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Regular paper

Application of the BIOLOG system for characterization of *Serratia marcescens ss marcescens* isolated from onsite wastewater technology (OSWT)*

Joanna Chojniak[1], Łukasz Jałowiecki[1], Elmar Dorgeloh[2], Berta Hegedusova[2], Helene Ejhed[3], Jörgen Magnér[3] and Grażyna Płaza[1]✉

[1]Department of Environmental Microbiology, Institute for Ecology of Industrial Areas, Poland; [2]Development and Assessment Institute in Waste Water Technology at RWTH Aachen University, Germany; [3]Natural Resources & Environmental Effects, IVL Swedish Environmental Research Institute, Sweden

The scope of this study was to apply the Biolog system to identify and characterize a *Serratia* strain isolated from the surface of black plastic pieces which constitute the fluidized bed filter (onsite wastewater technology, OSWT). The preliminary isolation of the strain was done in the medium with tetracycline at a 16 mg/l concentration. To characterize the isolated strain, the following Biolog methods were applied: (1) EcoPlates microplates for evaluation of physiological profiling, (2) GEN III OmniLog® ID System for identification of the isolate, and (3) phenotypic microarrays (PM) technology for evaluation of sensitivity to antibiotics (PM11 and PM12). Results were recorded using the original OmniLog® software. The *Serratia* strain was identified as *Serratia marcescens ss marcescens* with similarity index 0.569. The same identification was obtained by the 16S rDNA analysis. PM analysis showed an enhancement of phenotype (resistance or growth) of this strain to 35 antibiotics. The loss of phenotype (sensitivity or non-growth) was observed only for 5 antibiotics: lomefloxacin (0.4 µg/ml), enoxacin (0.9 µg/ml), nalidixic acid (18.0 µg/ml), paromomycin (25.0 µg/ml) and novobiocin (1100 µg/ml). This study acknowledges that the methods proposed by the Biolog system allow correct and complete identification and characterization of the microbes isolated from different environments. Phenotypic microarrays could be successfully used as a new tool for identification of the multi-antibiotic resistance of bacteria and for determination of the minimal inhibition concentrations (MIC).

Key words: onsite wastewater treatment; Biolog system; *Serratia* spp.; antibiotic resistance

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INTRODUCTION

Serratia spp are motile, non-endospore forming Gram-negative rods belonging to the *Enterobacteriacea* family. In the laboratory, *Serratia* species are routinely isolated from bloodstream and wound sites using blood agar culture, or from respiratory and urinary sites using selective culture methods. Common selective agar cultures include MacConkey agar, which categorizes *Serratia* isolates with the other non-lactose fermenting *Enterobacteriaceae,* or chromogenic agars, which classify them into a broad *Klebsiella*, *Enterobacter*, *Serratia* and *Citrobacter* (KESC) grouping (Hejazi & Falkiner, 1997). It is well known for producing a red pigmentation, called prodigiosin. Prodigiosin is a member of the prodiginines family which is produced only by some *Serratia* species (Venil *et al*., 2009; Sundaramoorthy *et al*., 2009; Casullo *et al*., 2010). *S. marcescens,* as a human pathogen, is involved in hospital-acquired infections (HAIs), particularly catheter-associated bacteremia, urinary tract infections and wound infections. Due to its abundant presence in the environment, and its preference for damp conditions, *S. marcescens* is commonly found growing in bathrooms (especially on tile grout, shower corners, toilet water line, and basins), where it manifests as a pink, pink-orange, or orange discoloration and slimy film feeding on phosphorus-containing materials or fatty substances, such as soap and shampoo residue (Grimont & Grimont, 1992). Once considered a harmless saprophyte, *Serratia marcescens* is now recognized as an important opportunistic pathogen combining a propensity for healthcare-associated infection and antimicrobial resistance. In accordance with its role as an agent of opportunistic infection, *S. marcescens* was traditionally associated with low intrinsic pathogenicity. Whilst almost all isolates produce extracellular products, such as DNase, chitinase, lecithinase, lipase, gelatinase and siderophores, it appears that in *S. marcescens*, these products do not act as potent virulence factors. As a typical member of the *Enterobacteriaceae* family, and complementary to its capacity for survival, *S. marcescens* characteristically demonstrates a propensity to express antimicrobial and antibiotic resistance. *S. marcescens* are uniformly resistant to a wide range of antibiotics, including narrow-spectrum-penicillins and cephalosporins, cefuroxime, cephamycins, macrolides, tetracycline, nitrofurantoin and colistin (Sleigh, 1983; Traub, 2000; Stock *et al*., 2003).

