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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

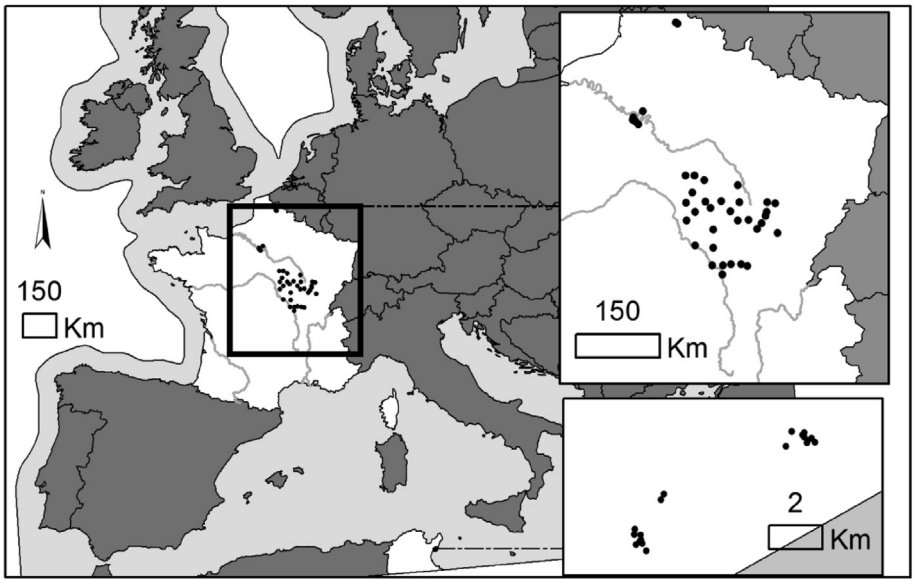

Fig. 1. Sampling areas. Sampling sites in France and Tunisia are positioned on a map.

Table 1
Soil and clinical strains used in the study.

Origin		Strain name	Putative anthropogenic pressure
Soil			
France	Feucherolles	BPOE5102, BPOE5103, BPOE5104, BPOE5105, BPOE5101, BPOE5100, BPOE5108, BPOE5112, BPOE5107, BPOE5113, BPOE5109, BPOE5114, BPOE5106, BPOE5110, BPOE5111, BPOE5123, BPOE5124, BPOE5125, BPOE5126, BPOE5127	Use of organic amendments
	Pierrelaye	BPOE5156, BPOE5157, BPOE5158	
	control	BPOE5159, BPOE5171, BPOE5172 BPOE5173	
	moderately contaminated	BPOE5166, BPOE5167	Former use of wastewater from Paris for irrigation for a century
	highly contaminated	BPOE5154, BPOE5155, BPOE5160, BPOE5161, BPOE5162, BPOE5163, BPOE5164, BPOE5165, BPOE5168, BPOE5169, BPOE5170	
Tunisia	Nabeul	BPOE5128, BPOE5131, BPOE5134, BPOE5135, BPOE5136, BPOE5137, BPOE5138, BPOE5139, BPOE5140, BPOE5141, BPOE5142, BPOE5143, BPOE5144, BPOE4145	Use of wastewater for irrigation
		BPOE5130, BPOE5132	Use of groundwater for irrigation
	Souhil	BPOE5133, BPOE5149	Use of wastewater for irrigation
		BPOE5129, BPOE5147, BPOE5148, BPOE5150, BPOE5151, BPOE5152 BPOE5153	Use of groundwater for irrigation
Reference strain		R551.3 [23]	
Clinical			
CF individuals		BPOE5189, BPOE5190, BPOE5191, BPOE5192, BPOE5193, BPOE5194, BPOE5195, BPOE5196, BPOE5197, BPOE5198, BPOE5199, BPOE5200	Antibiotic treatments, antiseptics used for disinfection
Infected patients		BPOE5174, BPOE5176, BPOE5177, BPOE5178, BPOE5179, BPOE5180, BPOE5181, BPOE5182, BPOE5183, BPOE5184, BPOE5185, BPOE5186, BPOE5187, BPOE5188	Antibiotic treatments, antiseptics used for disinfection
Reference strain		K279a [36]	

performed using VIA (vancomycin–imipenem–amphotericin) medium [32] supplemented with cycloheximide (200 mg l^{-1}). Homogenized soil suspensions were serially diluted in sterile saline solution. One hundred microliters of the 10^{-3} to 10^{-5} dilutions were spread on TSA agar plates and 100 µl of the 10^{0} to 10^{-2} dilutions were spread on VIA agar plates. Three plates were inoculated per dilution. Bacterial colonies were counted after 5 days or 48 h of incubation at 28 °C for TSA and VIA plates, respectively. For each soil sample, 10–20 green colonies on VIA plates were collected and further confirmed as being *S. maltophilia* as previously described by *sme*D gene PCR [33]. Data was expressed as CFU (colony-forming unit) per gram of dry soil.

2.4. Genetic characterization by pulsed field gel electrophoresis (PFGE)

A collection of 74 soil isolates of *S. maltophilia* were analyzed (Table 1). Among them, 22 were from the Feucherolles site, 23 from the Pierrelaye site and 29 from Tunisian sites (20 from Nabeul and 9 from Souhil). Molecular typing of *S. maltophilia* chromosomal DNA was performed by PFGE after digestion of genomic DNA with the *Xba*I enzyme as previously described [34]. Briefly, genomic DNA was digested with 25 U/ml of *Xba*I (Fermentas, Saint-Rémy-lès-Chevreuse, France). Macrorestriction fragments were separated using a CHEF-DR III apparatus (Bio-Rad), with pulse time ramped from 5 to 35 s over 20 h at 14 °C and 6 V/cm. Genomic relatedness was established using criteria defined by Tenover et al. [35]. Macrorestriction patterns were considered identical if they shared all bands. A PFGE cluster was arbitrarily defined as banding patterns that showed more than 90% similarity. Strains were considered genetically different when they exhibited distinct banding patterns. One representative of each PFGE profile was selected for further analyses (i.e. resistance properties) to ensure that the analysis was performed on isolates belonging to different strains.

2.5. Bacterial strains

Clinical strains (26 strains, Table 1) were provided by the University Hospital of Toulouse (France). These strains were isolated from CF individuals (12 strains) and from infected patients (14 strains). *S. maltophilia* K279a [36] and *S. maltophilia* R551.3 [23] strains were added to the set of strains.

