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Minireview

The rhizosphere as a reservoir for opportunistic human pathogenic bacteria

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Summary

During the last years, the number of human infections caused by opportunistic pathogens has increased dramatically. One natural reservoir of opportunistic pathogens is the rhizosphere, the zone around roots that is influenced by the plant. Due to a high content of nutrients, this habitat is a 'microbial hot-spot', where bacterial abundances including those with strong antagonistic traits are enhanced. Various bacterial genera, including *Burkholderia*, *Enterobacter*, *Herbaspirillum*, *Ochrobactrum*, *Pseudomonas*, *Ralstonia*, *Staphylococcus* and *Stenotrophomonas*, contain root-associated strains that can encounter bivalent interactions with both plant and human hosts. Mechanisms responsible for colonization of the rhizosphere and antagonistic activity against plant pathogens are similar to those responsible for colonization of human organs and tissues, and pathogenicity. Multiple resistances against antibiotics are not only found with clinical strains but also with strains isolated from the rhizosphere. High competition, the occurrence of diverse antibiotics in the rhizosphere, and enhanced horizontal gene transfer rates in this microenvironment appear to contribute to the high levels of natural resistances. While opportunistic bacteria from the rhizosphere have some properties in common, each of these emerging pathogens has its own features, which are discussed in detail for *Burkholderia*, *Ochrobactrum* and *Stenotrophomonas*.

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Introduction

Opportunistic [Lat. = highly adaptable] or facultative human pathogenic bacteria are pathogens which cause diseases only in patients with a strong predisposition to illness, particularly in those who are severely debilitated, immunocompromised or suffering from cystic fibrosis (CF) or HIV infections (Parke and Gurian-Sherman, 2001; Steinkamp *et al*., 2005). This group of bacteria cause the majority of bacterial infections associated with significant case/fatality ratios in susceptible patients in Europe and Northern America. A special group are those bacteria responsible for hospital-acquired diseases which are called nosocomial infections. For example, in intensive care units in Europe 45% of the patients were infected by opportunistic pathogens (Vincent *et al*., 1995). In Germany alone, approximately one million nosocomial infections occur per year, of which about 40 000 are fatal. In the last two decades, the impact of opportunistic infections on human health has increased dramatically. Despite this fact, little is known about the ecology and pathogenesis of these emerging pathogens. While some opportunists live as human commensals or in aquatic habitats, other bacteria originate from terrestrial ecosystems like the rhizosphere, which is the zone around plant roots. In this review we will focus on indigenous rhizobacteria that bear the potential to be risky to human health.

Many genera, including *Burkholderia, Enterobacter, Herbaspirillum, Ochrobactrum, Pseudomonas, Ralstonia, Staphylococcus* and *Stenotrophomonas*, contain root-associated bacteria that enter bivalent interactions with plant and human hosts. Several members of these genera show plant growth promoting as well as excellent antagonistic properties against plant pathogens and were therefore utilized for the development of biopesticides (Weller, 1988; Whipps, 2001). Another striking feature is their common ability to degrade a wide range of environmental

pollutants (Binks *et al*., 1995; Parke and Gurian-Sherman, 2001; Lee *et al*., 2002). However, many strains also successfully colonize human organs and tissues and thus cause diseases. The development of biotechnological applications in the biological control of plant pathogens and bioremediation of xenobiotics is now gaining momentum but more risk assessment needs to be carried out on these products to ensure that they do not inadvertently pose a threat to human health. The problems with biopesticides based on strains of the genus *Burkholderia* as discussed below underlines the importance of thorough risk assessment studies prior to registration (Govan *et al*., 2000).

This review discusses key aspects of rhizobacteria as possible facultative pathogenesis to humans: (i) the rhizosphere as an environment for opportunistic bacteria, (ii) the occurrence and diversity of opportunistic bacteria in the rhizosphere, (iii) characteristics of the opportunistic pathogens essential for infection and patient factors affecting predisposition, and (iv) general and specific characteristics on examples such as *Burkholderia, Ochrobactrum* and *Stenotrophomonas*.

Which biotic and abiotic parameters characterize the rhizosphere?

The rhizosphere, a term introduced by Hiltner already in 1904, is defined as the layer of soil influenced by root metabolism. In comparison to root-free soil, the rhizosphere forms a nutrient-rich niche for microorganisms as a result of exudation of compounds (Lynch, 1990). The rhizosphere and its inhabiting microorganisms fulfil important ecological functions, e.g. for nutrient cycles, and are responsible for plant growth and health (Sørensen, 1997). Additionally, this microenvironment is described as 'microbial hot-spot' where diverse interactions between organisms, beneficial as well as pathogenic, take place (Whipps, 2001). However, in practice the distinction between harmless and harmful bacteria is not always clear-cut and is strongly host dependent.