In recent years, more attention is focused on application of the BIOLOG™ system to identify and characterize microorganisms (bacteria, yeasts and fungi), and its wide usage for microbial communities' analysis based on community-level physiological profiles (CLPP) can provide insight into microbial roles in changing ecosystems. Direct high-throughput assessment of phenotypes (phenome) using the Phenotype MicroArray (PM) system (Biolog) has stirred much attention for molecular biol-

✉e-mail: pla@ietu.katowice.pl
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ogy, genomic, and population studies of microorganisms. A large number of cellular phenotypes can be tested by PM technology (Biondi et al., 2009; Bochner, 2009; Zhang & Biswas, 2009; Mesak & Davies, 2009; Line *et al.*, 2010; Decorosi *et al.*, 2011; Mazur *et al.*, 2013; Lucas & Manna, 2013; Park & Rafii, 2014). The Phenotype MicroArray consists of preconfigured well arrays, in which each well tests a different cellular phenotype, and an automated instrument that continuously monitors and records the response of the cells in the wells of the arrays (Bochner *et al.*, 2001). The reduction of a tetrazolium dye over time as the plates are being incubated, is recorded (the intensity of the subsequent color change is proportional to bacterial growth).

Biolog Ecoplates have been used for characterizing community level physiological profiles (CLPP) to determine differences in microbial community structure and carbon utilization intensity. These plates were developed especially for bacterial community analysis of aquatic and terrestrial samples (for examples: soils; surface and ground waters, wastewater) and have already been proven useful for pinpointing differences in microbial activity and community structure among and within environmental samples (Garland & Mills, 1991; Gomez *et al.*, 2006; Preston-Mafham *et al.*, 2002; Bucher & Lanyon, 2005; Zhang *et al.*, 2010; Kong *et al.*, 2013; Chen *et al.*, 2013).

The scope of this study was to apply the Biolog system to identify and characterize a *Serratia* strain isolated from the surface of black plastic pieces which constitute the fluidized bed filter in onsite wastewater technology (OSWT).

MATERIALS AND METHODS

Description of onsite wastewater treatment facility and sampling. Bacteria were isolated from the aerated filter system (the fluidized bed reactor). The wastewater flows freely through the small wastewater treatment plant. The separation of materials occurs in the first primary clarifier in which the primary sludge accumulates. In the second clarifier, the remaining solid parts of the sewage are separated. In the third tank which is the bioreactor, the biological treatment takes place during which wastewater is aerated and mixed. The air is being dosed into the reactor to support oxygenation and mixing. Discharge of growth bodies into secondary treatment is prevented with a plastic slot. In the final clarification, the biofilm is separated from the cleaned wastewater. The biofilm settles as excess sludge on the floor of the container and is pumped back with a sewage pump into the pretreatment tank. This technology operates on a principle of a fluidized bed biological reactor, with fluidized media providing a high active surface for microorganisms growing on it. The bioreactor functions with continuously moving media. Microorganisms are immobilized on the small, fluidized units of carrier media which enables the treatment process to operate with a minimal biomass wash-out. Wastewater is pumped upward through a bed of media resulting in fluidization of the carrier media. There are suspended microorganisms in the bioreactor as well, which are released from the fluidized media. Microorganisms which in addition are sloughed from the surface of carrier media were collected in the form of a liquid sample. For analysis of those microorganisms which create the biofilm growing attached to the carrier media that remain fluidized, a biofilm covered carrier media were taken.

