6. Diversity and role of rhizobacteria associated to reed stands (*Phragmites australis*)

Lorenzo Brusetti
Luigimaria Borruso

6.1 Introduction

Rhizobacteria are microorganisms intimately associated to root systems. They are mainly involved in plant growth promotion, supply of nutrients, defence against phytopathogens and insect biocontrol. A particular importance is recognised with the biodiversity of rhizobacteria associated to plants involved in phytoremediation. These bacteria can play an important role in pollutants and xenobiotic transformation, detoxification and mineralisation. In this chapter, we review the biodiversity of these bacteria, underlining their role in the phytoremediation processes, specifically focusing on *Phragmites australis*. Case studies regarding the study sites in China are discussed.

The rhizosphere of submerged plants is defined as the narrow area of sediments characterised by peculiar chemical-physical properties, directly affected by root secretions (root exudates) released by living plants. Plant exudates include amino acids, carbohydrates, sugars, vitamins, mucilage and proteins (Bais et al. 2004), responsible for chemotactical attraction of microorganisms to the plants. Nutrient availability stimulates bacterial proliferation on roots' surface (rhizoplane) and in the surrounding area (Figure 1) (Hale et al. 1971). Microbial population density in the rhizosphere can be 1,000–2,000 times higher than in the surrounding bare soil, while metabolic activity of some taxa can be incremented to more than 1,000-fold. This phenomenon is known as the 'rhizosphere effect' (Egamberdieva et al. 2008; Berendsen et al. 2012).

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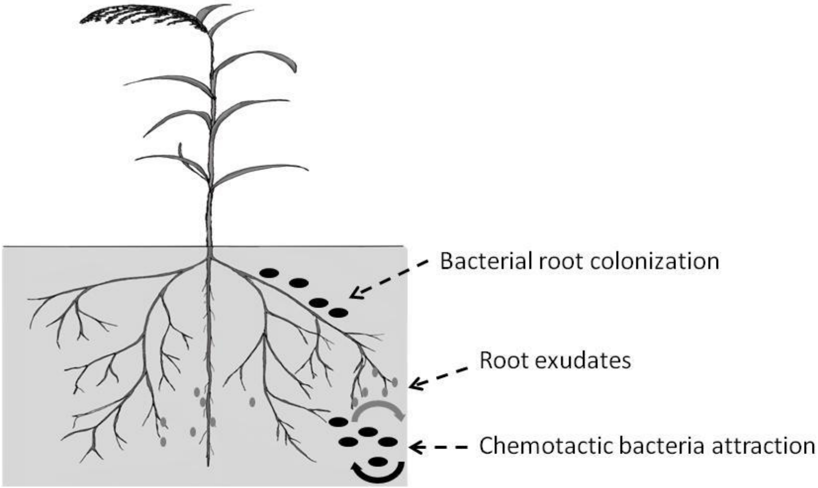

Figure 1 – Bacterial communities associated with plant roots and bacterial attraction via root exudates (Borruso 2014).

It has been reported that plant root exudation differs among species. Consequently, several experiments have found that microbial communities differ according to plant species. For example, a species-specificity of rhizobacterial communities associated to *Solanum tuberosum*, *Fragaria ananassa* and *Brassica napus* were found in an agricultural field (Smalla et al. 2001).

Other works reported that species-specificity of microbial communities due to the rhizosphere effect may be masked or partially masked by a variety of environmental matrices (Boeuf-Tremblay et al. 2005). In this case, particular environments such as hypersaline ponds (Lozupone & Knight 2007), where salt concentration is a stronger stressor and major responsible in shaping of rhizobacterial communities of *P. australis*, even if their root played an important role in the increment of microbial community diversity (Borruso 2014). Even other important abiotic parameters, such as soil or sediment texture (Schutter et al. 2001) or pH (Lauber et al. 2008) can sometimes play a more important role in shaping rhizobacterial communities than roots' exudates.

