediation of contaminants, especially remediation of metals through aquatic plant-microbial interaction. [8] Plant enzymes establish the degradation of pollutants during phytoremediation; whereas, during bioaugmentation, the indigenous microbial population performs the degradation. In many of these studies, an important contribution to the degradation of pollutants goes to microbes present in the rhizosphere of plants. This contribution of the rhizomicrobial population is referred to as rhizoremediation (Anderson *et al.* 1993; Schwab and Banks 1994). [9] [10]

In some cases, rhizosphere microbes are even the main

contributors to the degradation process. A plant can be considered to be a solar-driven biological pump and treatment system, attracting water with its root system, accumulating water-soluble pollutants in the rhizosphere, and concluding with the degradation or translocation of the pollutant (Erickson 1997). [11]

REVIEW OF LITERATURE

While many plants and bacteria have their own mechanisms for dealing with heavy metal contaminants, the interaction of plants and microorganisms may increase or decrease the heavy metal accumulation in plants, depending on the nature of the plant-microbe interaction. Because phytoremediation is a relatively new technology, understanding mechanisms of plant-microbe interactions in removing contaminants from the environment is still not well characterized. There have, however, been several ideas about the nature of plant-microbe interactions in metal accumulation. [12]

De Souza *et al.*, proposed several possible mechanisms, including bacterial stimulation of plant metal uptake compounds such as siderophores; bacterial root growth promotion increasing the root surface area and bacterial transformation of elements into more soluble forms; [13]. Van der Lelie related the basis of this plant-microbe interaction to bacterial metal resistance, since the bioavailability of metals could be altered by bacterial expression of resistance systems [14]. Bacteria promote plant growth, thus increasing surface area of the plant and allowing more metal uptake. [12]

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Certain compounds produced by bacteria have been shown to promote plant growth, including siderophores [15, 16], 1-aminocyclopropane-1-carboxylic acid deaminase (ACC deaminase) [17] [18]. Rajkumar & Freitas [19] suggested that indole-3-acetic acid (IAA) indirectly promotes metal accumulation in plants by increasing plant biomass. The increased degradative capability of rhizosphere microbial communities is not limited to terrestrial plants [20] [21]. Federle & Schwab [22] and Federla & Ventullo [23] have made similar observations of the increased microbial degradation of surfactants in the rhizospheres of aquatic plants. Microbial communities associated with duckweed (*Lemna minor*) readily mineralized LAE, but not LAS. Similar results on microbial degradation of LAS and LAE by the microbiota of submerged plant detritus were obtained by Federle & Ventullo. [23]

To access the possible additional benefits of microbial filters (biofilms), containing aquatic vegetation in biotransformation of hazardous organic compounds, Wolverton and McDonald-McCaleb [24] compared removal of a variety of EPA priority pollutants in nonvegetated filters removed 61-99% and 39-81% of the aromatics (benzene, biphenyl, chlorobenzene, dimethylphtalate, ethylbenzene, naphthalene, p-nitrotoluene, toluene, p-xylene) and aliphatics (bromoform, chloroform, 1-2-dichloroethane, tetrachlorosystem increased the removal of both the aromatics (81->99%) and rates as well as possible abiotic degradation and adsorption mechanisms were not performed, losses due to volatilization appeared to be minor in these systems.

RESULT AND DISCUSSION

Bacteria resistant to Cu^{2+} , Ni^{2+} or Zn^{2+} were isolated from the rhizosphere of water hyacinth (*Eichhornia crassipes* (Mart.)) and their metal ion removal capacities (RCs) were determined. The Ni^{2+} and Zn^{2+} RCs of the respective metal ion-resistant bacteria were less than 4.1 mg g^{-1} , while one of the Cu^{2+} -resistant bacteria (Strain CU-1) showed a significant high Cu^{2+} RC of 10.6 mg g^{-1} . The effect of inoculating water hyacinth with Strain CU-1 on its Cu^{2+} RC was further studied. Water hyacinths were treated with an antibiotic, oxytetracycline (OTC), to remove most rhizospheric bacteria of plant roots. Inoculation of Strain CU-1 increased the Cu^{2+} RC of the plant root by 1.91 (OTC-treated) and 1.56 (OTC-untreated) folds respectively when compared with the control. Results also showed that Strain CU-1 colonized onto the plant root and led to the increase of Cu^{2+} RC of the roots of water hyacinth. [25]