The black plastic carrier media were collected from the bioreactor of the facility which is located in the testing field at PIA (Development and Assessment Institute in Waste Water Technology, RWTH Aachen University, Germany). The samples were collected into prepared sterile polypropylene (PP) bottles by the trained/skilled field worker taking the test material at one time, wearing protective gloves and using sterile equipment. All the samples collected consisted of grab samples which were stored in bottles at 4°C for microbiological analysis within 24 h from the sampling time.

Isolation of *Serratia* spp. Culturable bacteria were evaluated in a series with 10-fold dilutions of the liquid sample, i.e. 1 mL of the liquid sample was dispersed in 9 mL of sterilized physiological solution (0.85% NaCl) by shaking for 2 min. 1 mL of aliquots of the different dilutions were pipetted onto plates, and pour-plate method was used for isolation of the microorganisms. Bacteria were incubated on SMA medium (Standard Methods Agar, BioMerieux) containing different concentrations of antibiotics (kanamycin — 16 mg/L; streptomycin — 30 mg/L; tetracycline — 16 mg/L) at 30°C for 48–72 hours. After incubation, several colonies were selected according to common and morphological characteristics, and streaked onto fresh SMA medium for 24 h at 30°C to obtain uncontaminated colonies.

Identification of *Serratia* spp. by 16S rDNA. Bacterial DNA was extracted from bacterial cultures according to the manufacturer's protocol of Roche PCR Master Kit Ref. 11 636 103 001, and it was stored in –20°C. PCR reaction was carried out to amplify the target DNA fragments using the universal primers: forward primer: AGA GTT TGA TCC TGG CTC A and reverse primer: GTG TGA CGG GCG GTG TGT AC. After amplification, the reaction mixtures were resolved on a 1.5% agarose gel. PCR products were excised from the agarose gel and purified by ExtractMe DNA Gel-Out (Blirt, Poland). The 16S rDNA gene sequence was compared with GenBank NCBI database, using BLAST software to determine the genetic similarities of the isolates.

Identification of *Serratia* spp. by the BIOLOG™ GEN III microplates. The identification of isolated bacteria was performed by new GEN III MicroPlate™ test panel of the Biolog system. The test panel provides a "Phenotypic Fingerprint" of the microorganism which can then be used to identify them to a species level. The GEN III MicroPlates™ enable testing of Gram-negative and Gram-positive bacteria in the same test panel. The test panel contains 71 carbon sources and 23 chemical sensitivity assays. GEN III dissects and analyzes the ability of the cell to metabolize all major classes of compounds, in addition to determining other important physiological properties such as pH, salt and lactic acid tolerance, reducing power, and chemical sensitivity. All the reagents applied were from Biolog, Inc. (Hayward, CA, USA). Fresh overnight cultures of the isolates were tested as recommended by the manufacturer. Bacterial suspensions were prepared by removing bacterial colonies from the plate surface with a sterile cotton swab and agitating it in 5 ml of 0.85% saline solution. Bacterial suspension was adjusted in IF-0a to achieve a 90–98% transmittance (T90) using a Biolog turbidimeter. 150 μL of the suspension was dispensed into each well of a Biolog GEN III microplate. The plates were incubated at 26°C in an Omnilog Reader/Incubater (Biolog). After incubation, the phenotypic fingerprint of purple wells is compared to the Biolog's extensive species library. If a match is found, a species level identification of the isolates could be made.

Table 1. Extensive biochemical analysis of a *Serratia* strain using the BIOLOG™ GEN III microplate