The rhizosphere effect describes the phenomenon that in rhizospheres, in comparison to bulk soil, the biomass and activity of microorganisms is enhanced as a result of the exudation of compounds. A long list of diverse substances, such as organic acids, sugars, amino acids, vitamins, polymeric carbohydrates is known to release by plant roots (Neumann and Römheld, 2001). However, the nutrient content is not constant and the different amounts of nutrients lead to changing osmolarities in the rhizosphere (Miller and Wood, 1996). Sloughing-off of root cap cells also contribute to rhizodeposition (Lynch and Whipps, 1991). In comparison to bulk soil, the high content of nutrients in the rhizosphere is an outstanding parameter of the rhizosphere. Thus, the competition between microorganisms for this nutrient-rich ecological site is very high. Bacteria, which are highly competitive, for example due to the production of antibiotic substances, can colonize the rhizosphere. Many rhizobacteria produce an extended list of antibiotics, like the fluorescent pseudomonads and *Streptomyces* species (Fravel, 1988; Raaijmakers *et al*., 2002; Weller *et al*., 2002). Antibiotics produced by rhizobacteria include 2,4-diacetylphloroglucinol, pyrrolnitrin, pyoluteorin and herbicolin A, all of which have also been detected directly in the rhizosphere (Thomashow *et al*., 1997). The occurrence and production of diverse antibiotic substances in the rhizosphere explains the frequent detection of bacteria with multiple antibiotic resistances in this microenvironment. Furthermore, in a recent study by Riesenfeld and colleagues (2004) antibiotic resistance genes were analysed by cultivation-independent methods using a metagenome approach. They found that soil samples harbour antibiotic resistance genes in a greater genetic diversity than has been accounted for by previous studies based on cultivation dependent studies.

So far, whenever antibiotics have been detected in aquatic or terrestrial habitats before, it has been in material obtained from these microhabitats, which are localized areas of intense microbial interaction. Indeed, highly complex interactions that can occur in the rhizosphere as has been reviewed by Whipps (2001). In addition, it was shown that bacteria which have antifungal or/and antibacterial activity are greatly enriched in the rhizosphere of different plants (Berg *et al*., 2002; Berg *et al*., 2005). Like a protection shield, they are able to prevent infections by plant pathogens (Weller, 1988). In addition to the advantage for stable colonization of the rhizosphere, production of antibiotics can protect rhizobacteria against grazing by protozoa, which was demonstrated for prodigiosin, a red, antifungal antibiotic produced by *Serratia* species (Groscop and Brent, 1964) and violacein, a purple pigment produced by *Chromobacterium violacaeum* (Matz *et al*., 2004). Some bacterial species with antagonistic activity towards other microorganisms including eukaryotic ones can also cause opportunistic infections in humans. Hence, the number of opportunistic pathogens may also be increased in the rhizosphere in comparison to bulk soil. The high content of nutrients results not only in an enhanced microbial activity it is also the reason why the rhizosphere is a preferential place for gene transfer (Knudsen *et al*., 1988). Due to horizontal gene transfer (HGT), the composition of bacterial genomes can be changed rapidly. Evidence for the conjugative transfer of chromosomal genes of the biocontrol strain *Pseudomonas fluorescens* CHA0 and the clinical strain *Pseudomonas aeruginosa* PAO1 in the rhizosphere of wheat in contrast to bulk soil was presented by Troxler and colleagues (1997). Alonso and colleagues (2000) showed that a *Stenotrophomonas* strain has acquired a cluster of

antibiotic and heavy metal resistance genes from gram-positive bacteria. Most of these genes are isoforms of genes previously found on *Staphylococcus aureus* plasmids. In conclusion, due to the high microbial population density and the rapidly changing conditions, the rhizosphere forms a unique habitat in terrestrial ecosystems, which may select for opportunistic pathogens, as these organisms are fiercely competitive for nutrients and produce many antimicrobial metabolites.

Which bacterial species potentially pathogenic to humans occur in the rhizosphere?

Bacterial colonization of the rhizosphere is highly influenced by the plant species (Smalla *et al*., 2001; Kowalchuk *et al*., 2002; Garbeva *et al*., 2004). In addition, the composition and proportion of bacteria that are antagonistic towards plant pathogens is strongly plant dependent (Berg *et al*., 2002). Table 1 summarizes examples of bacteria with a potential risk to humans. These bacterial species originate from the rhizospheres of diverse plants and were detected by cultivation-dependent as well as independent techniques. They were identified by partly sequencing of 16S rRNA genes or other identification systems, like FAME. For grouping of bacteria into risk groups the public databases, e.g. those by the German Collection of Microorganisms and Cell Cultures (http://www.dsmz.de) are used. These examples demonstrate that opportunistic pathogenic bacteria belong to many different species. Altogether, 27 different genera with 36 species were reported until now. Some species, e.g. *Burkholderia cepacia* and *Stenotrophomonas maltophilia* were detected in most of the investigated rhizospheres whereas others, e.g. the occurrence of *Serratia* spp.,

Table 1. Examples for the occurrence of potentially human pathogenic species in the rhizosphere of diverse plants.