6.2 Microorganisms beneficial for plants

In the rhizosphere, there are a number of pathways linked to biogeochemical cycles. The exchange taking place between plants and microorganisms within the soil-root interface leads to several decomposition and mineralisation processes. Among them, the most important are symbiotic nitrogen fixation, phosphorous solubilisation, production of phytohormones, siderophores and biocontrol agents. These processes cause an increment of nitrogen and phosphorous assimilation at the root level, and a stimulation of the plant growth due to phytohormones (Glick 2012; Prashar et al. 2013).

6.2.1 Biological N_2 fixation

The most studied microorganisms involved in nitrogen fixation belong to the rhizobia group, nitrogen-fixing bacteria in symbiosis with plants of the family *Papilionaceae*. Rhizobia are a paraphyletic group ranging from α-Proteobacteria (i.e. *Sinorhizobium*, *Rhizobium* and *Azorhizobium*) to β-Proteobacteria (i.e. *Burkholderia*, *Herbaspirillum*) (Cocking 2003; Valverde et al. 2003; Udvardi & Poole 2013). Other species belonging to the genus *Azospirillum* are in symbiosis with the family of the Graminaceae, whereas *Frankia* sp. has an actinorhizal symbiosis with a nodular rhizosphere and rhizoplan of geni, e.g. *Alnus*, *Myricae*, *Betula* or *Coriaria* (Benson & Silvester 1993; Kennedy et al. 1997; Simonet et al. 1999).

6.2.2 Nutrient acquisition

Despite the high amount of phosphorous found in soil and sediments, most of it is not available (i.e. bound to calcium, aluminium or iron) for plant nutrition and growth. In this context, plants benefit of phosphate-solubilising bacteria such as *Enterobacter agglomerans* and *Azotobacter chroococcum*, which are able to convert phosphorous from an inorganic mineral form to a bioavailable form for plants (Kole et al. 1988; Kim et al. 1998). *Actinomycetae*, *Bacillus* and *Clostridium* play a key role in the humic acids formation by degrading lignin and cellulose plant tissues, therefore increasing soil fertility and bacterial activity (Rösch et al. 2002). In the rhizosphere, fungi, plants and bacteria compete for iron. Some bacteria, such as *Serratia*, *Pseudomonas*, *Rhodococcus* and *Acinetobacter*, are able to produce siderophores, molecules with a high affinity with ferric ions that cause iron solubilisation therefore improving its bioavailability (Prashar et al. 2013).

6.2.3 Biocontrol of plant diseases

The potential use of rhizobacteria able to stimulate plant growth and to protect plants from diseases and stresses has become a well-studied issue in the last decades. Bacteria involved in biocontrol are associated with a number of plant species widespread in many environments. These microorganisms are able to compete for space and resources, invading the pathogens ecological niche. Some strains belonging to *Pseudomonas*, *Bacillus* and *Stenotrophomonas* are involved in the production of antibiotics that affects phytopathogen growth and diffusion (Compant et al. 2005). For instance, *Pseudomonas fluorescens* and *P. chlororaphis* produce phenazine, responsible for the fungal disease suppression in plants (Kim et al. 2011).

6.2.4 Phytohormones production

Phytohormones are crucial in plant growth, development and response to environmental factors. Their production is a strategy adopted by bacteria to improve the interaction with roots. The production of gibberellic acid and cytokinins stimulates an increment of the dimension of root surface and length, which determines plant development positively (Kloepper et al. 2007).