2.6. Antibiotic resistance test

In vitro antimicrobial susceptibility of *S. maltophilia* was routinely determined using the Vitek®2 system with a card (NO93) dedicated to non-fermenting Gram-negative bacteria (Biomérieux, Marcy l'Etoile, France) according to the manufacturer's instructions. Minimal inhibitory concentrations of 16 antibiotics [ticarcillin (TIC 16, 32, 64 µg/ml), ticarcillin/clavulanic acid (TIM 8/2, 32/2, 64/2 µg/ml), piperacillin (PIP 4, 16, 64 µg/ml), piperacillin/tazobactam (TZP 4/4, 16/4, 128/4 µg/ml), ceftazidime (CAZ 1, 2, 8, 32 µg/ml), cefepime (FEP 2, 8, 16, 32 µg/ml), imipenem (IPM 2, 4, 16 µg/ml), meropenem (MEM 0.5, 4, 16 µg/ml), amikacin (AMK 8, 16, 64 µg/ml), gentamicin (GEN 4, 16, 32 µg/ml), isepamicin (ISP 4, 8, 32 µg/ml), tobramycin (TOB 8, 16, 64 µg/ml), ciprofloxacin (CIP 0.5, 2, 4 µg/ml), pefloxacin (PEF 0.5, 2, 8 µg/ml), colistin (CS 4, 16, 32 µg/ml) and trimethoprim/sulfamethoxazole (SXT 0.5/9.5, 2/38, 16/304 µg/ml)] were determined. Interpretations were established following the recommendations of the antibiogram committee of the French Society of Microbiology, http://www.sfm-microbiologie.org).

2.7. Metal resistance profiles

The metal resistance of *S. maltophilia* was determined using TSA medium diluted 10-fold (TSA 1/10) and supplemented with 5, 10, 20 and 50 mM Zn^{2+} ($ZnCl_2$), 0.5, 1, 2, 5 mM Cu^{2+} ($CuCl_2$), 0.6, 1.25, and 2.5 mM Cd^{2+} ($CdCl_2$), 10 and 50 µM Hg^{2+} ($HgCl_2$) as previously performed in Deredjian et al. [37]. Preliminary tests enabled us to determine suitable concentrations for each metal. The *Pseudomonas aeruginosa* PAO1 strain and the BPOE5174 *S. maltophilia* clinical strain were used as controls for each experiment. A suspension was prepared from a 24-h pure culture on 1/10-TSA in a saline suspension ($NaCl_2$ 0.8%). One hundred microliters of the suspension were inoculated on supplemented and non-supplemented 1/10-TSA plates. The cultures were incubated at 28 °C for seven days. A strain was considered resistant when its growth on metal-supplemented medium was equivalent to its growth on the same metal-free medium. When growth was slower, resistance was intermediate. When no growth was obtained, the strain was considered sensitive. The experiments were performed in duplicate.

2.8. Statistical analysis

To investigate the relationship between *S. maltophilia* abundance and soil characteristics, principal component analysis was performed using a table containing 83 rows (soil samples), and 12 variables including all properties (physicochemical properties, granulometry, metal content and CFU) except land use.

To investigate the distribution of *S. maltophilia* strains according to their antibiotic and metal resistance capacities, antibiotic and metal resistance profiles of *S. maltophilia* strains were grouped into one table composed of 93 rows (strains) and 22 variables (16 antibiotics, 6 metals). The phenotypic data was encoded as R for resistant, I for intermediate, and S for sensitive. This qualitative data was submitted to a multiple correspondence analysis (MCA) [38].

To assess the relationships between antibiotic resistance and metal resistance in *S. maltophilia* strains, co-inertia analysis was conducted [39]. To that purpose, 2 tables were constructed with the same rows (93 strains). One table contained the results of antibiotic resistance phenotypes and the other contained those of metal resistance phenotypes. Separate MCAs were computed from each table, and co-inertia analysis was conducted on each of them. The significance of the

Table 2
Number of CFU (Colony Forming units) of *S. maltophilia* in organic amendments.

Sites (Region and city)	Description	Number of treated samples	CFU × 10^3 (g drywt sample)$^{-1}$ (±standard deviation)
Ile de France			
Versailles	Green waste and animal powder	2	0
	Poultry dropping	2	0
	Dried pig manure	2	0
	Compost of horse manure with wood chip (farm 1)	2	13.9 (±3.92)
	Compost of horse manure with farm wheat straw (farm 2)	2	1.71 (±1.21)
	Compost of horse manure with commercial wheat straw (farm 3)	2	2.96 (±1.05)
Feucherolles	1 month wet bovine manure year 2006	3	13.9 (±7.08)
	1 month wet bovine manure year 2007	3	
	Compost of municipal solid waste (MSW)	3	0
	Compost of fermentable fraction of municipal wastes and green wastes (BW)	3	0
	Compost of sewage sludge, green wastes, and wood chips	3	0
Rhône-Alpes			
Versailleux	6 month dry bovine manure year	2	5.79 (±3.20)
	1 month wet bovine manure year	2	75.0 (±42.4)
Joyeux	6 month dry bovine manure year	2	0.294 (±0.509)
St Olive	1 month horse manure year	2	880 (±33.4)

relationships highlighted by co-inertia analysis was tested by a Monte-Carlo permutation test.

All these analyses were carried out with ade4 software, a package for the R statistical environment [40].

3. Results

3.1. Prevalence of S. maltophilia in organic amendments

S. maltophilia were screened from organic amendments and isolated only from bovine manure at Feucherolles [14 (±7.1) × 10^3 CFU (g drywt)$^{-1}$ of sample], and from each of the manure collected at farms from the Dombes region [from 0.29 (±0.51) to 880 (±33) × 10^3 CFU (g drywt)$^{-1}$ of sample] and Versailles area [from 1.7 (±1.2) to 14 (±3.9) × 10^3 CFU (g drywt)$^{-1}$ of sample] (Table 2).