Properties	Results	Properties	Results
Dextrin	-	Glycyl-L-Proline	+
D-Maltose	+	L-Alanine	+
D-Trehalose	+	L-Arginine	-
D-Cellobiose	-	L-Aspartic Acid	+
Gentiobiose	-	L-Glutamic Acid	+
Sucrose	+	L-Histidine	+
D-Turanose	-	L-Pyroglutamic Acid	-
Stachyose	-	L-Serine	+
pH 6	+	Lincomycin	+
pH 5	+	Guanidine HCl	+
D-Raffinose	-	Niaproof 4	+
α-D-Lactose	-	Pectin	-
D-Melibiose	-	D-Galacturonic Acid	+
β-Methyl-DGlucoside	+	L-Galactonic Acid Lactone	+
D-Salicin	+	D-Gluconic Acid	+
N-Acetyl-D-Glucosamine	+	D-Glucuronic Acid	+
N-Acetyl-β-D-Mannosamine	-	Glucuronamide	+
N-Acetyl-D-Galactosamine	+	Mucic Acid	-
N-Acetyl Neuraminic Acid	-	Quinic Acid	-
1% NaCl	+	D-Saccharic Acid	-
4% NaCl	-	Vancomycin	+
8% NaCl	-	Tetrazolium Violet	+
α-D-Glucose	+	Tetrazolium Blue	+
D-Mannose	+	p-HydroxyPhenylacetic Acid	+
D-Fructose	+	Methyl Pyruvate	+
D-Galactose	+	D-Lactic Acid Methyl Ester	-
3-Methyl Glucose	-	L-Lactic Acid	+
D-Fucose	-	Citric Acid	+
L-Fucose	+	α-Keto-Glutaric Acid	-
L-Rhamnose	-	D-Malic Acid	-
Inosine	+	L-Malic Acid	+
1% Sodium Lactate	+	Bromo-Succinic Acid	+
Fusidic Acid	+	Nalidixic Acid	-
D-Serine	+	Lithium Chloride	+
D-Sorbitol	+	Potassium Tellurite	-
D-Mannitol	+	Tween 40	+
D-Arabitol	-	γ-Amino-Butryric Acid	-
myo-Inositol	+	α-Hydroxy Butyric Acid	-
Glycerol	+	β-Hydroxy-D,L-Butyric Acid	-
D-Glucose- 6-PO4	+	α-Keto-Butyric Acid	-
D-Fructose- 6-PO4	+	Acetoacetic Acid	-

Determining of physiological profile of *Serratia* spp. by BIOLOG™ EcoPlates. Biolog EcoPlates are 96-well plates, containing three replicate sets of 31 different substrates, which are ecologically relevant and structurally diverse compounds. These substrates are widely used to assess functional diversity of soil microbial communities, and are based on community-level carbon source utilization patterns (Preston-Mafham *et al.*, 2002). 10 mL of water were shaken in 90 ml of distilled sterile water for 20 min at 25°C. Next 150 μL of each sample was inoculated into each well of Biolog EcoPlates and incubated at 26°C. The rate of utilization was indicated by the reduction of the tetrazolium salt, a redox indicator dye that changes from colorless into purple in the wells. The color development was monitored every 24 h as absorbance with Microstation (Biolog Inc.) at a wavelength of 590 and 750 nm. The data were collected using Microlog Data Collection Software 1.2 (Biolog Inc.).

The five guilds of carbon substrates proposed by Weber & Legge (2009) were used: 1) carbohydrates (Carb), 2) carboxylic and acetic acids (C & AA), 3) amino acids (AA), 4) polymers (Poly), and 5) amines and amides (A & A). For each guild, corrected absorbance values of the substrates were summarized and expressed as a percentage of total absorbance value of the plate (Weber & Legge, 2009).

Evaluation of antibiotic resistance of *Serratia* spp. by using BIOLOG™ PM microplates. PM panels are 96-well microplates containing different substrates in each well. PM11 and PM12 assays were used to determine the antibiotic resistance of the bacteria. In addition to a unique substrate (antibiotics), each well of the panels also contains the needed minimal medium components and a specific dye.

The strain was grown overnight at 30°C on LB agar medium, and then cells were picked up with a sterile cotton swab and transferred into a sterile, capped tube containing 20 ml of the inoculation fluid (IF-0, Biolog Inc.). Cell density was adjusted to 81% transmittance on the Biolog turbidimeter. The PM11 and PM12 plates were inoculated with the cell suspension (100 μL/well), and then incubated at 30°C for 48 h in the Omnilog Incubater/Reader (Biolog Inc., Hayward, USA). The changes of color in the wells were measured every 15 min, which provided both, amplification and quantitation of the phenotype. Analysis was carried out using OmniLog® phenotype microarray software v 1.2 to determine the phenotypic differences. The data were collected using OmniLog® MicroArray™ Data Collection Software Release 1.2 (Biolog Inc.), which generated a tetrazolium color development as a function of time.