No.	Origin	Species	References
1.	Rhizosphere of oilseed rape (*Brassica napus*)	*Aeromonas salmonicida, Bacillus cereus, Burkholderia cepacia, Chromobacterium violaceum, Chryseomonas luteola, Chryseobacterium indologenes, Cytophaga johnsonnae, Enterobacter intermedius, Pantoea agglomerans, Proteus vulgaris, Pseudomonas aeruginosa, Salmonella typhimurium, Serratia grimesii, S. liquefaciens, S. proteamaculans, S. rubidaea, Sphingomonas paucimobilis, Stenotrophomonas maltophilia*	Berg *et al*. (2002) Berg *et al*. (1996) Graner *et al*. (2003)
2.	Rhizosphere of potato (*Solanum tuberosum*)	*Achromobacter xylosoxidans, Alcaligenes faecalis, Bacillus cereus, Burkholderia cepacia, Chromobacterium violaceum, Cytophaga johnsonae, Enterobacter amnigenus, E. cloacae, E. intermedius, Flavimonas oryzihabitans, Francisella philomiragia, Janthinobacterium lividum, Kluyvera cryorescens, Ochrobactrum anthropi, Pantoea agglomerans, Proteus vulgaris, Pseudomonas aeruginosa, Serratia grimesii, Sphingobacterium spiritivorum, Sphingomonas paucimobilis, Staphylococcus epidermis, S. pasteuri, S. xylosus, Stenotrophomonas maltophilia*	Gupta *et al*. (2001) Lottmann and Berg (2001) Berg *et al*. (2002) Reiter *et al*. (2002) Krechel *et al*. (2004) Sessitsch *et al*. (2004) Berg *et al*. (2005) Lottmann *et al*. (1999)
3.	Rhizosphere of strawberry (*Fragaria x ananassa)*	*Acinetobacter baumannii, A. calcoaceticus, Burkholderia cepacia, Pantoea agglomerans, Proteus vulgaris, Salmonella typhimurium, Serratia grimesii, S. proteamaculans, Staphylococcus epidermis*	Berg *et al*. (2002) Berg *et al*. (2005)
4.	Rhizosphere of *Medicago sativa*	*Stenotrophomonas maltophilia, Flavobacterium johnsonae*	Schwieger and Tebbe (2000)
5.	Rhizosphere of *Chenopodium album*	*Stenotrophomonas maltophilia, Flavobacterium johnsonae*	Schwieger and Tebbe (2000)
6.	Rhizosphere of sunflower (*Helianthus*) *annuus*	*Burkholderia cepacia, Stenotrophomonas maltophilia, Flavobacterium odoratum*	Hebbar *et al*. (1991)
7.	Rhizosphere of maize (*Zea mays*)	*Burkholderia cepacia, Klebsiella pneumoniae, Serratia liquefaciens, Sphingomonas paucimobilis, Stenotrophomonas maltophilia*	Lambert *et al*. (1987) Dalmastri *et al*. (1999) Chelius and Triplett 2000)
8.	Rhizosphere of rice (*Oryza sativa*)	*Aeromonas veronii, Alcaligenes xylosoxidans, Enterobacter cloacae, Ochrobactrum anthropi, Pseudomonas aeruginosa, Serratia marcescens*	Mehnaz *et al*. (2001) Gyaneshwar *et al*. (2001) Tripathi *et al*. (2001)
9.	Rhizosphere of wheat (*Triticum sativum*)	*Burkholderia cepacia, Enterobacter agglomerans, Ochrobactrum anthropi, Ochrobactrum tritici, Pseudomonas aeruginosa, Salmonella typhimurium, Staphylococcus aureus, Stenotrophomonas maltophilia, Streptococcus pyogenes*	Morales *et al*. (1996), Germida and Siciliano (2001)

seem to be specific for the rhizosphere of oilseed rape. Many bacteria closely related to important nosocomial pathogens like *P. aeruginosa, Serratia marcescens, B. cepacia* and *S. maltophilia* are very prominent rhizosphere colonizers. Furthermore, the rhizosphere harbours bacterial species that have been reported to cause skin and wound infections (*Bacillus cereus, Proteus vulgaris, Pseudomonas* spp.), urinary tract infections (*P. vulgaris, B. cepacia*), and to be associated with CF patients (*B. cepacia, P. aeruginosa*). Although these data provide evidence for the occurrence of opportunistic pathogens in the rhizosphere very little information on their virulence relative to the one of their clinical counterparts is available. Therefore, the risk assessment for each strain is necessary and will be a very important objective for microbiologists in the future. For example, strains could be analysed for the occurrence of specific virulence determinants.

Bacteria living in the rhizosphere can have a neutral, pathogenic or beneficial interaction with their host plant. In healthy plants, the occurrence of pathogenic bacteria is low and controlled by the plant defence system and plant beneficial bacteria enriched by the rhizosphere conditions. The latter group is comprised of plant growth promoting rhizobacteria (PGPR), which influence plant growth by producing phytohormons or enhancing the availability of nutrients, strains that induce systemic resistance in plants, and truly antagonistic bacteria (Van Loon *et al*., 1998, Whipps, 2001). Antagonists are naturally occurring organisms that express traits that enable them to interfere with pathogen growth, survival and infection. Bacteria antagonistic to plant pathogens represent an important part of the rhizosphere communities. The proportion of antagonistic strains amounts up to 35% of the culturable bacteria (Opelt and Berg, 2004). The majority of rhizobacterial species which emerged as pathogens belongs to the group of antagonistic bacteria, e.g. *P. aeruginosa, S. aureus*, *Burkholderia* spp. and *S. maltophilia*. To our knowledge, only for *Ochrobactrum* spp. no antagonistic activity has been reported.