3.2. Distribution of S. maltophilia in soils from France and Tunisia

S. maltophilia isolates were detected in 83% of the samples (104 out of 124 samples). No *S. maltophilia* colonies were obtained from 3 samples from Ile de France sites or 17 samples (i.e. sites) from the Burgundy region. Among the other samples, *S. maltophilia* abundance varied from 3.3 (±5.7) × 10^2 to 1.9 (±0.80) × 10^5 CFU (Feucherolles site) per gram of dry soil (Table 3). The *S. maltophilia* population represented between 0.001% and 1.2% of the total heterotrophic microflora. The abundance of *S. maltophilia* also varied within sites. In Pierrelaye, abundance varied from 0 to 4.7 (±2.5) × 10^4 CFU/g of dry soil and in Burgundy, from 0 to 7.2 (±0.50) × 10^3 CFU/g. In Tunisia, although maximum abundances were 3.7 (±0.1) × 10^4 and 9 (±0.7) × 10^4 CFU/g in Souhil and Nabeul sites respectively, intra-site variability was also observed. At Feucherolles, intra-site variability was also observed, reaching a 10^2 factor between samples. PCA performed on the physic-chemical properties of soils with *S. maltophilia* abundances (Fig. 2) revealed no relationship between soil characteristics and the distribution and abundance of *S. maltophilia* in soil from France and Tunisia (CFU arrow on PCA; size of squares were not linked to any parameter such as metals, silt, sand, clay, CEC or pH).

3.3. Antibiotic resistance profile

The antimicrobial susceptibility of *S. maltophilia* was studied on 20 strains from bovine and horse manure, 65 strains from our soil samples, 26 strains from clinical origin and reference strains K279a and R551.3. A high diversity of antibiotic resistance profiles was observed among manure and soil strains, both considering the number of antibiotics a strain was resistant to, i.e. multi-resistance (Fig. 3), and the antibiotic type (data not shown). Resistance to 1–12 antibiotics was obtained in all strains whatever their origin (only clinical strains were able to resist more than 13 antibiotics) (Fig. 3). Only 36% of soil strains exhibited resistance to more than 3 antibiotics (Fig. 3), whereas more than 85% and 92% of manure and clinical strains, respectively, were found to be resistant to more than 3 antibiotics. Most of the soil strains (38 out of 42) belonging to a sensitive phenotype were isolated from the French sites of Feucherolles and Pierrelaye. Most soil strains resisting 4–12 antibiotics were isolated from Tunisia. Among the 13 strains resisting 7 antibiotics or more, 10 were isolated from soils irrigated with wastewater and 3 from soils irrigated with groundwater. Among the 12 Tunisian strains resisting less than 7 antibiotics, 7 strains originated from wastewater-irrigated soils and 5 from groundwater-irrigated soils. Most manure strains that resisted more than 7 antibiotics originated from compost from bovine manure and horse manure.

Table 3
Number of CFU of *S. maltophilia* in soils.

Sites (Region and city)	Description	Number of treated samples	CFU $\times 10^3$ (g drywt sample)$^{-1}$ (±standard deviation)
Ile de France			
Chavenay	Rapeseed	1	0.740 (±1.05)
	Wheat, horse manure	2	0
Fontenay le Fleury	Wheat	1	0
	Wheat, horse manure	1	0.361 (±0.511)
Crespierre	Wheat	1	0.354 (±0.501)
Les Alluets le Roy	Maize	3	5.43 (±0.578)
Feucherolles	Non amended soil	4	186.7 (±84.9)
	1 month wet bovine manure year 2006	4	7.79 (±3.30)
	1 month compost of municipal solid waste (MSW)	4	414.48 (±52.30)
	1 month compost of fermentable fraction of municipal waste and green wastes (BW)	4	13.51 (±2.80)
	1 month compost of sewage sludge, green wastes, and wood chips	4	17.25 (±3.90)
Pierrelaye-control	Unplanted	2	0.5 (±0.24)
Pierrelaye-2 (moderately metal contaminated)	Unplanted	5	18 (±5.03)
Pierrelaye-3 (highly metal contaminated)	Corn	12	9.45 (±3.60)
Nord Pas de Calais			
Dourges	Miscanthus	5	1.19 (±1.03)
Courcelles les Lens	Miscanthus	6	2.47 (±1.03)
	Miscanthus, rhizosphere samples	6	0.784 (±0.679)
	Wheat	2	13 (±5.2)
Tunisia			
Nabeul		15	12 (±22.02)
Souhil		10	14.5 (±9.19)
Burgundy			
Cudot		1	0
Joigny		1	0
Brienon sur Armancon		1	0
Balot		1	0
Treigny		1	0
Merry sur Yonne		1	0
Angely		1	0
Venarey les Laumes		1	0
Is sur Tille		1	2.6 (±0.284)
Bourberain		1	2.85 (±1.39)
Saint Père		1	0.433 (±3.05)
Courcelles		1	0.4 (±0.49)
Dompierre en Morvan		1	0
Ruffey les Echirey		1	7.16 (±0.538)
Nannay		1	0
Hery		1	4.37 (±0.832)
Marcilly-Ogny		1	6.67 (±0.353)
Commarin		1	4 (0)
Blisms		1	1.33 (±1.73)
Echevronne		1	5.88 (±1.02)
Moulins		1	0
Sougy sur Loire		1	0
Remilly		1	0
Gilly sur Loire		1	0
Rigny sur Arroux		1	3.33 (±0.49)
Palinges		1	0
La Guiche		1	0.333 (±0.122)
Salornay sur Guye		1	0
L'Hopital le Mercier		1	0
Morey saint Denis		1	1.19 (±0.11)
Saint Aubin		1	0.803 (±0.069)
ENESAD		1	3.21 (±0.139)