6.3 Bioremediation and phytoremediation as potential tool for freshwater and sediment restoration

Freshwater sediments are considered a hot spot for the accumulation of a wide range of organic and inorganic contaminants. Metallic contaminants and nutrients represent a dramatic issue in several freshwater sediments, where the growing use of fertilisers and pesticides in agriculture has led to an increasing presence of nitrogen and phosphorous. The development of metallurgical industries has caused an increment in the level of heavy metals pollution in the environment (Su et al. 1994; Wang et al. 2001; Cheng 2003; Borruso 2014). Heavy metals negatively influence the quality of crops, atmosphere and water, or affect human and animal health (Zhang 1999; Liao

1993). Moreover, the significant presence of carbon and energy sources in sediments tends to determine an increase in biomass and diversity of microbes potentially able to degrade organic pollutants (Perelo 2010). Xenobiotics can be immobilised in sediments or transformed and degraded through biological processes carried out by microorganisms. In particular, bioremediation is the use of the metabolic potential of organisms, primarily microorganisms, to prevent or remove pollutants. There are two fundamental approaches that can be used in bioremediation, i.e. (1) the biostimulation of indigenous microbial communities through the addition of a suitable electron donor/acceptor or nutrients (Morgan & Watkinson 1989; Margesin & Schinner 2001), (2) the bioaugmentation, which is the use of an exogenous microbial community isolated from other environments and capable to break down or render harmless various contaminants (Vogel 1996).

Bioremediation can be applied only where environmental conditions permit proliferation and activity of microbial communities on site. The application often involves the variation of chemical-physical parameters to both optimise microbial growth and contaminants degradation. For example, some species of microorganisms are able to reduce metals toxicity through a number of metabolic pathways. Metals can be extracted from sediments thanks to organic acids produced by microorganisms, which can cause metals leaching by extracting them from ores into solution. *Geobacter* sp. and *Thiobacillus* sp. have the capability to transform mercury into methylated-mercury generating metal-leaching and sulphuric acid from the oxidation of elemental sulphur (Lovley 1995; Gomez & Bosecker 1999). *Penicillium simplicissimum* has the capability to transform the insoluble form ZnO into the soluble form Zn (Franz et al. 1991).

In the literature, it is reported that the role of bacteria in the bioremediation of toxic organic compounds includes polychlorinated biphenyls compounds (PCBs) and polycyclic aromatic hydrocarbons (PAHs). PCBs dechlorination is predominantly carried out by reductive processes operated by Cloroflexi. The dechlorination process has been found in different sediments affected by

PCBs contamination (Abramowicz 1995; Sowers & May 2013) and it causes a reduction in carcinogenicity potential of the PCBs (Abramowicz 1995). For example, anaerobic bacteria belonging to *Dehalococcoides* were isolated from river sediments and resulted to dechlorinate 64 PCB congeners (Bedard et al. 2007). The aromatic ring that characterises PAHs, has been recently demonstrated to be hydrogenated in anoxic conditions by bacteria that use nitrate and sulphate as terminal electron acceptors (Dou et al. 2009). *Pseudomonas* sp. and *Vibrio* sp. isolated from sediments were found to be able to degrade PAHs by using naphthalene as growth substrate (Rockne et al. 2000).

Efficiency of bioremediation may be increased by the interaction between bacteria and plant roots. Corn, wheat, macrofitae, soybean and common grasses are extensively used for bioremediation purposes due to their extensive fibrous root system extending into the rhizosphere. Usually, plants exposed to contaminants show a change in morphological and physiological traits. For instance, the growth reduction is mostly due to the increment in stress ethylene, which inhibits root elongation, nodulation and auxin transport. This effect can be mitigated via the presence of plant-grow-promoting bacteria that limit the inhibitory effects of various stresses (Glick 2010). Indeed, microbial communities strictly associated to the plant rhizosphere play a key role. Microbes play an important role in degradation, immobilisation and reduction of contaminants bioavailability (Glick 2010). It is well known that bacteria in the plant root zone break down pollutants such as organic toxins, chlorinated molecules, fuels and solvents (Mothes et al. 2010; Glick 2010).