What turn rhizobacteria into opportunistic pathogens?

Rhizobacteria with antagonistic activity against eukaryotes are able to interact with their hosts by various mechanisms. These mechanisms include (i) inhibition of pathogens by antibiotics, toxins and bio-surfactants [antibiosis], (ii) competition for colonization sites and nutrients, (iii) competition for minerals, e.g. for iron through production of siderophores or efficient siderophore-uptake systems, (iv) degradation of pathogenicity factors of the pathogen such as toxins, and (v) parasitism that may involve production of extracellular cell wall-degrading enzymes such as chitinases and β-1,3 glucanase (Fravel, 1988; Bloemberg and Lugtenberg, 2001; Raaijmakers *et al*., 2002; De Souza *et al*., 2003a). Furthermore, the importance to recognize and adhere to plant roots for all plant-associated bacteria is underlined in many biocontrol studies. An early step in the establishment of a plant–bacterium interaction is attachment of cells to plant roots, in which for example fimbriae and cell-surface proteins are involved (Lugtenberg and Dekkers, 1999). For the colonization of plant roots, flagella, O-antigen of lipopolysaccharides (LPS), the growth rate and the ability to grow on root exudates are important (Lugtenberg and Dekkers, 1999). Other factors that contribute to rhizosphere fitness include the ability to use seed and root exudates as carbon sources or, more in general, ecological and nutritional versatility. In addition, synthesis of compatible solutes by bacteria contributes to survival under changing osmolarities, which occur in the rhizosphere (Miller and Wood, 1996).

Pathogenic bacteria can cause damage to the host. Steps of pathogenesis include invasion, colonization and growth, and several strategies to establish virulence, the relative ability of a pathogen to cause disease in the host. In addition, recognition and adherence to human cells is necessary to establish pathogenicity. Many mechanisms involved in the interaction between antagonistic plant-associated bacteria and their host plants are similar to those responsible for pathogenicity of bacteria (Rahme *et al*., 1995). In addition, these mechanisms may also be involved in colonizing the human body (Cao *et al*., 2001), as shown in Fig. 1. An additional important feature, which is necessary to survive on/in humans, is the ability to grow at 37°C. Interestingly, we found that the majority of rhizobacteria isolated from oilseed rape and strawberry in Northern Germany is able to grow at 37°C (G. Berg, unpublished results). Concerning the induction of resistance in plants by rhizosphere bacteria, the stimulation of the plant's innate immunity by bacterial components like flagella or lipopolysaccharide resembles in many ways the response of the mammalian innate immune system towards pathogens. This systemic acquired resistance (SAR) was reviewed by Van Loon and colleagues (1998). However, also a systemic response of plants towards non-pathogens was described and termed induced systemic resistance (ISR) (Van Loon *et al*., 1998). Recently, *N*-acyl homoserinelactone signalling compounds of *Serratia liquefaciens* involved in quorum sensing were shown to induce components of the innate immune system of tomato plants, like the pathogenesis-related protein and chitinase (Hartmann *et al*., 2004) which possibly points to an additional response pattern. In contrast, the interaction of rhizobacteria with plants via phytohormons like indole-3-acetic acid appear to be a result of coevolution and thus are unique to plant-associated strains. However, indepen-

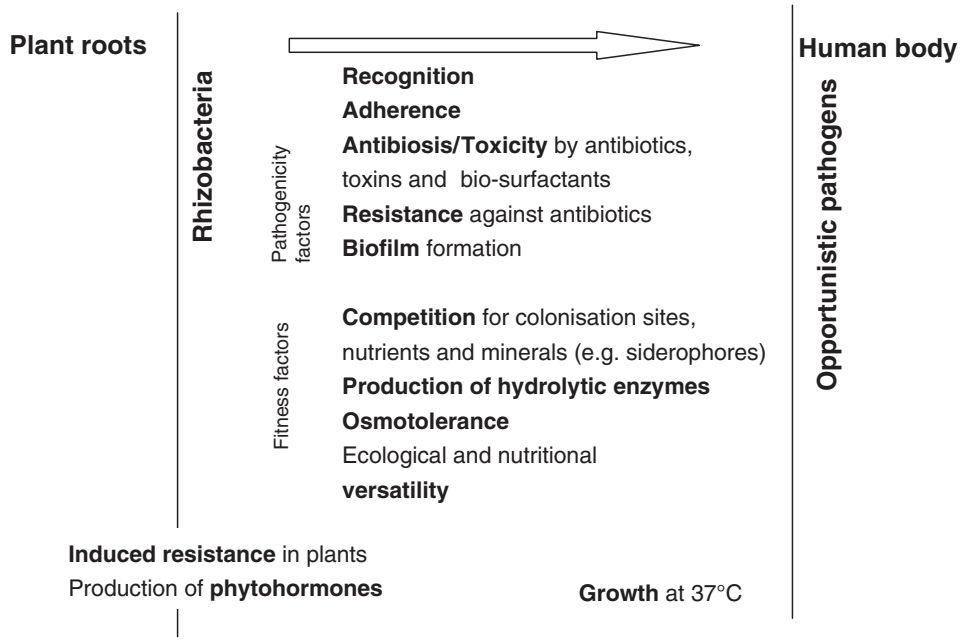

Fig. 1. Supposed mechanisms of rhizobacteria responsible for colonization of plant roots and humans and antagonism/pathogenicity.

dent of their origin, clinical and rhizosphere-associated *Stenotrophomonas* strains can produce indole-3-acetic acid although environmental strains tend to produce a higher amount of the phytohormone (Suckstorff and Berg, 2003).