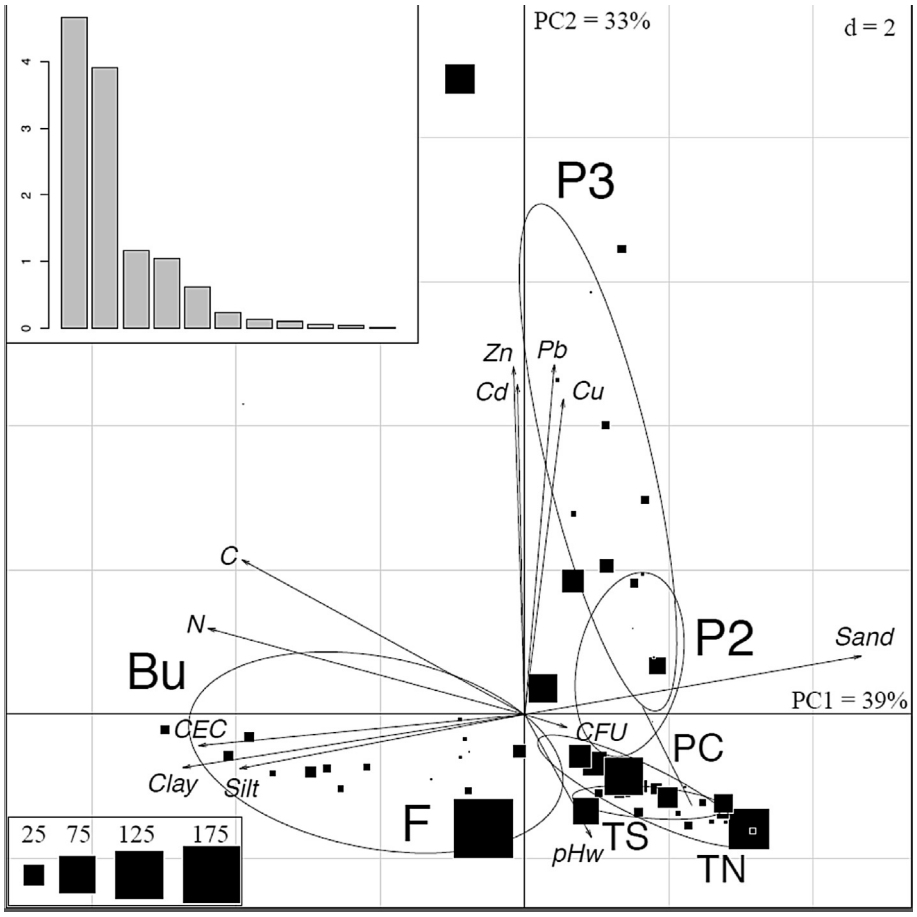

Fig. 2. PCA of physic-chemical characteristics of soils and *S. maltophilia* abundance. The data table contains *S. maltophilia* CFUs with soils in rows and physicochemical characteristics in columns. The soils are not plotted individually; only groups are displayed, using inertia ellipses (i.e. P3 for Pierrelaye highly contaminated 3). Square sizes are proportional to the absolute value of *S. maltophilia* abundance (in CFUs $\times 10^3$). The groups are based on soil origin: TS = Souhil (Tunisia), TN = Nabeul (Tunisia), F = Feucherolles, PC = Pierrelaye control, P2 = Pierrelaye moderately contaminated 2. The first axis presents soils characterized by a high CEC, clay, silt, N and C content on the left versus sandy soils on the right; the second axis compares soils highly contaminated by metals: P3 = Pierrelaye 3, Zn: zinc, Pb: lead, Cu: copper, Cd: cadmium. CFU: colony forming unit of *S. maltophilia* in number $\times 10^3$.

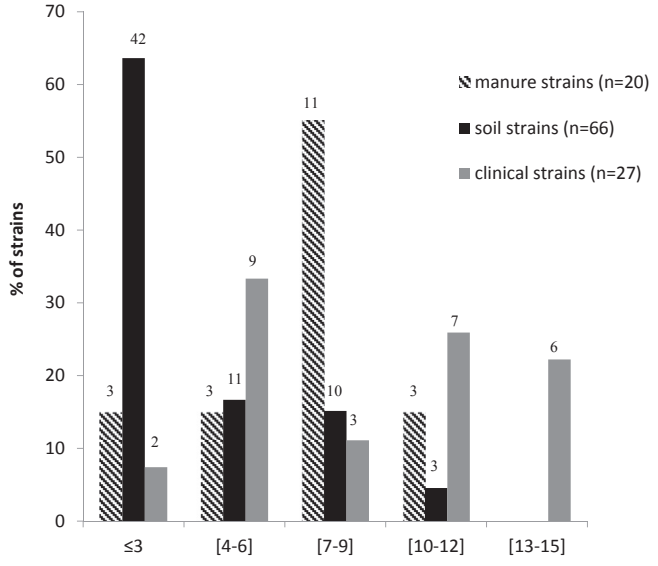

Fig. 3. Antibiotic multiresistance in 20 manure strains, 66 soil strains and 27 clinical strains of *S. maltophilia*; n = number of strains in each category; [ ] = number of antibiotics. Effectives are indicated above the bar graph.

Resistance to all 16 antibiotics was observed in clinical and soil strains, except for the trimethoprim/sulfamethoxazole association, that was active against all soil strains (data not shown). Strains isolated from Feucherolles and Pierrelaye resisted the β-lactams ticarcillin, piperacillin, imipenem and meropenem. Resistance to the quinolones ciprofloxacin and pefloxacin was also observed in strains from Feucherolles and resistance to the β-lactam cefepime was observed in strains from Pierrelaye. For strains isolated from Tunisian soils, resistance was observed for most antibiotics except for trimethoprim/sulfamethoxazole, and the quinolones ciprofloxacin and pefloxacin (data not shown).

3.4. Metal resistance

To examine the role of metal content as a selective pressure upon antibiotic resistance, we focused our investigation on isolates from Feucherolles, Pierrelaye and Tunisia, as these sites exhibit varying metal contents, and this led us to observe the extent of diversity towards antibiotic resistance among their indigenous *S. maltophilia* populations (above data). Prior

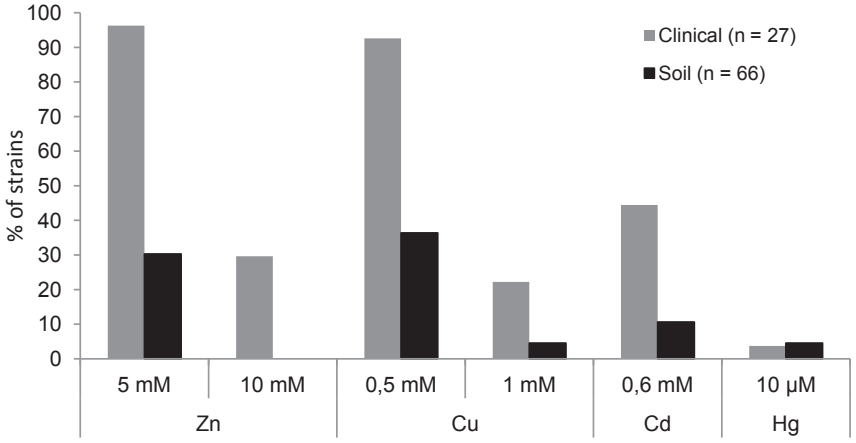

Fig. 4. Heavy metal resistance in 66 soil strains and 27 clinical strains of *S. maltophilia*; n = number of strains in each category.

to this study, we analyzed our set of isolates using PFGE to ensure that non-redundant isolates were screened for metal resistance. A set of 74 soil isolates was selected, and R551-3 and K279 strains were included as reference strains. Among the 74 isolates, 65 PFGE profiles were obtained. Few identical profiles were observed within each site. Twenty profiles were detected at Feucherolles and Pierrelaye, and only 2 and 3 profiles, respectively, were shared by no more than 2 isolates. Similarly, the 20 isolates from Nabeul showed 16 distinct profiles. No identical profile was detected between sites. No common profile was detected between soil isolates and the 2 reference strains.

