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Mercury affects the distribution of culturable species of *Pseudomonas* in soil

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Abstract

Pseudomonas bacteria isolated during 52 days on Gould's S1 agar from soil spiked with 0, 3.5 and 15 mg Hg(II) kg soil^{-1} were characterised to reveal whether mercury affected them differently. Isolates from the treatments with 0 and 15 mg Hg kg^{-1} were characterised using FT-IR characterisation and subsequent 16S rDNA partial sequencing of representative isolates. To verify the selectivity of Gould's S1 agar and the FT-IR characterisation, all 450 isolates were subjected to the following tests: Gram-determination, catalase and oxidase activity, pigment production on PDA and growth at different temperatures. Furthermore, the isolates were tested for their ability to grow on agar amended with 10 mg Hg kg^{-1} as an indication of mercury resistance. We found that up to 80% of the isolates in soil amended with 15 mg Hg kg^{-1} were mercury-resistant, whereas only up to 20% were resistant in the treatments with 0 and 3.5 mg Hg kg^{-1}. We found two groups of *Pseudomonas*, which probably represent non-described species since they did not group closely with any known species of *Pseudomonas* in the dendrogram. Hg-enhanced isolates were closely related to *P. frederiksbergensis*. Furthermore, Hg resistance was almost exclusively restricted to *P. frederiksbergensis* and *P. migulae* groups. We conclude that Hg caused a shift in the dominating species of culturable *Pseudomonas*.

Keywords: 16S rDNA partial sequencing; FT-IR; Mercury; *Pseudomonas*; Resistance

1. Introduction

Mercury (Hg) is mainly found in the terrestrial environment due to anthropogenic activities, such as burning of fossil fuels, industry and the use of fungicides (Dhawale et al., 1996; Boening, 2000).

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Although the industrial use of mercury has been reduced in recent years due to stricter regulations, high concentrations are still present in the environment. In a Danish topsoil and in Minamata Bay sediment 511 and 46 mg Hg kg^{-1} soil or sediment was found, respectively; but typically concentrations around 5–10 mg Hg kg^{-1} soil or sediment are found (Nakamura et al., 1986; Barkay and Olson, 1986; Rasmussen and Sørensen, 1998; Müller et al., 2001b). Hg as an element cannot be mineralised or be otherwise totally removed from the environment, but some Hg-resistant bacteria are capable of transforming Hg(II) to the less toxic Hg(0) (Foster, 1987; Summers and Barkay, 1989). This could be relevant since Hg, when found as a co-contaminant in soils heavily polluted with organic compounds, could impair bioremediation of the organic contaminant due to the high toxicity of Hg (Foster, 1987; Giller et al., 1998; Baldrian et al., 2000). One way to optimise the bioremediation of co-contaminated soils could be by using a microbial consortium consisting of bacteria capable of transforming Hg(II) to the less toxic Hg(0), thereby permitting the activity of the other part of the consortium: degraders specific to the organic contaminant. However, for such bioremediation to be successful more information about Hg-resistant culturable bacteria is needed.

The effects of mercury on the species distribution of culturable bacteria have largely been neglected (Bååth et al., 1998). In contrast, the effects of heavy metals on bacterial populations in soil have been studied at the ecosystem level by measuring different C and N cycling processes (Bååth, 1989; Brookes, 1995), thymidine incorporation into DNA (Díaz-Raviña and Bååth, 1996), and sole carbon source utilisation patterns (Müller et al., 2001a). In recent years, the use of molecular methods to investigate diversity in bacterial communities has prevailed (Ranjard et al., 2000; Rasmussen and Sørensen, 2001). Previous studies showed that Hg causes an increased relative abundance of Hg-resistant bacteria but further characterisation of resistant isolates has rarely been carried out (Osborn et al., 1993; Ranjard et al., 1997, 2000; Smit et al., 1998; Rasmussen et al., 2000; Rasmussen and Sørensen, 2001).

Fourier transform infrared spectroscopy (FT-IR) is a relatively easy way to characterise bacterial isolates. The basic principle of FT-IR is that all components of bacterial cells, including cell walls, proteins, membranes, and nucleic acids, give specific absorption spectra for strains cultured under standardised conditions (Naumann et al., 1991; Helm et al., 1991a, 1991b). Subjecting environmental isolates of *Pseudomonas* spp. to FT-IR showed a correlation with repetitive extragenic palindromic (REP)-PCR and carbon source utilisation patterns (Johnsen and Nielsen, 1999; Aagot et al., 2001). REP-PCR is a well-known reproducible method to distinguish closely related organisms on the basis of DNA diversity (de Bruijn, 1992). FT-IR can therefore be used to characterise pseudomonads on a subspecies level. Identification cannot be obtained by FT-IR, unless extensive databases are available, but isolates can be quickly sorted into groups (Tindall et al., 2000). Subsequently, representative isolates of the groups can be characterised further, e.g. by 16S rDNA partial sequencing.

In a previous study, Hg did not affect the number of culturable bacteria on *Pseudomonas*-specific Gould's S1 agar plates even though we saw a decreasing number of bacteria on the general medium 1/100 tryptic soy agar (Holtze et al., 2003). Gould's S1 medium is selective for *Pseudomonas* bacteria (Gould et al., 1985; Kragelund et al., 1996; Johnsen and Nielsen, 1999). Pseudomonads are fast-growing, Gram-negative bacteria and, hence, they are good candidates to be selectively favoured in Hg-spiked soils (Palleroni, 1984; Kelly and Reanney, 1984; Campbell et al., 1995). The present study was designed to investigate whether Hg equally affected all of the culturable bacteria or if only a few types of isolates were enhanced over time. To investigate the distribution of isolates we used FT-IR characterisation combined with traditional biochemical tests and subsequent 16S rDNA partial sequencing of representative isolates. Furthermore, we tested the ability of the isolates to grow on agar amended with Hg to determine whether resistance was randomly spread among the groups of isolates.

2. Materials and methods

2.1. Isolation of bacteria

Bacterial isolates were isolated during a previous study presented elsewhere (Holtze et al., 2003). The