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Application of Biolog Microarrays Techniques for Characterization of Functional Diversity of Microbial Community in Phenolic-contaminated Water

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ABSTRACT: The impact of phenolic-contaminated water on microbial community structure was assessed using Biolog microarrays techniques. The following Biolog plates were used: GEN III plates, new test panel for identification of both Gram-negative and Gram-positive bacteria, EcoPlates microarray for evaluation of functional diversity of microbial communities and phenotype microarrays (PMs) for characterization of the selected bacterial strains. Most of the isolated strains were identified as: *Paenibacillus castaneae*, *Chryseobacterium indoltheticum*, *Pseudomonas fluorescens*, *Acinetobacter johnsonie*, *Mycobacterium flavescens*, *Ralstonia pickettii*, *Acinetobacter schindleri*, *Microbacterium maritypicum*. The mean value of substrate richness (S) was high (30.67). Also, microbial activity in contaminated water evaluated by AWCD and AUC was high. The mean values of AWCD and AUC were 1.5 and 740.10, respectively. Instead, the mean values of Shannon-Weiner functional diversity index (H) and Shannon Evenness index were low, 1.46 and 0.978, respectively. The carbohydrates (Carb) and carboxylic and acetic acids (C & AA) were the most utilized carbon sources by the microbial communities of phenol-contaminated water. The proprieties of *Pseudomonas fluorescens* and *Paenibacillus castaneae* to oxidize 190 different substrates as sole carbon sources (PM1 and PM2), and the sensitivity to various toxic chemical compounds at 4 different concentrations (PM11, PM12 and PM13) were evaluated. Phenotypic microarrays used identified the differences between species. Both studied bacterial strains showed high ability to metabolize aminoacids as well as carbohydrates. Among carboxylic acids *Pseudomonas fluorescens* was able to use more of substrates as a sole of carbon in comparison with *Paenibacterium castaneae*.

Key words: Phenolic compounds, Biodiversity, Biolog system, GEN III identification system, EcoPlates microarray, phenotype microarrays (PM)

INTRODUCTION

The Water Framework Directive (2000/60/EC) aims to establish a legal framework for the protection of water quality in European countries. The directive recognizes that specific measures have to be adopted at a European level against water pollution by individual pollutants, or groups of pollutants, presenting a significant risk to the aquatic environment and water used for the production of drink water. The Annex II of the Directive on Priority Substances (Directive 2008/105/EC; a daughter directive of the WFD) replaces Annex X of the WFD contains the list of 33 priority substances including phenolic compounds. The emissions, discharges and losses of the phenols need to be ceased or phased out. Phenol pollution comes mainly from waste from pharmaceutical and metallurgical industries, and petrochemical refinery. Phenols are also industrial commodity as a precursor material in production of herbicides, nylon, epoxies, explosives, perfumes, numerous pharmaceutical drugs and detergents. This class of aromatic chemicals is common in nature such as: amino acids, hormones or obtained from plants: antioxidants or phytohormones. These substances imposes severe risks to human health and the environment. The toxicity and accumulation of phenolic compounds in human, animals and plants tissues have been observed (Basha *et al*., 2010).

Biolog™ system combines the biolog's microbial identification, functional diversity of microbial communities and phenotype microarray

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testing approaches. These capabilities enable a wide range of studies, from routine species-level identification to projects in detailed strain characterization, such as fingerprinting and tracking strains that cause product contamination, comparison of nonpathogenic and pathogenic strains, gene and mutant characterization and bioprocess improvement. With the automated instrumentation, phenotypic properties can be measured quantitatively and kinetically. The researches are able to obtain the information on genetic differences, environmental change, and exposure to drugs and other chemicals. They can correlate genotypes with phenotypes, determine metabolic and chemical sensitivity properties of cells, discover new targets for antimicrobial compounds, optimize growth and culture conditions in bioprocess development. Borglin *et al*. (2012) in their review describe applications of phenotype arrays to environmental microbiology. Phenotypic microarray technology enables a screen of the phenotypic characteristics of microorganisms, either pure culture, community, or consortia (Bochner *et al*. 2001).