Both soil and clinical strains were able to resist the 4 metals, i.e. Zn, Cu, Cd and Hg (Fig. 4). Some soil strains were found to be able to grow at maximal concentrations of 5 mM Zn, 1 mM Cu, 0.6 mM Cd and 10 µM Hg. Clinical strains grew at the same concentrations of Cu, Cd and Hg, but some of them also grew in the presence of 10 mM Zn. None of the strains, irrespective of their origin, grew in the presence of the highest concentrations tested, i.e. 20 and 50 mM Zn, 2 and 5 mM Cu, 1.25 and 2.5 mM Cd and 50 µM Hg. Resistance frequencies were lower in the soil strain group than in the clinical one except for Hg, as no difference between soil and clinical groups was observed (Fig. 4).

Among soil strains, metal resistance profiles greatly differed depending on the strain origin. The Pierrelaye strains, whatever the field of origin and the metal content of soil (France), were the least resistant, since no resistance was observed for Zn, Cd and Hg, and only a few strains were resistant to Cu. Twenty percent and 5% of Feucherolles strains (France) were resistant to Zn and Cu, respectively. Finally, strains isolated from Tunisia were resistant to all 4 metals (data not shown).

3.5. Strain grouping based on antibiotic and metal resistance

MCA conducted on antibiotic and metal resistance phenotypes of the 93 *S. maltophilia* strains separated the strains according to their resistance properties (Fig. 5). The projection of the strains was mainly driven by the first and second axes, which accounted for, respectively, 43% and 13% of the total inertia of our data. On the first axis, we observed that the strains were distributed along a gradient of antibiotic and metal resistance from sensitivity on the left to resistance on the right. Moreover, strains were grouped depending on their origin. The strains found to be sensitive to most antibiotics and metals were mainly those isolated from the French sites of Feucherolles and Pierrelaye. It should be noted that sensitive profiles were observed among strains from both non-contaminated sites (Feucherolles and Pierrelaye control field) and highly metal-contaminated sites (field Pierrelaye-3 at the Pierrelaye site). Most clinical strains showed high metal and antibiotic resistance capacity. Strains isolated from Tunisian sites were distributed along the gradient. Some of them had a sensitive phenotype, close to strains from Pierrelaye and Feucherolles, while others had a resistant phenotype close to clinical strains. Again, it should be noted that the occurrence of both sensitive and resistant strains was observed in samples from non-contaminated fields. On the second axis, we observed that resistant strains could be divided into 2 groups, one characterized by aminoglycoside and colistin resistance (and Hg resistance) and composed of clinical strains and Tunisian strains from Nabeul, and the other characterized by resistance to other antibiotics such as β-lactams and quinolones and composed of clinical strains and Tunisian strains (Nabeul and Souhil). The K279a clinical strain belonged to the aminoglycoside- and colistin-resistant group, whereas the R551-3 environmental strain was closer to sensitive strains.

Co-inertia analysis of antibiotic resistance and metal resistance of *S. maltophilia* strains showed that cross-covariances were high for resistant and intermediate metal and antibiotic phenotypes and also for sensitive metal and antibiotic phenotypes. This suggests a positive relationship between antibiotic and metal resistance. The Monte-Carlo permutation test confirmed the existence of a significant association between antibiotic and metal resistance phenotypes (p-value = 0.001).