Identification of virulence factors is particularly difficult in many opportunistic human pathogens because of the lack of adequate animal models. However, for some of these bacteria alternative, often non-mammalian infection models were developed. For *Pseudomonas, Stenotrophomonas* and *Burkholderia* pathogenesis models using the slime mould fungus *Dictyostelium discoideum* and the nematode *Caenorhabditis elegans* are available (Köthe *et al*., 2003; Alonso *et al*., 2004). Several studies provided evidence that similar or even identical functions are responsible for beneficial interactions with plants and virulence in humans. For example, the involvement of siderophore-uptake systems or extracellular enzymes is common to both beneficial bacteria and human pathogens (Tan *et al*., 1999). Dörr and colleagues (1998) reported that type IV pili of the plant-associated *Azoarcus* sp. BH72 are responsible for the adhesion on plant and fungal cells. Furthermore, the amino acid sequence of the pilus showed a high similarity to pili of the human-associated strains of *P. aeruginosa* and *Neisseria gonorrhoeae*. While a mutant of *Pseudomonas fluorescens* deficient in a laurolyl transferase involved in lipid A biosynthesis resulted in an impaired root colonization (Dekkers *et al*., 1998), a similar mutant of *Salmonella typhimurium* is limited in its ability to colonize organs of the lymphatic system of mice (Jones *et al*., 1997). In a study published by Alonso and colleagues (1999) it was shown that clinical and environmental isolates of *P. aeruginosa*, which is the major cause for morbidity and mortality in CF patients, share several phenotypic traits with respect to both virulence and environmental properties. Several studies support the view that the environmental strains are indistinguishable from those from clinical sources in terms of genotypic, taxonomic or metabolic properties (Kiewitz and Tümmler, 2000; Finnan *et al*., 2004). Wolfgang and colleagues (2003) used a whole-genome DNA microarray to determine the genome content of 18 *P. aeruginosa* strains from different sources. While a remarkable conservation of genes including those encoding nearly all known virulence factors was observed, preferential sites for the integration of novel genetic material were also detected. Data of recent analyses revealed that the *P. aeruginosa* genome is made up of a conserved core and variable accessory segments (Ernst *et al*., 2003; Wolfgang *et al*., 2003). Restriction fragment length polymorphism based on 14 single nucleotide polymophisms (SNPs) of conserved loci in 111 *P. aeruginosa* isolates of diverse habitats allowed specific fingerprinting and a discrimination of strains (Morales *et al*., 2004). Interestingly, the highly virulent clinical strain CHA shared their SNP genotype with two environmental strains, which again supports the view that *P. aeruginosa* isolates that thrive in non-clinical habitats possess all functions to potentially infect mammals. In addition, differences between environmental strains and those which cause infections may occur at the level of regulation of genes, rather than their pres-

ence or absence (Parke and Gurian-Sherman, 2001). Production of antagonistic compounds by rhizobacteria has been shown to be regulated by two-component systems (Keel and Défago, 1997) or by quorum-sensing systems (Pierson and Pierson, 1996; Steidle *et al*., 2002). Interestingly, the response regulator gene *gacA* influence the production of secondary metabolites in pathogenic and antagonistic *Pseudomonas* strains (De Souza *et al*., 2003b). Furthermore, mutations in *gac*A and *gac*S are the basis for phase variation and different regulation of secondary metabolites and enzymes in *Pseudomonas* sp. PFL1171 (Van den Broek *et al*., 2005).

A further hint that similar traits are involved in the interaction between bacteria and eukaryotes was obtained from studies which analyse the rhizosphere competence of human pathogenic bacteria. Due to the idea that plants may serve as reservoirs of human-associated bacteria in long-term space missions containing bioregenerative life support systems, Morales and colleagues (1996) analysed the ability of five pathogens to colonize hydroponically grown wheat. While *B. cepacia* and *P. aeruginosa* strains showed considerable growth in the rhizosphere, *Escherichia coli* and *S. aureus* survived without substantial growth and *Streptococcus pyogenes* cells died in the rhizosphere although all these bacterial species were also found in the wheat rhizosphere. Troxler and colleagues (1997) also reported that the human pathogen *P. aeruginosa* is an excellent colonizer of the wheat rhizosphere and able to protect wheat and cucumber rhizospheres from the fungal pathogens *Gaeumannomyces graminis* and *Pythium ultimum*. Another study shows that clinical and root-associated *Stenotrophomonas* strains are able to colonize the strawberry rhizosphere and increase plant growth by stimulating root growth and root hair development (Suckstorff and Berg, 2003). There is no clear evidence that strains from the rhizosphere directly colonize the human body, but the appearance of unique clones in individual patients suggests that they may be independently acquired from the environment (Parke and Gurian-Sherman, 2001).