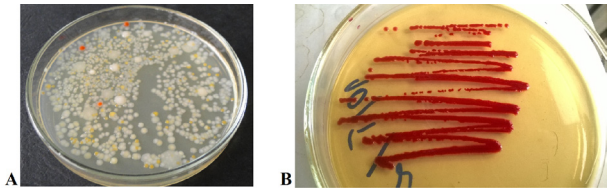

Figure 1. *Serratia marcescens* growing on the solid media in a consortium of bacteria — red colonies (A) and uncontaminated culture (B).

RESULTS AND DISCUSSION

Serratia spp. was isolated from the carrier media - the surface of black plastic pieces. Under laboratory conditions, it was isolated from a solid medium with tetracycline at 16 mg/l concentration. The basic cellular morphological properties are shown in Fig. 1A and B. The bacterium was rod shaped with smooth edged red colonies. The *Serratia* spp. isolated, was identified as *Serratia marcescens ss marcescens* by the Biolog system with similarity index 0.569. Characteristics of biochemical reactions obtained from GEN III are described in Table 1. The 16S rDNA analysis showed that the sequence of the strain aligned with the sequence of *Serratia marcescens* from the data base with a probability of 99–100 %. Complete sequence (1500 bp) of the 16S rDNA of the strain was determined and similarity search by BLASTn was done. The BLASTn results are shown in Table 2. The BIOLOG identification and 16S rDNA sequencing gave a close evolutionary relationship with *S. marcescens*. The new GEN III microplates are applicable to an unprecedented range of both Gram-negative and Gram-positive bacteria. Before, the identification of Gram-positive and Gram-negative bacteria were identified separately by two different plates named GP (Gram-positive) and GN (Gram-negative). The GEN III microplates can be used to evaluate the ability of the cell to metabolize all major classes of biochemicals, in addition to determining other important physiological properties such as pH, salt, and lactic acid tolerance, reducing power, and chemical sensitivity. Biolog's technology gives a possibility to identify environmental and pathogenic microorganisms by producing a characteristic pattern or "metabolic fingerprint" from discrete test reactions, performed within a 96 wells microplate. More than 2650 identification profiles of environmental and fastidious organisms of interest in diverse fields of microbiology can be determined by the system. Five databases are available for a broad spectrum of aerobic and anaerobic bacteria, yeasts and filamentous fungi.

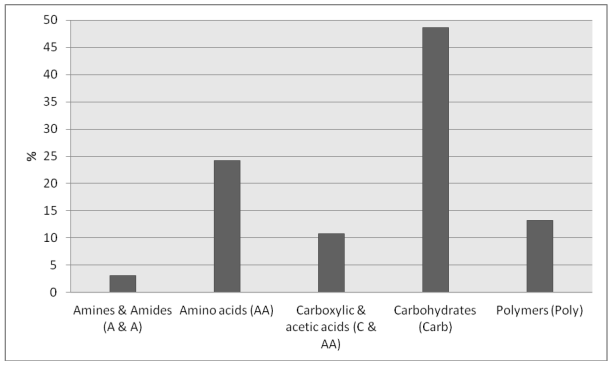

Figure 2. Pattern of total carbon source utilization for the five guilds proposed by Weber & Legge (2009) obtained from Eco-Plates.

Ventura & Zink (2002) stated that 16S rDNA genes sequencing and analysis has become one of the cornerstones of modern microbial taxonomy. Therefore, these sequences are used to define genus specifics for rapid detection of bacterial species. However, 16S rDNA gene sequences are normally not sufficient to differentiate strains within a species, which the BIOLOG™, however, sometimes can. In contrast, this molecular tool was helpful in assessing the overall phylogenetic relationship between the most typical bacteria. In a previous study, it has been demonstrated that BIOLOG™ could be a valuable complement to other methods used for strain verification. However, using it as a single method for identification could be misleading. The major advantage of the 16S rDNA gene sequencing is that the 16S rDNA gene is present in all bacteria, and it is a universal target for bacterial identification and provides high accuracy, reliability and reproducibility for identification of any bacterial organism (Drancourt *et al.*, 2004).