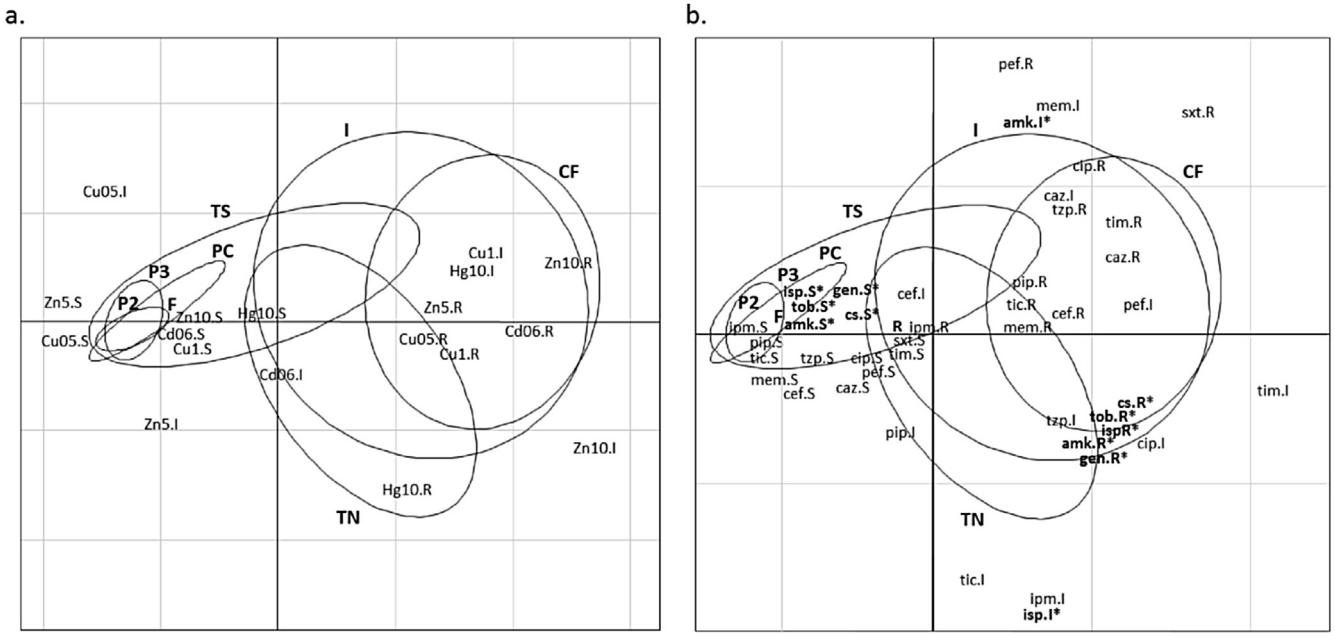

Fig. 5. MCA biplot of antibiotic and metal resistance phenotypes of *S. maltophilia* strains. The first axis accounts for 43% of total inertia and the second axis for 13%. The data table contains phenotypes (R/I/S), with strains in rows and metals and antibiotics in columns. Metals (Fig. 5a.) and antibiotics (Fig. 5b.) are represented on 2 different plots for better clarity, but the two graphs could actually be superimposed. The 93 strains are not plotted individually; only groups are displayed, using inertia ellipses. The groups are based on strain origin: I = infected patients, CF = cystic fibrosis patients, TS = Souhil (Tunisia), TN = Nabeul Tunisia, F = Feucherolles, PC = Pierrelaye control, P2 = Pierrelaye 2 (i.e. moderately contaminated), P3 = Pierrelaye 3 (i.e. highly contaminated). The first axis presents sensitive strains on the left versus resistant ones on the right; the second axis compares strains resistant to particular antibiotics (marked with an asterisk). TIC = ticarcillin, TIM = ticarcillin-clavulanic acid, PIP = piperacillin, TZP = piperacillin-tazobactam, CAZ = ceftazidime, FEP = cefepime, IPM = imipenem, MEM = meropenem, AMK = amikacin, GEN = gentamicin, ISP = isepamicin, TOB = tobramycin, CIP = ciprofloxacin, PEF = pefloxacin, CS = colistin, SXT = trimethoprim/sulfamethoxazole; R = resistant, I = intermediate, S = sensitive.

4. Discussion

S. maltophilia has wide environmental distribution and is readily isolated from water, soil and sewage. However, little is known about its abundance within these environments. In the present work, we enumerated *S. maltophilia* within the bulk soil of agricultural sites that are physically, chemically and geographically different from each other, and we determined whether soil characteristics influenced its distribution. Whatever their characteristics, most soil samples from France (Feucherolles, Pierrelaye, and Burgundy) and Tunisia led to the detection of *S. maltophilia*, confirming its widespread presence and as the fact that this species is a natural inhabitant of soil. This result was expected, since soil is considered to be a reservoir of *S. maltophilia* [41]. *S. maltophilia* showed a high frequency of occurrence in the rhizosphere [21] and a recent report on its presence as a rice endophyte led the authors to conclude that this species is highly adapted to the plant niche [42]. Our data evidenced that *S. maltophilia* can also survive in bulk soil far from the influence of plant roots. Furthermore, several of our samples were collected whereas the soil was not planted, or else long after crop harvest. Nevertheless, we showed that the success of isolation and the abundance of *S. maltophilia* differed both within and between sites. Sites from Burgundy showed the lowest abundance of *S. maltophilia* and the site of Feucherolles the highest. At the latter, *S. maltophilia* represented up to 1.2% of total heterotrophic microflora. We then tested the role of soil characteristics on *S. maltophilia* abundance and observed that it was not related to any of the tested characteristics, i.e. pH, CEC, clay, silt or metals. We expected pH to influence *S. maltophilia* prevalence, since this parameter is known to shape the diversity of soil bacterial communities [43]. Similarly, metal concentration could be a factor that favored growth of *S. maltophilia*, as it has been demonstrated that metal-resistant *S. maltophilia* populations are selected in soils exposed to chromium concentrations [27]. However, when considering soil pH, it should be mentioned that our samples may not cover a range of pH (from 4.91 in a Burgundy sample to 8.64 in a Nabeul sample) wide enough to reveal such a relationship.

The presence of *S. maltophilia* in agricultural soils could also be the consequence of anthropogenic activities such as irrigation with wastewater or fertilization with animal-derived products. We then looked for the presence of *S. maltophilia* in various organic amendments derived from animal farms in the Ile de France and Rhône-Alpes regions. As previously reported for *P. aeruginosa* using the same set of samples [31], our data showed that *S. maltophilia* is present in bovine and horse manures and that composting did not eliminate *S. maltophilia* cells. Furthermore, *S. maltophilia* was found to be more abundant than *P. aeruginosa*, and able to survive in both wet and dry manures. These observations confirmed the high adaptability of *S. maltophilia* to environmental conditions. Screening for the presence of *S. maltophilia* in soil that

received or not organic amendment showed that no relationships could be seen between its prevalence and the addition of manures, since soils that did not receive manure (i.e. control soil from Feucherolles, Pierrelaye and Tunisian soils) showed a high abundance of that species. Unfortunately, at the time of sampling, we did not have the opportunity to look for the presence of *S. maltophilia* in wastewater or sewage sludge. However, several reports from the literature indicated that *S. maltophilia* can be recovered from these waste samples. As Tunisian sites and Pierrelaye soils were irrigated with wastewater from municipal treatment plants or received sewage materials as organic amendments, it would be expected that *S. maltophilia* in these soils originated from exogenous sources. As abundance could not be directly related to irrigation or organic amendment, a diversity study would need to be performed in order to determine whether detected populations in soils are indigenous or originate from exogenous sources.

The observed differences in the prevalence of *S. maltophilia* at the various sites could also be related to potential bias in our sampling strategy. For instance, several samples over a limited area at Pierrelaye were analyzed, whereas only one sample per site over a large region, i.e. Burgundy, was considered. Similarly, differences could be related to the season and climatic conditions in which soil samples were collected, as well as to the presence and type of vegetation cover at the time of sampling. Samples from Burgundy and Pierrelaye were collected in spring, whereas Feucherolles samples were collected in autumn. Some samples in Pierrelaye and Burgundy were collected in fields without any vegetation cover, whereas others from some Burgundy sites and Tunisia were covered with grassland or citrus and orange trees, respectively. One could expect that the presence of plants could influence the abundance of *S. maltophilia*, even in bulk soil. Similarly, biotic parameters could also be involved in variation in the proportion of *S. maltophilia* between sites and within a given site. More analysis would then be required to resolve the causes of the differences in *S. maltophilia* prevalence observed here.

During recent decades, *S. maltophilia* has become a major opportunistic pathogen related to its high multidrug resistance capacity [2,3]. While antibiotic resistance has been fully investigated in *S. maltophilia* clinical isolates, few studies have been conducted thus far on environmental isolates. Ninety-three strains, representing the 91 different PFGE profiles plus K279a and R551.3 strains, were chosen for further analyses of resistance properties. High phenotypic diversity was revealed in both the number of antibiotics that the strains were able to resist and the diversity of the antibiotic families (Fig. 3). Resistance to all antibiotics was observed, except for trimethoprim/sulfamethoxazole. Our manure and soil strains were able to resist between 1 and 12 antibiotics, whereas clinical strains were able to resist up to 15 antibiotics (Fig. 3). Resistant strains were observed in manure samples and in amended and non-amended soils. Such a high diversity of antibiotic resistance profiles among *S. maltophilia* strains has already been observed [22]. Antibiotic use at the hospital and for treating CF patients explained the frequent resistant phenotype observed among our set of clinical strains. The high frequency of antibiotic resistance among manure strains could also be explained by antibiotic presence in manure, since farm animals are often treated with antibiotics. Manure used as a fertilizer could then be a source of antibiotic-resistant bacteria in soil. In our study, no resistant strains were detected in the amended soil at the Feucherolles site, suggesting that manure-originating soil did not survive in soil and/or isolated sensitive strains were indigenous soil populations.