The use of Biolog™ microtiter plates for expressing the functional diversity of microbial communities was originally described by Garland and Mills (1991). Biolog system have been extensively used in applied ecological research to identify microbes and to detect changes in soil microbial communities (Preston-Mafham *et al*., 2002; Kirk *et al*. 2004; Bradley *et al*., 2006; Stefanis *et al*. 2013). However, no information is known about the impact of contaminated waters on the diversity of microbial communities.

The aim of the study was to apply the Biolog microarray technologies to characterize the microbial community diversity in phenolic contaminated water and to present the broad possibility of using of Biolog system in environmental microbiology. The following microarray techniques were used: GEN III identification system, EcoPlates microplates and phenotype MicroArrays™ (PMs).

MATERIALS & METHODS

The 5 water samples were taken from phenolic contaminated pond "Kalina" (Upper Silesia, Poland) and collected in a 1-liter polyethylene container sterilized by autoclaving (121 °C, 15 min). After transportation to the laboratory, the samples were mixed and divided into sterilized smaller polyethylene flasks under sterile conditions, in the safety cabinet. The mixed samples were stored in the bottles at 4 °C for microbiological analysis before 24 h of sampling. The concentrations of phenolic compounds in the contaminated water are presented in Table 1.

Culturable bacterial and fungal numbers were evaluated by serial 10-fold dilutions of the water. 1 mL of the water was dispersed in 10 mL of sterilized physiological water (0.8% NaCl) by shaking for 2 min. After suitable dilutions, 1 mL of aliquots of the different dilutions was pipetted onto plates, and pour-plate method was used for isolation of microorganisms. Psychrophilic bacteria was incubated

Table 1. Concentrations of various phenolic compounds in the water sample

Phenolic compounds	Concentrations	
	(ug/L)	%
Phenol	5.38	9.84
2-methylphenol	1.90	3.47
3-methylphenol+4-methylphenol	23.4	42.71
2,6-dimethylphenol	0.74	1.34
2-etylphenol	1.48	2.70
2,4-dimethylphenol	0.98	1.79
2,5-dimethylphenol	0.85	1.56
3-ethylphenol	2.12	3.88
3,5-dimetylofenol	2.89	5.28
2,3-dimethyphenol	1.57	2.87
3,4-dimethylphenol	4.62	8.44
2,3,6-trimethylphenol	4.01	7.32
2,3,5-trimethylphenol	4.80	8.78
sum	54.71	100.00

on SMA medium (Standard Methods Agar, BioMerieux) containing 100 mg cycloheximide/L at 22°C for 72 hours. After incubation the bacterial colonies were counted and colony forming unit (CFUs) were calculated. All microbiological analyses were performed in triplicate. The percentile of the 3 measurements was ± 2 %.

The bacteria were selected from the plates according to frequently and morphological characteristics. The bacteria which were occurred with the high frequency were chosen for the future analysis. The identification of isolated bacteria was performed by new GEN III MicroPlate™ test panel of the Biolog system. The GEN III MicroPlates™ are 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. The 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. The bacterial suspension was adjusted in IF-0a to achieve a 90-98% transmittance (T90) using a Biolog turbidimeter. The 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).

Biolog EcoPlates are 96-well plates, containing three replicate sets of 31 different substrates, which are ecologically relevant, structurally diverse compounds. These substrates are widely used to assess functional diversity of soil microbial communities, and are based on community-level carbon sources 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 were inoculated into each well of Biolog EcoPlates and incubated at 26°C. The rate of utilization was indicated by the reduction of the tetrazolium, a redox indicator dye that changes from colorless into purple. The color development was read as absorbance every 24 h with Microstation (Biolog Inc.) at a wavelength of 590 nm. The data were collected using Micrologg Data Collection Software 1.2 (Biolog Inc.).