Shanab et al., studied chromate-tolerant bacteria for enhanced metal uptake by *Eichhornia Crassipes*. A total of 85 chromate-resistant bacteria were isolated from the rhizosphere of water hyacinth grown in Mariout Lake, Egypt, as well as the sediment and water of this habitat. Bacterial isolates RA1, RA2, RA3, RA5, and RA8 had 16 S rRNA gene sequences that were most similar to *Pseudomonas diminuta*, *Brevundimonas diminuta*, *Nitrobacteria irancium*, *Ochrobactrum anthropi*, and *Bacillus cereus*, respectively. Water hyacinth inoculated with RA5 and RA8 increased Mn accumulation in roots by 2.4- and 1.2-fold, respectively, compared to uninoculated controls. The highest concentrations of Cr (0.4 g kg^{-1}) and Zn (0.18 g kg^{-1}) were accumulated in aerial portions of water hyacinth inoculated with RA3. Plants inoculated with different bacterial isolates showed higher Cr concentrations in roots compared to the control (Table 1). These bacterial isolates are potential candidates in phytoremediation for chromium removal. [26]

Table 1. Effect of bacterial isolates on Cr concentration: [26]

Bacterial isolates inoculated with plant	Increased Cr Concentration (%)
RA1	7
RA2	11
RA3	24
RA5	29
RA7	35
RA8	21

Das et al., studied the release of Chromium enriched effluent in many water bodies around leather tannery complex in East Kolkata. In the lentic ecosystem of that area the Chromium concentration varies between 5 mg/L to 15 mg/L , which is highly toxic. *Lemna* was grown in such Chromium contaminated system in vitro and the plant was found to be tolerant to a maximum Chromium concentration of 60 mg/L . It also showed efficient Chromium uptake with highest efficiency of 91.67% at a concentration of 10 mg/L in 50 hours after which the plants showed partial chlorosis but continued to complete its life cycle. Bacteria isolated from the rhizosphere water of this plant also showed tolerance to a maximum of 130 mg/L of Chromium concentration though its efficiency in removing Chromium remained comparatively lower than the plant at around 65% for all different concentrations up to 50 mg/L in 12 hours. Hence they together can serve as potential bioremediator for Chromium pollution. [27]

In one another study *Eichhornia crassipes* was introduced into a high rate algal pond (HRAP), in which the green alga *Chlorella vulgaris* was the dominant species. In this glass-house experiment, the combined algae \pm water hyacinth system removed 23% more nitrogen than did the normal HRAP. [28]

Rhizospheric micro-organisms are well known for their coexistence with plants and for providing nutrition to plants. [29] It was recently reported that these organisms facilitate the uptake of essential elements, such as iron, copper and zinc. [30] *Eichhornia crassipes* showed increased removal efficiency of heavy metals through the activity of its rhizospheric bacteria [31]. Zhang et al., studied a spherical copper-resistant bacterium, namely ACU, strain isolated from the rhizosphere of *E. crassipes* for its ability to increase the copper-resistant capability of another aquatic macrophyte, namely *Potamogeton crispus* L.; this plant has been widely used in purifying heavy metalpolluted water, and is sensitive to excessive copper exposure. These findings indicated that ACU grew by anchoring itself on the surface of *P. crispus* and could increase the ability of *P. crispus* to resist copper toxicity [32]. Rhizosphere-enhanced degradation has also been reported to occur with genetically engineered microorganisms. [33]