Functional diversity, defined by the number and types of carbon substrates used by the *Serratia* strain, was evaluated by the Biolog EcoPlates. In the majority of peer reviewed articles, the Biolog EcoPlates were used to evaluate the functional diversity of whole communities in various environments (Paul *et al.* 2006; Stefanowicz, 2006; Weber & Legge, 2009; Al-Mutairi, 2009; Sala *et al.*, 2010; Garcia *et al.* 2013; Kenarova *et al.*, 2014; Gryta *et al.*, 2014). Studies have been done in diverse applications of microbial ecology and have demonstrated the fundamental utility of Eco microplates in detecting population changes in soil, water, wastewater, activated sludge, compost, and industrial wastes. Communities of organisms

Table 2. The BLASTn results, according to the NCBI database

Description	Accession No	Max Identity (%)	Max score	Total score
Serratia mercescens strain C3	GU212864.1	99	2433	2433
Serratia mercescens strain LEP9	AB635400.1	100	2431	2431
Serratia mercescens strain N4-5	EF035134.1	100	2431	2431
Serratia mercescens strain N4.1	AY514435.1	100	2431	2431
Serratia mercescens strain N1.14	AY514433.1	100	2431	2431
Serratia mercescens strain N1.6	AY514431.1	100	2431	3431
Serratia mercescens strain PS-1	KF258679.1	99	2425	2425
Serratia mercescens strain GR6	KC009686	99	2425	2425

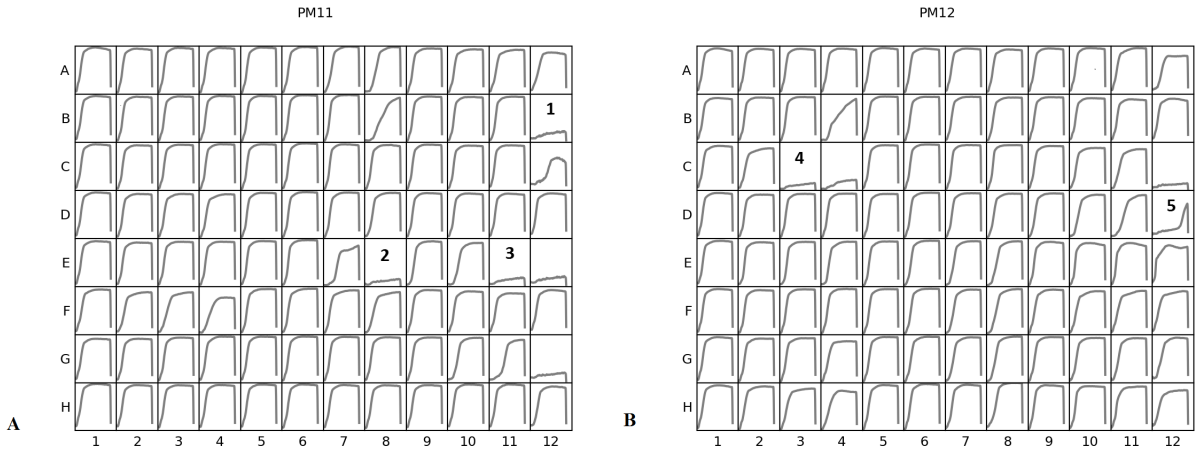

Figure 3. Growth kinetics of *Serratia marcescens* in the individual wells of PM11 (A) and PM12 (B). 1 — lomefloxacin (0.4 µg/ml); 2 — enoxacin (0.9 µg/ml); 3 — nalidixic acid (18 µg/ml); 4 — paromomycin (25 µg/ml); 5 — novobiocin (1100 µg/ml). A–H — number of rows in a plate; 1–12 — number of columns in a plate

result in a characteristic reaction pattern called a metabolic fingerprint. This approach, called community-level-physiological profile (CLPP) has been demonstrated to be effective in evaluation of changes in microbial communities. In this study, physiological profile of a *Serratia* strain was evaluated by using EcoPlates. The results obtained from the EcoPlates demonstrated that the *Serratia* strain used an average of 48% of the 31 available carbon