Microbial response in each microplate that expressed average well-color development (AWCD) was determined as follows (Garland & Mills, 1991; Gomez *et al*., 2004):

$$AWCD = \Sigma\ ODi/31 \quad (1)$$

where ODi is optical density value from each well, corrected subtracting the blank well (inoculated, but without a carbon source).
AUC (Area Under the Curve) was calculated as:

$$AUC = \Sigma\ (A_n + A_{n+1})/2 \times (t_{n+1} - t_n) \quad (2)$$

where A_n and A_{n+1} is the absorbance of each individual well at two consecutive measurements at two different measurement times for t_n and t_{n+1}.
Biolog data incubated for 72 h were analyzed according to Zak *et al*. (1994) to give substrate richness (catabolic richness) (S) values, e.g. total number of oxidized C substrates = total number of wells with absorbance over 0.25, and catabolic diversity index (Shannon-Weiner functional diversity index, H). The Shannon-Weiner functional diversity index was calculated as:

$$H = - \Sigma pi(lnpi) \quad (3)$$

Where pi is the ratio of the activity on each substrate (ODi) to the sum of activities on all substrates "ODi. Shannon Evenness (E) index was calculated from Shannon-Weiner diversity index (H) and substrate richness (S) index as follows:

$$E = H/ln\ S \quad (4)$$

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). The carbon sources, selected as miscellaneous by Zak *et al*. (1994), were included into carbohydrates category according to Weber and Legge (2009). For each guild the corrected absorbance values of the substrates were summarized and expressed as a percentage of total absorbance value of the plate (Weber & Legge, 2009).

PM panels are 96-well microplates containing different substrates in each well. PM1 and PM2 are carbon source panels. PM3, 6, 7 and 8 are nitrogen source panels. PM4 contains various phosphate and sulfur sources; PM5 contains various biosynthesis pathway endoproducts and nutrient supplements. PM11-PM20 are chemical sensitivity assays including toxic compounds and ions. In addition to a unique substrate, each well of these metabolic panels also contains the needed minimal medium

components. Strains were grown overnight at 26°C on LB 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 following plates PM1, PM2, PM11, PM12 and PM13 plates were inoculated with the cell suspension (100 µL/well), and then incubated at 26°C during 48h in the Omnilog Incubater/Reader (Biolog Inc., Hayward, USA). The cell respiration was measured every 15 min 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 opacity response was recorded as a positive integer named OmniLog™ units (OL units). An average height threshold of 100 OmniLog unit (OU) was chosen to evaluate the results obtained from PMs (Mara *et al*., 2012).

The data were collected using OmniLog® MicroArray™ Data Collection Software Release 1.2 (Biolog Inc.). The Statistica 10.0 PL software package was used for graphic of Biolog OUs (OmniLog units). Each data point in plots is represented as a rectangular region, with different colors corresponding to the values.

RESULTS & DISCUSSION

Based on the different cultural-morphological properties, about 20 strains were selected. The most isolated strains belonged to the species: *Paenibacillus castaneae* (ID: 0.677), *Chryseobacterium indoltheticum* (ID: 0.646), *Pseudomonas fluorescens* (ID: 0.589), *Acinetobacter johnsonie* (ID: 0.511), *Mycobacterium flavescens* (ID: 0.549), *Ralstonia pickettii* (ID: 0.799), *Acinetobacter schindleri* (ID: 0.514), *Microbacterium maritypicum* (ID: 0.535). Metabolic fingerprint of selected bacterial strains isolated from phenolic-contaminated water performed by GEN III identification system is presented in Fig. 1.

The values of ecological indices evaluated from Eco plates are presented in Table 2. The Shannon-Weiner diversity index (H) and Shannon Evenness index (E) yield additional information about the microbial functional diversity based on the structure of the substrates used. AWCD and AUC are mostly used as indicators of general microbial activity (Kong *et al*., 2006). According to the values of AWCD and AUC the microbial activity in contaminated water was high. The microbes adopted to the environmental conditions in the contaminated water. But, the values of Shannon-Weiner diversity index (H) and Shannon Evenness index (E) were low, 1.46 and 0.978, respectively. Only the substrates from two groups, the carbohydrates (Carb) and carboxylic and acetic acids (C & AA), were the most utilized by the microbial community (Fig. 2).