There are different kinds of phytoremediation (Figure 2); they include:

- transportation and concentration of the substances from the environment into plant tissues (phytoextraction);
- degradation or breakdown of organic contaminants in metabolic processes carried out by plants and their associated microorganisms (phytodegradation);
- removal and absorption of toxins or excess of nutrients by plant roots from waters (rhizofiltration);
- reduction in the mobility and bioavailability of contaminants by plant roots and their associated bacteria in soil and/or groundwater (phytostabilisation); and
- plants uptake and transpiration of pollutants from soil or water, which are then released into atmosphere (phytovolatilisation) (Salt et al. 1995; Pilon-Smits 2005; Glick 2010).

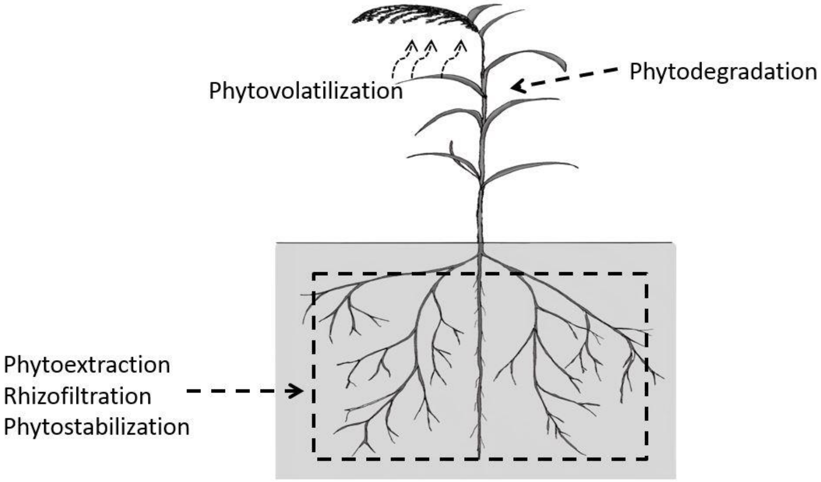

Figure 2 – Different types of phytoremediation processes involving removal and containment of contaminants (Borruso 2014).

For instance, *P. australis* stands are commonly used for phytoremediation, as they are able to collect and store in its tissues a number of toxic compounds as well as heavy metals (Southichak et al. 2006; Vymazal et al. 2009). *P.*

australis is indeed widespread in extremely polluted environments since it contains large amounts of lignin and cellulose, which are known to be able to absorb many heavy-metal ions from aqueous solutions (Vymazal et al. 2009).

6.4 Diversity and role in phytoremediation of the microorganisms associated with reed

The diversity of microorganisms associated with the rhizosphere is enormous, more than tens of thousands of species. Among bacteria, the most represented taxa are α/β/γ-Proteobacteria, Actinobacteria, Acidobacteria, CFB group and Firmicutes. Several studies regarding bacterial diversity associated with the rhizosphere of *P. australis* in natural and contaminated environments and its implications in the remediation process are reported in the literature. Sulphate-reducing rhizobacterial communities inhabiting Lake Valenci in Hungary highlighted a dominant presence of taxa affiliated to *Desulfovibrio* sp. and *Desulfotomaculum* sp. Moreover, the authors noticed a higher abundance of sulphate-reducing bacteria in the rhizosphere than in the surrounding bulk sediments probably due to the partly presence of oxygen in the rhizosphere and the root exudates (Vladár et al. 2008). In the same lake, culturable bacteria from the inner and outer rhizome surfaces were studied to assess the differences in the microbial composition between the healthy and the declining *P. australis* reed stands. A shift in the bacterial communities' composition in healthy and declining reed stands was found. Bacteria characterised by a fermentative metabolism, such as *Erwinia billingiae*, *Aeromonas sobria*, *Pantoea agglomerans*, *Pseudomonas azotoformans*, *Kocuria rosea* and *Bacillus* spp., resulted to be predominant in healthy *P. australis* reed stands. Differently, in declining reed stands bacteria with a saprotrophic metabolism such as *Acinetobacter* spp., *Aeromonas hydrophila*, *Curtobacterium luteum* and *Agrobacterium vitis* were isolated (Micsinai et al. 2003).

