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Original article

Occurrence of *Stenotrophomonas maltophilia* in agricultural soils and antibiotic resistance properties

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Abstract

The occurrence of *Stenotrophomonas maltophilia* was monitored in organic amendments and agricultural soils from various sites in France and Tunisia. *S. maltophilia* was detected in horse and bovine manures, and its abundance ranged from 0.294 (±0.509) × 10^3 to 880 (±33.4) × 10^3 CFU (g drywt)$^{-1}$ of sample. *S. maltophilia* was recovered from most tested soil samples (104/124). Its abundance varied from 0.33 (±0.52) to 414 (±50) × 10^3 CFU (g drywt)$^{-1}$ of soil and was not related to soil characteristics. Antibiotic resistance properties of a set of environmental strains were compared to a clinical set, and revealed a high diversity of antibiotic resistance profiles, given both the numbers of resistance and the phenotypes. Manure strains showed resistance phenotypes, with most of the strains resisting between 7 and 9 antibiotics. While French soil strains were sensitive to most antibiotics tested, some Tunisian strains displayed resistance phenotypes close to those of clinical French strains. Screening for metal resistance among 66 soil strains showed a positive relationship between antibiotic and metal resistance. However, the prevalence of antibiotic resistance phenotypes in the studied sites was not related to the metal content in soil samples.

Keywords: *Stenotrophomonas maltophilia*; Distribution; Abundance; Soil; Antibiotic resistance; Metal resistance

1. Introduction

Stenotrophomonas maltophilia, previously known as *Pseudomonas maltophilia* and later *Xanthomonas maltophilia*, has been described in the last decades as an environmental globally emerging Gram-negative multi-drug-resistant organism that is commonly associated with respiratory infections in humans, and that is increasingly isolated from cystic fibrosis (CF) patients [1–3]. This species has been implicated in a variety of infections alongside respiratory tract infections, including bacteremia, bone and joint infections, urinary tract and eye infections, endocarditis and meningitis [1]. It has also

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been shown to cause infections in animals, such as respiratory infections with chronic coughing in horses, canines and felines [4–7].

As previously mentioned, *S. maltophilia* is classified as multi-drug-resistant bacteria and is characterized by a high intrinsic capacity to resist a wide range of antimicrobial molecules. Its intrinsic resistance is particularly due to the presence of broad-spectrum efflux pumps, enzymes such as L1 metallo-β-lactamase, L2 Ambler class A β-lactamase and AAC(6′)-Iz and APH(3′)-IIa aminoglycoside-modifying enzymes [8]. Various studies have also revealed the capacities of clinical strains to develop antibiotic resistance mechanisms due to mutation or acquisition of mobile elements [9–11]. *S. maltophilia*, as a multi-drug-resistant opportunistic pathogen, resists antibiotics and biocides like hypochlorite cleaners, triclosan, SDS and antiseptics containing quaternary ammonium compounds [12].

Before being recognized as an emerging opportunistic pathogen, *S. maltophilia* was primarily known to be an ubiquitous environmental microorganism described in a variety of natural and anthropogenic environments such as soil [13], water [14] and sediment [15]. Its presence has been reported in extreme ecosystems such as deep sea or high altitudes [16,17], as well as polluted sites [18]. Considering terrestrial environments, its isolation from industrial and agricultural soils [19,20], the rhizosphere [21,22] and internal plant tissue [23] has been reported. In these niches, *S. maltophilia* can then act as a degrader of a variety of xenobiotic compounds [24,25], and hydrocarbons [26], thus playing a significant role in bioremediation of polluted sites [27,28], as well as a plant growth promoter or biological control agent of plant pathogens due to their production of phytohormones [29] and chitinolytic activities [30], respectively.

Although *S. maltophilia* has been found worldwide in soils, the link between prevalence and soil characteristics and anthropogenic constraints has not yet been investigated. For instance, the presence of *S. maltophilia* in various water sources and sewage raises questions about the potential dispersion in soil through common agricultural practices, i.e. irrigation and organic amendment and factors driving its survival. To fill in this knowledge gap, the objectives of this study were: i) to evaluate the distribution and abundance of *S. maltophilia* in various agricultural soils from France and Tunisia; and ii) to characterize antibiotic resistance profiles of a set of soil- and manure-originating isolates and to compare these properties to those of clinical strains. As several studies reported co-resistance to antibiotics and metal among clinical and environmental bacteria, a secondary objective was to evaluate the metal phenotypes of *S. maltophilia* in order to better appreciate the role of soil metal content in selection of antibiotic resistance.

2. Materials and methods

2.1. Sampling sites

Samples (n = 124) were collected from 42 sites in France and 2 sites in Tunisia (Fig. 1). French sites were located in regions in Burgundy (32 sites in the French RMQS 'Réseau de Mesures de la Qualité des Sols = a French soil quality monitoring network'), the Ile de France (8 sites, Chavenay, Fontenay le Fleury, Crespierre, Les Alluets le Roy, Feucherolles, Pierrelaye control, Pierrelaye moderately metal contaminated and Pierrelaye highly metal contaminated) and the Nord Pas de Calais (2 sites, Dourges and Courcelles les Lens) (Fig. 1). Soil characteristics are listed in Supplementary Table 1. The occurrence of *S. maltophilia* was measured based on analysis of one or several samples per site, as indicated in Supplementary Table 1. For French sites from the national RMQS program, each sample was constituted of ten samplings per field collected from the upper layer (0–5, 0–10 or 0–20 cm), sifted through a 2 mm mesh [31]. They were collected during various campaigns between 2006 and 2011. In Pierrelaye sites, samples were collected in 3 fields, mostly distinguished based on their metal content (Cd, Cu, Pb and Zn), due to long-term amendment with sewage sludge and irrigation with wastewaters. These fields were moderately contaminated (Pierrelaye-2), highly contaminated (Pierrelaye-3) or non-contaminated, i.e. nearby fields that had never been irrigated with wastewater (Pierrelaye-Control) as classified based on total metal concentrations. Eighteen samples were collected in the three areas chosen according to their level of heavy metal contamination during a campaign conducted in April 2009. Five samplings per plots made up one sample. In Tunisia, soils were sampled from 2 distinct sites, Nabeul and Souhil, planted with orange and citrus trees irrigated with either wastewater or groundwater over 25 and 19 years, respectively. Samples were collected from 15 and 10 nearby fields from Nabeul and Souhil sites, respectively (Fig. 1). Each sample was composed of 5 samplings per field and was collected from the upper layer (0–20 cm), sifted through 2 mm mesh sieves and stored at room temperature for no longer than 1 week.

2.2. Sources of organic amendments

We included 1- or 6-month-old bovine and horse manure obtained from 5 farms in the Dombes area (Rhône-Alpes), as well as various organic amendments, i.e. bovine manure, horse manure, poultry droppings, dehydrated pig manure and various municipal composted wastes used on an INRA experimental site at Feucherolles (Ile de France) (Table 1) or in various fields around Versailles (Ile de France). Some of these amendments were provided by the INRA of Grignon. A total of 35 samples were studied.

2.3. Bacterial counts

Bacterial cells from soils were extracted by blending 5 g of soil with 50 ml of a saline solution (NaCl 0.8%) for 90 s in a Waring blender (Eberbach Corporation, New Hampshire, USA). The total heterotrophic microflora was enumerated on tryptic soy agar diluted 10-fold (TSA1/10) (Oxoïd, Dardilly, France) supplemented with cycloheximide (200 mg l^{-1}) to impair growth of fungi. *S. maltophilia* enumeration was