Up to now, the most common plates used in ecological studies were the GN microplates However, GN microplates were developed specifically for the identification of Gram-negative bacteria and contain carbon sources appropriate to that group. Only a few of carbon sources in GN plates seem to contribute to microbial community in environmental samples and many of them are redundant to the analysis. EcoPlates are now produced and are tailored to ecological applications. They comprise three replicate sets of 31 environmentally applicable substrates. Ecoplates, using more ecologically relevant structurally diverse compounds, are likely to provide a more useful test for microbial analysis. Preston- Mafham *et al*. (2002) reported use of different types of Biolog plates in the literature. In around 88% papers reporting community analysis by Biolog system GN plates have been used. Before only the plates have been available. In contrast, Ecoplates were only used in 7 papers; it is around 6% from all 122 papers published.

Only few reports describe the application of Biology system to detect substrates profiles of microbial communities in the water samples. Lawley and Bell (1998) used the Biolog GN microplate community profiles to detect the effect of chlorine and *E. coli* supplementation on inoculum density and species diversity of the river communities. The *E. coli* supplements increased the inoculum density and decreased the species richness. The principle component analysis with a reduced set of substrates suggested some influence of *E. coli* on the community profile. The obtained results gave the suggestion that supplementing the autochtonus population of the water river with large amounts of *E. coli* can generate a profile similar to pure *E. coli*. Both chlorine treatments and *E. coli* supplements changed the functional diversity of the river communities. Grover and Chrzanowski (2000) utilized 95 carbon sources of Biolog-GN microtiter plates to evaluate the seasonal changes in bacteriplankton community of the waters among 4 lakes of different trophic status and climate. Community-level physiological profiles (CLPPs) obtained suggested some differences among lakes and sampling time. The substrates pattern found by the authors reveled that carbohydrates were used by bacterioplankton in mid-to late summer under relatively warm conditions. On the other hand, during the cooler times of the year microbial communities

Table 2. Soil microbial activity (AWCD and AUC) and functional diversity indices (H, E, S) based on 72-h incubation (means ± standard deviation, n = 3) for 31 sole carbon source substrates of ECO plates

Indices	Mean values	Stand. Dev.
AWCD (average well-color development)	1.5	0.523
AUC (Area Under the Curve)	740.10	0.085
H (Shannon-Weiner diversity index)	1.46	0.004
E (Shannon Evenness)	0.98	0.002
S (Substrate richness)	30.67	0.6

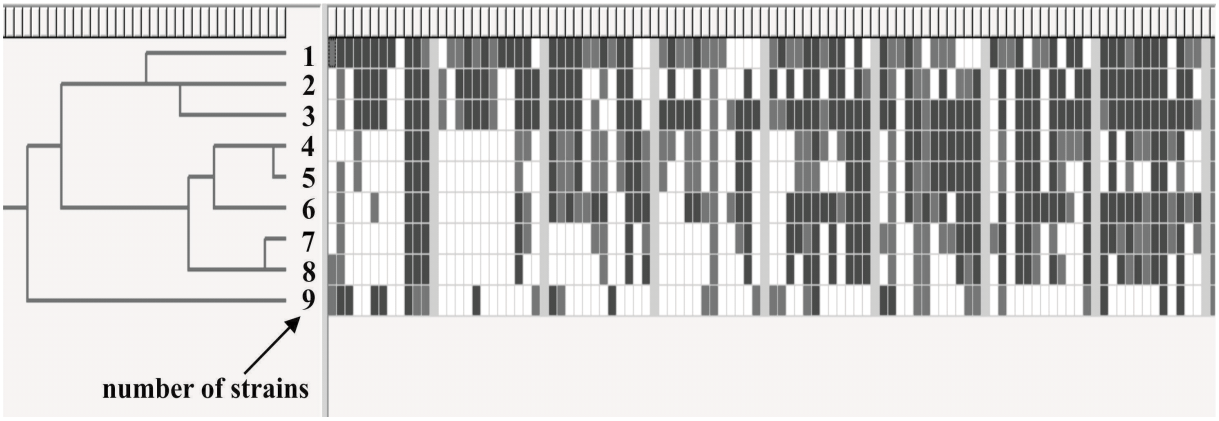

Fig. 1. Metabolic characterization and dendrogram of identified strains obtained from Biolog GEN III identification system