Underground and wastewater could also be a source of antibiotic-resistant bacteria in soil through irrigation, since both are known to harbor *S. maltophilia* [44]. Since the Tunisian sites were irrigated, resistant *S. maltophilia* strains might then have originated from exogenous sources. The potential presence of antibiotics in the wastewater could also favor the selection and/or maintenance of resistant strains. In contrast, at Pierrelaye, irrigation was not a source of resistant strains. Similarly, the presence of antibiotics in these soils [45] did not exert selective pressure. Due to the significant co-resistance to metals and antibiotics observed among strains and the high amount of metals in soil, we expected the strains to be resistant. One explanation for the observed absence of resistance among strains could lie in the non-availability of metals and antibiotics due to the presence of poorly degradable organic compounds entrapping metals and antibiotics (Van Ort, personal communication).

In conclusion, since we observed that *S. maltophilia* was present in various soil types from geographically different agricultural lands, we concluded that different biotic and abiotic conditions enable its survival, pointing to the high adaptability of that species. Further investigations are thus needed to evaluate whether specific populations are associated with specific habitats and identify local environmental conditions and/or alternative niches (plant roots, soil macrofauna) that participate in this specificity. High antibiotic resistance was observed among soil strains. However, further studies are needed to better appreciate whether exogenous sources and environmental selective pressure contribute to the prevalence of this phenotype in agricultural soils.

Conflicts of interest

We have no conflict of interest.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.resmic.2016.01.001.

References

[1] Brooke JS. *Stenotrophomonas maltophilia*: an emerging global opportunistic pathogen. Clin Microbiol 2012;25:2–41.
[2] Jones RN, Sader HS, Beach ML. Contemporary *in vitro* spectrum of activity summary for antimicrobial agents tested against 18569 strains non-fermentative Gram-negative bacilli isolated in the SENTRY Antimicrobial Surveillance Program (1997–2001). Int J Antimicrob Agents 2003;22:551–6.
[3] Razvi S, Quittell L, Sewall A, Quinton H, Marshall B, Saiman L. Respiratory microbiology of patients with cystic fibrosis in the United States, 1995–2005. Chest 2009;136:1554–60.
[4] Albini S, Abril C, Franchini M, Hüssy D, Filioussis G. *Stenotrophomonas maltophilia* isolated from the airways of animals with chronic respiratory disease. Schweiz Arch Tierheilkd 2009;151:323–8.
[5] Winther L, Andersen RM, Baptiste KE, Aalbæk B, Guardabassi L. Association of *Stenotrophomonas maltophilia* infection with lower airway disease in the horse: a retrospective case series. Vet J 2010;186:358–63.
[6] Ohnishi M, Sawada T, Marumo K, Harada K, Hirose K, Shimizu A, et al. Antimicrobial susceptibility and genetic relatedness of bovine *Stenotrophomonas maltophilia* isolates from a mastitis outbreak. Lett Appl Microbiol 2012;54:572–6.
[7] Petridou E, Filioussis G, Karavanis E, Kritas SK. *Stenotrophomonas maltophilia* as a causal agent of pyogranulomatous hepatitis in a buffalo (*Bubalus bubalis*). J Vet Diagn Invest 2010;22:772–4.
[8] Sanchez MB, Hernandez A, Martinez JL. *Stenotrophomonas maltophilia* drug resistance. Future Microbiol 2009;4:655–60.
[9] Avison MB, von Heldreich CJ, Higgins CS, Bennett PM, Walsh TR. A TEM-2 beta-lactamase encoded on an active Tn1-like transposon in the genome of a clinical isolate of *Stenotrophomonas maltophilia*. J Antimicrob Chemother 2000;46:879–84.
[10] Chang LL, Chen HF, Chang CY, Lee TM, Wu WJ. Contribution of integrons, and SmeABC and SmeDEF efflux pumps to multidrug resistance in clinical isolates of *Stenotrophomonas maltophilia*. J Antimicrob Chemother 2004;53:518–21.
[11] Toleman MA, Bennett PM, Bennett DM, Jones RN, Walsh TR. Global emergence of trimethoprim/sulfamethoxazole resistance in *Stenotrophomonas maltophilia* mediated by acquisition of sul genes. Emerg Infect Dis 2007;13:559–65.
[12] Ledder RG, Gilbert P, Willis C, McBain AJ. Effects of chronic triclosan exposure upon the antimicrobial susceptibility of 40 ex-situ environmental and human isolates. J Appl Microbiol 2006;100:1132–40.
[13] Juhnke ME, des Jardin E. Selective medium for isolation of *Xanthomonas maltophilia* from soil and rhizosphere environments. Appl Environ Microbiol 1989;55:747–50.
[14] Romano G, Stampi S, Zanetti F, De Luca G, Tonelli E. Occurrence of gram-negative bacteria in drinking water undergoing softening treatment. Zentralbl Hyg Umweltmed 1997;200:152–62.
[15] Dungan RS, Yates SR, Frankenberger Jr WT. Transformations of selenate and selenite by *Stenotrophomonas maltophilia* isolated from a seleniferous agricultural drainage pond sediment. Environ Microbiol 2003;5:287–95.
[16] Romanenko LA, Uchino M, Tanaka N, Frolova GM, Slinkina NN, Mikhailov VV. Occurrence and antagonistic potential of *Stenotrophomonas* strains isolated from deep-sea invertebrates. Arch Microbiol 2008;189:337–44.
[17] Flores MR, Ordoñez OF, Maldonado MJ, Farias ME. Isolation of UV-B resistant bacteria from two high altitude Andean lakes (4,400 m) with saline and non saline conditions. J Gen Appl Microbiol 2009;55:447–58.
[18] Matyar F, Kaya A, Dincer S. Antibacterial agents and heavy metal resistance in Gram-negative bacteria isolated from seawater, shrimp and sediment in Iskenderun Bay, Turkey. Sci Total Environ 2008;407:279–85.
[19] Franco AR, Calheiros CS, Pacheco CC, De Marco P, Manaia CM, Castro PM. Isolation and characterization of polymeric galloyl-ester-degrading bacteria from a tannery discharge place. Microb Ecol 2005;50:550–6.
[20] Sturz AV, Matheson BG, Arsenault W, Kimpinski J, Christie BR. Weeds as a source of plant growth promoting rhizobacteria in agricultural soils. Can J Microbiol 2001;47:1013–24.
[21] Berg G, Eberl L, Hartmann A. The rhizosphere as a reservoir for opportunistic human pathogenic bacteria. Environ Microbiol 2005;7:1673–85.
[22] Berg G, Roskot N, Smalla K. Genotypic and phenotypic relationships between clinical and environmental isolates of *Stenotrophomonas maltophilia*. J Clin Microbiol 1999;37:3594–600.
[23] Taghavi S, Garafola C, Monchy S, Newman L, Hoffman A, Weyens N, et al. Genome survey and characterization of endophytic bacteria exhibiting a beneficial effect on growth and development of poplar trees. Appl Environ Microbiol 2009;75:748–57.
[24] Somaraja PK, Gayathri D, Ramaiah N. Molecular characterization of 2 chlorobiphenyl degrading *Stenotrophomonas maltophilia* GS-103. Bull Environ Contam Toxicol 2013;91:148–53.
[25] Dubey KK, Fulekar MH. Chlorpyrifos bioremediation in Pennisetum rhizosphere by a novel potential degrader *Stenotrophomonas maltophilia* MHF ENV20. World J Microbiol Biotechnol 2012;28:1715–25.
[26] Chen S, Yin H, Ye J, Peng H, Zhang N, He B. Effect of copper(II) on biodegradation of benzo[a]pyrene by *Stenotrophomonas maltophilia*. Chemosphere 2013;90:1811–20.
[27] Caliz J, Montserrat G, Martí E, Sierra J, Cruañas R, Garau MA, et al. The exposition of a calcareous Mediterranean soil to toxic concentrations of Cr, Cd and Pb produces changes in the microbiota mainly related to differential metal bioavailability. Chemosphere 2012;89:494–504.
[28] Meyer DD, Santestevan NA, Bücker F, Salamoni SP, Andreazza R, De Oliveira Camargo FA, et al. Capability of a selected bacterial consortium for degrading diesel/biodiesel blends (B20): enzyme and biosurfactant production. J Environ Sci Health A Toxic Hazard Subst Environ Eng 2012;47:1776–84.
[29] Peralta KD, Araya T, Valenzuela S, Sossa K, Martínez M, Peña-Cortés H, et al. Production of phytohormones, siderophores and population fluctuation of two root-promoting rhizobacteria in *Eucalyptus globulus* cuttings. World J Microbiol Biotechnol 2012;28:2003–14.
[30] Zhang Z, Yuen GY, Sarath G, Penheiter AR. Chitinases from the plant disease biocontrol agent, *Stenotrophomonas maltophilia* C3. Phytopathology 2001;91:204–11.
[31] Deredjian A, Colinon C, Hien E, Brothier E, Youenou B, Cournoyer B, et al. Low occurrence of *Pseudomonas aeruginosa* in agricultural soils with and without organic amendment. Front Cell Infect Microbiol 2014;29(4):53.
[32] Kerr KG, Denton M, Todd N, Corps CM, Kumari P, Hawkey PM. A new selective differential medium for isolation of *Stenotrophomonas maltophilia*. Eur J Clin Microbiol Infect Dis 1996;15:607–10.
[33] Pinot C, Deredjian A, Nazaret S, Brothier E, Cournoyer B, Segonds C, et al. Identification of *Stenotrophomonas maltophilia* strains isolated from environmental and clinical samples: a rapid and efficient procedure. J Appl Microbiol 2011;111:1185–93.
[34] Barchitta M, Cipresso R, Giaquinta L, Romeo MA, Denaro C, Pennisi C, et al. Acquisition and spread of *Acinetobacter baumannii* and *Stenotrophomonas maltophilia* in intensive care patients. Int J Hyg Environ Health 2009;212:330–7.