In 1995, Brazil and coworkers described the genetic construction of rhizosphere-competent pseudomonads which were engineered to contain the bacterial *bph* genes for biodegradation of PCBs. These strains have the potential to degrade PCBs in the rhizosphere and could be useful for bioremediation purposes. [34]

In addition, Crowley and co-workers [35] reported that a rhizosphere competent *Pseudomonas fluorescens* strain containing genes for 2,5-dichlorobenzoate degradation (2,5-DCB) had higher degradation rates in planted soil than non-planted ones. [36]

Five subgroups of sulfate-reducing bacteria (SRB) were detected by PCR in three macrophyte rhizospheres (*Polygonum densiflorum*, *Hymenachne donacifolia*, and *Ludwigia helminthoriza*)

and three subgroups in *Eichhornia crassipes* from La Granja, a floodplain lake from the upper Madeira basin. The SRB community varied according to the macrophyte species but with different degrees of association with their roots. The rhizosphere of the C4 plant *Polygonum densiflorum* had higher frequencies of SRB subgroups as well as higher mercury methylation potentials (27.5 to

36.1%) and carbon (16.06-5.40%), nitrogen (2.03-0.64%), Hg (94.50-6.86 ng Hg g⁻¹), and methyl mercury (8.25-1.45 ng Hg g⁻¹) contents than the rhizosphere of the C3 plant *Eichhornia crassipes*. Mercury methylation in *Polygonum densiflorum* and *Eichhornia crassipes* was reduced when SRB metabolism was inhibited by sodium molybdate. [36]

Table 2. Name of some microorganisms exhibiting resistance to metals

Aquatic macrophytes	Bacteria	Freshwater algae
<i>Arenicola christata</i>	<i>Agrobacterium</i>	<i>Anabaena cylindrica</i>
<i>Lemna minor</i> L. <i>trisolca</i>	<i>Bacillus</i> spp	<i>Chlamydomonas acidophila</i>
<i>Eichhornia crassipes</i>	<i>Desulfovibrio</i> spp	<i>Chlorella fusca</i> var <i>vacuolata</i>
<i>Hydrilla verticillata</i>	<i>Pseudomonas aeruginosa</i>	<i>Dictyococcus</i> sp
<i>Phragmites australis</i>	<i>Salmonella typhimurium</i>	<i>Euglena gracilis</i>
<i>Potamogeton pectinatus</i>	<i>Thiobacillus ferrooxidans</i>	<i>Hormidium rivulare</i>

FUTURE APPLICATIONS

The highly versatile metabolic capabilities of fungi and bacteria can be applied to reclaim polluted ecosystems and minimize the potential adverse effects of hazardous chemicals released to the environment. However, sufficient consortia of microorganisms, capable of degrading the contaminant(s), must be present, and environmental conditions conducive to degradation must be maintained. Environmental conditions onsite may significantly hinder microbial degradation of toxicants. In such cases, microbial degradation may be enhanced by altering conditions through nutrient additions, irrigation, or other interventions. The addition of external carbon sources may be especially important in such cases where the contaminant is degraded co-metabolically.

Vegetation may prove to be an important variable, affecting microbial degradation of unwanted chemicals these compounds. There is growing evidence that Rhizosphere treatment systems may be used successfully in the field. Establishing or selectively cultivating vegetation on a contaminated site is a relatively simple site management technique, which could have on ecosystems. Continued exploration of critical environmental variables, affecting the soil-plant-microbe-chemical relationship, will help to identify situations, in which bioremediation using vegetation may be appropriate.

CONCLUSIONS

The variety of plants and chemicals studied for evidence of microbial degradation in the rhizosphere strongly suggests that a diverse and synergistic microbial community, rather than a single species, is responsible for biotransformation of toxicants in the Rhizosphere.

Moreover, the information presented, herein illustrates the potential for Rhizosphere microbial communities to remediate systems through biotransformation of hazardous organic compounds in the root zone. Future research in this area should include investigations of the possible role and characterization of the microorganisms associated with different plant species and different histories of toxicant exposure.

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