(1–*Micrbacterium maritypicum*, 2-*Mycobacterium flavescens*, 3-*Paenibacillus castaneae*, 4 & 5-*Pseudomonas fluorescens*, 6-*Ralstonia picketti*, 7-*Acinetobacter johnsonii*, 8-*Acinetobacter schindleri*, 9-*Chryseobacterium indoltheticum)*

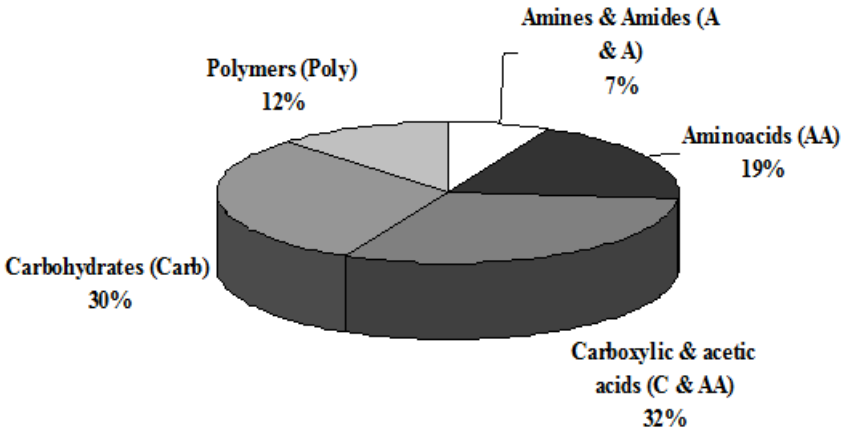

Fig. 2. Percent of total carbon source utilization response for the different guilds – amines and amides (A&A), amino acids (AA), carboxylic and acetic acids (C&AA), carbohydrates (Carb), and polymers (Poly) (results from EcoPlates analysis)

utilized amino and carboxylic acids. High utilization of carbohydrates during the warm times was probably driven by seasonal variations in algal abundance.

The two species, *Paenibacillus castaneae* and *Pseudomonas fluorescens* were tested for utilization of a variety of carbon sources using the Biolog Phenotype MicroArray named PM1 and PM2. As shown in Table 3, carboxylic and amino acids were the two classes of compounds mainly used by both species as carbon sources. Both species used the same amino acids, in spite of L-threonine which was used by *Pseudomonas fluorescens*. The differences were observed in catabolic activities of amino acids between species. The bigger differences were determined in the utilization of carboxylic acids group. *Pseudomonas fluorescens* used more compounds belong to carboxylic acids (32 from 60 substrates) than *Paenibacillus castaneae* (25 from 60 substrates). However, 24 of the same compounds from carboxylic acids group were used by both species. The high activity on D-sacharic acid, succinic acid, D-glucuronic acid, D-gluconic acid, L-lactic acid, D-galactonic acid-γ-lactone, D,L-malic acid was showed by both bacteria. *Paenibacillus castaneae* used Tween 20 and Tween 80 molecules containing fatty acids moieties with long aliphatic chains (laurate and oleate, respectively). Both strains no utilized polymers, amines and amides. Both strains showed high activity on L-aspartic acid, L-proline, L-glutamic acid, L-asparagine, L-glutamine, L-isoleucine, L-pyroglutamic acid and L-valine except L-threonine that was no degraded by *Paenibacterium castaneae*. Furthermore *Pseudomonas fluorescens* was able to used 32 and *Paenibacterium castaneae* 25 carboxylic acids. From 33 studied carboxylic acids only bromo succinic acid was not metabolized by *P. fluorescens*, whereas *P. castaneae* was not able to metabolize acetic acid, α-hydroxy-glutaric acid-y-lactone, tricarballylic acid, δ-amino valeric acid, butyric acid, itaconic and malonic acids. Carbohydrates were also highly utilized in the presence of studied bacterial strains. Carbon substrates such as: glycerol, uridine and adenosine were not degraded by *P. castaneae*. Tween 20 and Tween 80 were slightly utilized only by *P. castaneae*. Among other compounds methyl pyruvate was metabolized only by *P. fluorescens* strain (Fig. 3).