An important mechanism by which harmless bacteria can behave as pathogens is change of host or host niche, upon which their virulence potential is frequently revealed to its full extend. This mechanism is clearly relevant for opportunistic pathogens from the rhizosphere. In addition, other mechanisms such as structural changes of the bacterial chromosome due to gene acquisition and loss, recombination and mutations can lead to bacterial pathogenicity (for a review see Hacker *et al*., 2003). Genes responsible for pathogenicity or fitness of bacteria often occur as genomic islands, which are blocks of DNA with signatures of mobile genetic elements (Hacker and Carniel, 2001). They are called 'fitness islands' or 'pathogenicity islands' according to their function.

Which group of patients get infections by opportunistic pathogens?

With advances in medical technology, and the growth of at-risk populations, such as those with AIDS, the incidence of infections due to opportunistic pathogens is expected to increase. The group of patients with a predisposition for infections with opportunistic pathogens also includes older patients with chronic diseases, patients with long-term antibiotic therapy, and those who are immuno-suppressed. Denton and Kerr (1998) review risk factors for *Stenotrophomonas* infections including long-term antibiotic therapy, catheter, neutropenia or cytotoxic chemotherapy, prolonged hospitalization, admission to intensive care units, and corticosteroid therapy. Patients with CF are particularly susceptible to infections, because they have sticky, dehydrated mucus that lines epithelial cells of the digestive tract and the lungs, leading to nutritional disorders and chronic lung infections caused by a succession of microorganisms. Pulmonary infections typically begin in infancy with *S. aureus*, followed by *Haemophilus influenzae* in early childhood, and *P. aeruginosa* and *Burkholderia* spp. in adolescence (Parke and Gurian-Sherman, 2001). These facts underline the importance of a sensitive balance between the pathogen and the host in its impact on disease severity.

Examples for opportunistic pathogens in the rhizospheres

Burkholderia *spp. – multitalented bacteria*

The genus *Burkholderia* comprises more than 30 species, many of which are important pathogens of plants, animals and humans (Coenye and Vandamme, 2003). The highly pathogenic species *Burkholderia pseudomallei* and *Burkholderia mallei* are the causative agents of melioidosis and glanders, respectively, and are deemed potential agents of bioterrorism. *Burkholderia pseudomallei* is a saprophytic organism that is routinely isolated from soil and particularly rice paddies (Brook *et al*., 1997). In addition to the primary human pathogens, several other *Burkholderia* species have emerged as important opportunistic pathogens. In fact, over the past two decades, the number of human infections caused by *Burkholderia*-like bacteria has increased markedly (Govan *et al*., 1996; Coenye and Vandamme, 2003). Polyphasic-taxonomic studies revealed that these organisms comprise a very heterogeneous group of strains, collectively referred to as the *B. cepacia* complex (Bcc). This complex consist of at least nine validly described species, which share a high degree of 16S rDNA (98–100%) sequence similarity, and only moderate levels of DNA-DNA hybridization: *B. cepacia*, *Burkholderia multivorans*, *Burkholderia cenocepacia*, *Burkholderia stabilis*, *Burkholderia vietnamiensis*,

Burkholderia dolosa, *Burkholderia ambifaria*, *Burkholderia anthina* and *Burkholderia pyrrocinia*. Bcc strains can cause life-threatening lung infections in patients requiring mechanical ventilation and in individuals with chronic granulomatous disease or CF. Cystic fibrosis patients usually acquire Bcc strains late in the course of the disease when the patients are already chronically colonized with *P. aeruginosa*. The clinical outcome of this coinfection is variable and unpredictable, ranging from asymptomatic carriage to a fulminant and fatal pneumonia, the so-called 'cepacia syndrome' (Isles *et al*., 1984). Even though all Bcc strains have been isolated from CF patients, *B. multivorans* and *B. cenocepacia* are most commonly found in clinical samples (Coenye and Vandamme, 2003). In contrast to *P. aeruginosa* very little is known about the pathogenic mechanisms and virulence determinants of Bcc strains.

Although Bcc strains are ubiquitously distributed in nature, they have been most frequently isolated from the rhizosphere of crop plants and grasses (for a review see Tabacchioni *et al*., 2002). Bcc strains have attracted considerable interest from the agricultural industry as biological control agents (Govan *et al*., 1996; Holmes *et al*., 1998) to repress soilborne plant pathogens and thus improve germination and crop yields (Hebbar *et al*., 1998). One of the best-investigated *Burkholderia* biocontrol strain is *B. ambifaria* AMMD, which exhibits strong antagonistic activity against the plant pathogens *Pythium aphanidermatum* and *Aphanomyces euteiches* (Parke *et al*., 1991; King and Parke, 1996; Heungens and Parke, 2000). The strong biocontrol activity observed with some Bcc strains is, at least in part, a result of the production of a large variety of compounds with antifungal activity, including cepacin, altericidins, pyrrolnitrin, cepacidines, siderophores and a non-ribosomally synthesized lipopeptide (see Kang *et al*., 1998 and references therein). Moreover, field tests revealed that Bcc strains can colonize the rhizosphere of several crops, regardless whether a pathogen was present or not (Parke *et al*., 1991; Tran Van *et al*., 2000). At present, the factors that are required for the colonization of plants are unknown. It was realized already in the 1960s that Bcc strains exhibit an extraordinary metabolic versatility, including its ability to degrade a wide range of organic compounds (Stanier *et al*., 1966). Several strains have been identified that show a great potential as bioremediation agents, degrading recalcitrant herbicides such as 2,4-dichlorophenoxyacetic acid or 2,4,5-trichlorophenoxyacetic acid (the principal component of 'Agent Orange') (Kilbane *et al*., 1982), herbicides and pesticides (Holmes *et al*., 1998), and trichloroethylene (Folsom *et al*., 1990).