In a wetland located in northern China, Zhang et al. (2013b) analysed the rhizobacterial communities in different sites and noticed a shift according to the different levels of wetland degradation with a reduction in the abundance of Acidobacteria, Cyanobacteria and Fusobacteria and an increment in Actinobacteria in the more degraded sites. Several studies cover the role of *P. australis* and its rhizobacteria involved in phytoremediation. For example, the role of endophytic bacteria associated to *P. australis* in the Beijing Cuihu Wetland, China was analysed. The cloning library revealed that the predominant class was Proteobacteria with *Pleomorphomonas*, *Azospirillum*, and *Aeromonas*. These genera are involved in the phytoremediation of nitrogen, phosphorus, sulphur and some other organic compounds found in the wetland systems. The authors demonstrated a considerable reduction of P (56 %) total N (48 %) and organic matter (13 %) due to the presence of *P. australis* (Li et al. 2010b). Explorative results, from the samples collected in rhizosphere sediments associated to *P. australis* along the main drainage channel of the Hetao Irrigation District (see Chapter 2 and 10 for additional details), showed that the most abundant taxa were *Thiobacillus*, Nitrosomonadaceae and *Desulfobacterium*. These taxa are usually used for their bioremediation potential in particular for nitrate removal of sewage waste, of hydrocarbons degradation and on sites contaminated with chlorate such as pulp and paper industry wastewater (Borruso 2014).

P. australis has been widely used to treat wastewater from industries, medium-sized municipalities and farms (De Maeseneer 1997; Leonard & Swanson 2001; Vymazal et al. 2009) to improve water quality in freshwater environments. The municipal sewage of seven cities in the Czech Republic was treated trough filtration beds of constructed wetlands composed by *P. australis*. After the treatment, metal concentration (cadmium, nickel, lead, copper, chromium and zinc) resulted to have been decreased and become similar in concentration of unpolluted wetlands (Vymazal et al. 2010). In another study, bacterial communities in a constructed wetland located in Shanghai Mengqing Park were analysed and correlated to the different nutrients levels. A reduction of about 50 % of the ammonia and BOD_5 was reported. Moreover, a higher abundance of ammonia nitrifying bacteria in the ammonia-rich sediments revealed the key role of the microorganisms in ammonia removal (Park 2009). Additional studies showed that *P. australis* could contribute to antibiotics remediation (Conkle et al. 2008; Park et al.

2009; Hijosa-Valsero et al. 2011). Conventional wastewater and a constructed wetland treatment plants were compared to test the efficiency of antibiotic removals. After the treatment procedure, concentrations of tetracycline and Trimethoprim were evident. Decreased levels resulted in the constructed wetland treatment plant rather than in the conventional wastewater treatment plant, with a most probable cause and effect via the adsorption processes of *P. australis*. The authors explained these results with the presence of rhizobacterial communities and explained the important role and usage of antibiotic degradation (Hijosa-Valsero et al. 2011).

6.5 Conclusion

In the last few decades there has been an increasing interest by public and private institutions to manage the dangerous presence of pollutants. The traditional methods such as sediment removal, sediment oxidation or sediment filling are expensive and cause a high level of disturbance to the environment. In this context, selected plants and rhizobacterial strains due to their natural capabilities to bioremediate polluted environments can play a key role in land restoration processes. Indeed, microbial bioremediation coupled with phytoremediation processes in *P. australis* is demonstrated to be highly feasible and a well-established technology for cleaning up metals, pesticides, solvents and xenobiotics. The effectiveness of the phytoremediation approach can be increased with a better understanding of physiological, biochemical, molecular and genetic bases of the microbial communities associated to the plants used for the remediation process.

Nevertheless, a prudent policy in local territory administration that attend to *P. australis* stands both in extensive zones (e.g. lakes, large-sized ponds or marshes) and in narrower environments (e.g. natural or artificial water channels or small streams) will contribute to detoxification and remediation of highly polluted environments.

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