[35] Tenover FC, Arbeit RD, Goering RV, Mickelsen PA, Murray BE, Persing DH, et al. Interpreting chromosomal DNA restriction patterns produced by pulsed-field gel electrophoresis: criteria for bacterial strain typing. J Clin Microbiol 1995;33:2233−9.

[36] Avison MB, Higgins CS, von Heldreich CJ, Bennett PM, Walsh TR. Plasmid location and molecular heterogeneity of the L1 and L2 beta-lactamase genes of *Stenotrophomonas maltophilia*. Antimicrob Agents Chemother 2001;45:413−9.

[37] Deredjian A, Colinon C, Brothier E, Favre-Bonté S, Cournoyer B, Nazaret S. Antibiotic and metal resistance among hospital and outdoor strains of *Pseudomonas aeruginosa*. Res Microbiol 2011;162:689−700.

[38] Tenenhaus M, Young FW. An analysis and synthesis of multiple correspondence-analysis, optimal-scaling, dual scaling, homogeneity analysis and other methods for quantifying categorical multivariate data. Psychometrika 1985;50:91−119.

[39] Dray S, Chessel D, Thioulouse J. Procrustean co-inertia analysis for the linking of multivariate datasets. Ecoscience 2003;10:110−9.

[40] Thioulouse J, Dray S. Interactive multivariate data analysis in R with the ade4 and ade4TkGUI packages. J Stat Softw 2007;22:1−14.

[41] Ryan RP, Monchy S, Cardinale M, Taghavi S, Crossman L, Avison MB, et al. The versatility and adaptation of bacteria from the genus *Stenotrophomonas*. Nat Rev Microbiol 2009;7:514−25.

[42] Hardoim PR, Hardoim CC, van Overbeek LS, van Elsas JD. Dynamics of seed-borne rice endophytes on early plant growth stages. PLoS One 2012;7:e30438.

[43] Fierer N, Jackson RB. The diversity and biogeography of soil bacterial communities. Proc Natl Acad Sci U S A 2006;103:626−31.

[44] Ivanov V, Stabnikov V, Zhuang WQ, Tay JH, Tay ST. Phosphate removal from the returned liquor of municipal wastewater treatment plant using iron-reducing bacteria. J Appl Microbiol 2005;98:1152−61.

[45] Tamtam F, van Oort F, Le Bot B, Dinh T, Mompelat S, Chevreuil M, et al. Assessing the fate of antibiotic contaminants in metal contaminated soils four years after cessation of long-term waste water irrigation. Sci Total Environ 2011;409:540−7.