Evidence that has accumulated over the past few years showed that the same *Burkholderia* clonal lines that occur in the environment are also capable of infecting CF patients. For example, the *B. cepacia* type strain ATCC 25416T, which was originally isolated from a rotten onion, was also isolated from a CF patient in the UK (Govan *et al*., 2000). Likewise, *B. cenocepacia* PHDC, which is frequently recovered from CF patients in the mid-Atlantic region of the USA, is also found in agricultural soils of this region (LiPuma *et al*., 2002). A recent phylogenetic analysis of the *recA* genes of a large number of Bcc strains resulted in the identification of several clonal pairs of strains each from distinct environmental and clinical sources (Payne *et al*., 2005). In conclusion, while *B. cepacia* strains have a great biotechnological potential, its commercial use is severely hampered by the potential threat these strains pose on human health. Further work will be required to identify virulence factors of these bacteria to eventually link the pathogenic potential of a strain to its genetic repertoire.

Ochrobactrum *spp. – ubiquitous and frequent inhabitant of rhizospheres and other environmental habitats*

The genus *Ochrobactrum* belongs to the family *Rhizobiaceae* in the alpha 2 subclass proteobacteria and was first described by Holmes and colleagues (1988). The genus *Brucella,* severe human pathogens, is its closest phylogenetic relative (Velasco *et al*., 1998). Until now, five different species of *Ochrobactrum* were described: *Ochrobactrum anthropi* (Holmes *et al*., 1988), *O. intermedium* (Velasco *et al*., 1998), *O. tritici* (Lebuhn *et al*., 2000), *O. grignonense* (Lebuhn *et al*., 2000) and *O. gallinifaecis* (Kaempfer *et al*., 2003). *Ochrobactrum anthropi* was originally known as bacterial pathogen isolated from human clinical specimens, especially from immunocompromised patients (Swings *et al*., 1992). However, *Ochrobacter* species were shown to exist in a large variety of habitats, such as arsenical cattle dipping fluid (Holmes *et al*., 1988), together with *S. maltophilia* as symbionts of *Steinernema scapterisci* (Aguillera *et al*., 1993), as endophytic bacteria in cotton (McInroy and Kloepper, 1994), on the surface of wheat roots (Sato and Jiang, 1996, Lebuhn *et al*., 2000), and, most recently, as endophytes of deep-water rice (Verma *et al*., 2004) and nodule symbionts of *Acacia mangium* (Ngom *et al*., 2004). Using the monoclonal antibody mAb 2.11 raised against *O. anthropi* 1a, isolated from agricultural soil in Scheyern, Germany, which was shown to have a genus-wide specificity, 10^4–10^6 reactive bacterial serotypes were quantified in different agricultural soils (Lebuhn *et al*., 2000) and also in river sediments (unpublished). As no specific enrichment medium was available for *Ochrobactrum*, the monoclonal antibody mAb 2.11 was used to immunotrap bacteria of this genus from different soils. The phylogenetic analysis using these isolates were performed using REP-PCR-fingerprinting, 16S rDNA sequencing, DNA-DNA-hybridization as well as phenotypic fingerprinting (BIOLOG) and demonstrated that *O. tritici* occurred only in the rhizosphere while *O.*

anthropi, *O intermedium* and *O. grignonense* were isolated from soil and rhizosphere (Lebuhn *et al*., 2000). However, it was not possible to clearly separate the isolates according to their habitat (place of isolation) within the *Ochrobactrum* spp. on the basis of genetic and phenotypic characters (Bathe *et al*., 2005). Even on the basis of the ITS1-region sequences clinical and environmental isolates of the *Ochrobactrum* spp. could not be separated successfully (M. Lebuhn, unpublished results). However, it is possible to clearly separate *Brucella* from *O. intermedium* on the basis of phenotypic and molecular analyses (Lebuhn *et al*., 2000). In their diverse environmental niches (rhizosphere, soils, sediments and activated sludge) *Ochrobactrum* spp. are very versatile; they are capable of denitrification and thus can use alternative electron acceptors for energy generation in the case of a lack of oxygen. Some *Ochrobactrum* spp. isolates were found to be able to degrade quite recalcitrant chemicals, like atrazine or 3-chloroaniline (Goris *et al*., 2003). The medical relevance of *O. anthropi* infections has been documented by Jelveh and Cunha (1999). Clinical problems after transplantations related to *O. intermedium* were also reported (Moller *et al*., 1999).