Table 3. Sensitivity of *Serratia marcescens* to various antibiotics and toxic ions (analysis of PM11 and PM12)

PMs	Compounds and their targets		Results
PM11	Amikacin	wall, lactam	R
	Chlorotetracycline	protein synthesis, 30S ribosomal subunit, tetracycline	R
	Lincomycin	protein synthesis, 50S ribosomal subunit, lincosamide	R
	Amoxicillin	wall, lactam	R
	Cloxacillin	wall, lactam	R
	Lomefloxacin	DNA topoisomerase	R
	Bleomycin	DNA damage, oxidation	R
	Colistin	membrane, cyclic peptide	R
	Minocycline	protein synthesis, 30S ribosomal subunit, tetracycline	R
	Capreomycin	protein synthesis	R
	Demeclocyline	protein synthesis, 30S ribosomal subunit, tetracycline	R
	Nafcillin	wall, lactam	R
	Cefazolin	wall, cephalosporin	R
	Enoxacin	DNA topoisomerase	S
	Nalidixic acid	DNA topoisomerase	S
	Chloramphenicol	protein synthesis, amphenicol	R
	Erythromycin	protein synthesis, 50S ribosomal subunit, macrolide	R
	Neomycin	protein synthesis, 30S ribosomal subunit, aminoglycoside	R
	Ceftriaxone	wall, cephalosporin	R
	Gentamicin	protein synthesis, 30S ribosomal subunit, aminoglycoside	R
	Potassium tellurite	toxic anion	S
	Cephalothin	wall, cephalosporin	R
	Kanamycin	protein synthesis, 30S ribosomal subunit, aminoglycoside	R
	Ofloxacin	DNA topoisomerase	R

	Penicillin G	wall, lactam	R
	Tetracycline	protein synthesis, 30S ribosomal subunit, tetracycline	R
	Carbenicillin	wall, lactam	R
	Oxacillin	wall, lactam	R
	Penimepicycline	protein synthesis, 30S ribosomal subunit, tetracycline	R
	Polymyxin B	membrane, cyclic peptide	R
	Paromomycin	protein synthesis, 30S ribosomal subunit, aminoglycoside	S
	Vancomycin	wall	R
	D,L-Serine hydroxamate	tRNA synthetase	R
	Sisomicin	protein synthesis, 30S ribosomal subunit, aminoglycoside	R
	Sulfamethazine	folate antagonist, PABA analog	R
PM12	Novobiocin	DNA topoisomerase	S
	2,4-Diamino-6,7-diisopropylpteridine	folate antagonist, vibriostatic agent	R
	Sulfadiazine	folate antagonist, PABA analog	R
	Benzethonium chloride	membrane, detergent, cationic	R
	Tobramycin	protein synthesis, 30S ribosomal subunit, aminoglycoside	R
	Sulfathiazole	folate antagonist, PABA analog	R
	5-Fluoroorotic acid	nucleic acid analog, pyrimidine	R
	Spectinomycin	protein synthesis, 30S ribosomal subunit, aminoglycoside	R
	Sulfamethoxazole	folate antagonist, PABA analog	R
	L-Aspartic acid b-hydroxamate	tRNA synthetase	R
	Spiramycin	protein synthesis, 50S ribosomal subunit, macrolide	R
	Rifampicin	RNA polymerase	R
	Dodecyltrimethyl ammonium bromide	membrane, detergent, cationic	R

R – resistant; S – sensitive; PM – phenotypic microarray

substrates. Figure 2 presents the percentage of total carbon source utilization for each guild. The results indicated that the carbohydrate (Carb) and amino acid (AA) groups were the most intensively metabolized by *Serratia marcescens*. The most used carbon substrates were: N-acetyl-D-glucosamine, D-mannitol, β-methyl-D-glucoside, and i-erythritol from the carbohydrates guild, and L-asparagine and L-serine from the amino acids guild. Only D-galacturonic acid and Tween 40 and 80 were metabolized from the carboxylic and acetic acids and polymer guilds, respectively. *Serratia marcescens* can metabolize only selected carbon sources during growth.

One goal of the present study was to extend the knowledge on the natural antibiotic susceptibility of *Serratia marcescens*. PM microplates were successfully applied and developed for this approach. The arrays provide the identification of resistance to 39 antibiotics belonging to different chemical classes. Each antibiotic is presented at 4 concentrations. The list of the antibiotics in PM 11 and PM 12 with their effects on *Serratia marcescens* growth are presented in Table 3. The loss of phenotype (sensitivity) was only observed for 5 antibiotics: lomefloxacin at a concentration of 0.4 μg/ml, enoxacin at 0.9 μg/ml, nalidixic acid at 18 μg/ml, paromomycin at 25 μg/ml, and novobiocin at 1100 μg/ml (Fig. 3A and B). Lomefloxacin and enoxacin antibiotics are from the fluoroquinolone class**.** Nalidixic acid is a synthetic quinolone antibiotic. Quinolones and fluoroquinolones are bactericidal drugs, eradicating bacteria by interfering with DNA replication. However, paromomycin is an aminoglycoside.

Metabolic studies have been facilitated in recent years by the development of phenotypic microarrays (PMs). The PM technique allows phenotypic testing to become a simple analysis of gene expression and allows to directly observe the consequence of a genetic change. In the research described by Zhang & Biswas (2009), a *lia*S mutant of *Streptococcus mutants* was subjected to phenotypic microarray analysis of about 2000 phenotypes, including utilization of various carbon, nitrogen, phosphate and sulfur sources, osmolytes, metabolic inhibitors, and susceptibility to toxic compounds, including several types of antibiotics. In another paper, soil bacteria that fix atmospheric nitrogen in plant roots, *Sinorhizobium meliloti,* have been the subject of extensive PM analysis (Biondi *et al*., 2009). The metabolic activities of the four *S. meliloti* environmental isolates were assayed with PM plates compared with the reference strain. Metabolic studies in which phenotypic microarrays were used to characterize bacteria, such as *Campylobacter jejuni*, *Staphylococcus aureus*, *Clostridium perfringens*, *Streptococcus thermophiles* and *Rhizobium leguminosarum* were described (Mesak & Davies, 2009; Line *et al*., 2010; Decorosi *et al*., 2011; Lu-

cas & Manna, 2013; Mazur *et al*., 2013; Park & Rafii, 2014). However, there are no papers focusing on metabolic properties of *Serratia* strains evaluated by Biolog methods.

In this paper, identification, metabolic and antibiotic sensitivity of a *Serratia* strain isolated from the surface of black plastic pieces which constitute the fluidized bed filter was characterized by the Biolog system. Special attention was paid to apply and develop PM microplates for evaluation of multi antibiotic resistance of the bacteria, and for determining the antibiotic minimal inhibition concentration (MIC). Up to now, the antibiotic susceptibility is mostly performed by the disc diffusion method or microdilution procedure, but E-tests are applied for MIC evaluation.

The Biolog™ system combines the microbial identification, functional diversity of microbial communities and phenotype microarray testing approaches. The correlation between genotypes with phenotypes, determination of metabolic and chemical sensitivity properties of cells, discovery of new targets for antimicrobial compounds, optimization of growth and culture conditions in bioprocess development can be evaluated by the system.

In conclusion, this study demonstrates for the first time the possibility of using the Biolog's microarrays for identification and characterization of *Serratia marcescens* as an example of the presence of environmental and pathogenic microbes. Special attention was taken to present the PM 11 and PM 12 microplates as a novel tool for identification of the multi-antibiotic resistance of bacteria and for determining the minimal inhibition concentrations (MIC) of antibiotics.

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