Stenotrophomonas *spp. – multiresistant rhizobacteria*

The genus *Stenotrophomonas*, a very heterogenous group within the γ-subclass of *Proteobacteria*, comprise four recognized species: *S. maltophilia* (Palleroni and Bradbury, 1993), *S. acidaminiphila* (Assih *et al*., 2002), *S. nitritireducens* (Finkmann *et al*., 2000) and *S. rhizophila* (Wolf *et al*., 2002). So far, all validly described *Stenotrophomonas* species have shown a high intrageneric diversity and heterogeneity at the phenotypic and genotypic level (Berg *et al*., 1999; Hauben *et al*., 1999). *Stenotrophomonas* species play an important role in nature, especially in the global element cycle (Ikemoto *et al*., 1980), and they are frequently used in applied microbiology and biotechnology, e.g. in biological plant protection (Berg *et al*., 1994; Kobayashi *et al*., 1995; Nakayama *et al*., 1999). Recent interest has focused on the *Stenotrophomonas'* capability to degrade xenobiotic compounds (Binks *et al*., 1995; Lee *et al*., 2002) and their potential for decontaminating soil (bioremediation). Furthermore, over the last decade multidrug-resistant *S. maltophilia* has become increasingly significant causing case/fatality ratios in certain patient populations, particularly in those who are severely debilitated or immuno-suppressed (for a review see, Denton and Kerr, 1998). *Stenotrophomonas maltophilia* is associated with a broad spectrum of clinical syndromes, e.g. bacteraemia, endocarditis, respiratory tract infections, etc. *Stenotrophomonas* isolates occur ubiquitous and cosmopolitan, and were preferentially isolated from the rhizospheres of diverse plants all over the world especially those of *Brassicacaea* (Juhnke and Des Jardin, 1989; Berg *et al*., 1996). In addition, the bacterium has been isolated from a wide range of nosocomial sources, e.g. contact lens care systems, dialysis machines, ice-making machines, nebulizers and inhalation therapy equipment (Denton and Kerr, 1998).

Presently, the virulence factors responsible for the pathogenicity of these organisms are not well understood but several traits have been implicated in antagonism/pathogenicity. *Stenotrophomonas* strains of environmental and clinical origin are able to adhere to many surfaces, e.g. to human epithelial respiratory cells (De Abreu Vidipó *et al*., 2001) and they are equally able to colonize the rhizosphere of strawberry (Suckstorff and Berg, 2003). Furthermore, it was reported that many strains have the capability to produce extracellular enzymes, e.g. DNase, RNase, proteases, lipases, chitinases and elastases. Antibiotics synthesized by *Stenotrophomonas* are involved in pathogen suppression on plants whereas their importance for pathogenicity is still unclear (Jacobi *et al*., 1996; Nakayama *et al*., 1999). *Stenotrophomonas maltophilia* strains are often highly resistant to multiple antibiotics (Alonso and Martinez, 1997). Results obtained by Minkwitz and Berg (2001) indicated that the antibiotic resistance profile of *S. maltophilia* isolates was not associated with their origin (e.g. clinical and environmental especially from the rhizosphere). Such findings would suggest that strains of *S. maltophilia* did not acquire their antibiotic resistance during antibiotic therapy in the clinic/hospital environment. Although a general method to differentiate between clinical and environmental *S. maltophilia* strains could not be established (Minkwitz and Berg, 2001), functional genes were used to differentiate between plant-associated strains of *S. rhizophila* and potentially human-pathogenic strains of *S. maltophilia* (G. Berg, unpublished results).

Rhizosphere bacterial genera without known occurrence of opportunistic pathogens

However, there are also genera of rhizosphere bacteria known without having examples for pathogenic potential in plants, animals and humans. To our knowledge, these are the genera *Azospirillum* (Hartmann and Baldani, 2003) and *Gluconacetobacter* (Munoz-Rojas and Caballero-Mellado, 2003) which both harbour a diversity of diazotrophic PGPR. Based on *Bacillus thuringiensis* a natural insecticide called '*Bt*' was developed, that has been used for decades by farmers to control crop-eating insects and by the World Health Organization to kill mosquitoes. The ecological behaviour of *B. thuringiensis* and their relatives' *Bacillus anthracis* and *B. cereus* (both human pathogens) is not clear but *B. thuringiensis* was reported also from the rhizosphere (Jensen *et al*., 2003).

Bacillus thuringiensis is non-toxic to beneficial insects, and during the long time of application no risk for humans has been reported. Long-term-sheep feeding with *B. thuringiesis*-based biopesticed preparations (10^{12} spores daily for 5 months) did not harm to animals (Hadley *et al*., 1987). Moreover, it can also be assumed that strains, which are unable to grow at 37°C, do not pose a risk on human health and thus may be considered safe for biotechnological applications.

Conclusions and further considerations

In future, problems with opportunistic infections will become even more severe due to the increasing numbers of at-risk individuals in the human population. Management strategies for opportunistic pathogenic bacteria are urgently in demand to reduce the burning problem of environmentally acquired infections. In particular, it is necessary to develop alternative therapeutic agents and to establish new models for pathogenicity testing and epidemiological forecast prediction models. In addition, it will be important to understand the biology of these ambivalent bacteria in better detail, especially the genetic basis of their pathogenicity in both their natural hosts and humans. More information about the colonization of the rhizosphere as reservoir for opportunistic human pathogens and their routes of transmission is urgently needed. This will help to assess the potential risk for the use of certain groups of rhizosphere bacteria for biotechnological applications.

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