Despite the high phenotypic similarity among these species, some degree of differentiation between them could be made on the basis of growth at different pH, assimilation of substrates and sensitivity to antibiotics. The growth kinetics obtained from the all analysed PMs for both strains are presented on Fig. 4. In Table 4 sensitivity of *Pseudomonas fluorescens* and *Paenibacterium castaneae* to various compounds (antibiotics and toxic ions), results from analysis of PM 11-13 is presented.

In the last years Phenotype MicroArray technology (Biolog, Inc) was used to characterize the differences between the strains of the same species, mutants, and anaerobes bacteria. Mara *et al*. (2012) used phenotype microarray analysis to characterize *Acinetobacter* strains. The diversity of the five *Acinetobacter venetianus* strains were tested on PM1-PM2 carbon sources and PM3 nitrogen sources. Organic acids and amino acids were the two classes of compounds mainly used by tested strains as carbon sources, whereas carbohydrates were not used. Mesak and Davies (2009) investigated of the differences between two mutants (resistant to ciprofloxacin) of *Staphylococcus aureus*. The changes in metabolism of carbon, nitrogen, phosphateand sulfur sources as well as response to other nutrients, pH, osmolytes, antibiotics and other metabolic inhibitors were observed. The research of Lucas and Manna (2013) focused on the phenotype characterization of "*sar*" family mutants of *Staphylococcus aureus* and compared their properties with wild type strain. The staphylococcal specific "*sar*" family of proteins are involved in expression of numerous genes involving virulence, autolysis, biofilm formation, antibiotic resistance, oxidative stresses and metabolic processes. The eight metabolic panels (PM1 – PM8) were tested. Line *et al*. (2010) evaluated the ability of *Campylobacter jejuni* to oxidize 190 different substrates as sole carbon sources at 37°C and 42°C using phenotype microarray technology (PM1 and PM2). The results indicated that the expected amino acids, L-serine, L-aspartic acid, L-aspargine and L-glutamic acid were utilized in addition to a number of organic acids. The oxidation of the substrates was greater at 42°C than at 37°C. Decorosi *et al*. (2011) evaluated chemically sensitive profile of *Streptococcus thermophilus* using PM technology. They developed a suitable protocol for the PM-chemical sensitivity panels (PM11-PM20; 960 compounds) different chemical stress conditions (240 toxic chemical compounds at 4 different concentrations, including antibiotics, biocides, heavy metals and antimetabolites). PM approach may be used as a tool to indicate variations in the chemical sensitivity of microorganisms (antibiotics and biocides resistance) that are used in industrial processes. Borglin *et al*. (2009) outlined challenges presented in studying the physiology and phenotype of a sulfate-reducing anaerobic delta proteobacterium, *Desulfovibrio vulgaris*. The anaerobic growth of the strains in PM plates was observed. All available PM plates (PM1-PM20) were used, and growth changes were evaluated.

Table 3. Classes of carbon compounds used by *Pseudomonas fluorescens* and *Paenibacterium castaneae* (analysis of PM1 and PM2)

C-sources	Tested	*Pseudomonas fluorescens*		*Paenibacterium castaneae*	
		Used		Used	
		Number	%	Number	%
Amino acids	30	16	53.33	15	50.00
Carboxylic acids	60	32	53.33	25	38.33
Carbohydrates	70	17	24.3	14	20,00
Polymers	11	0	0	0	0
Alcohols	6	0	0	0	0
Amines	5	0	0	0	0
Amides	3	0	0	0	0
Fatty acids	5	1	20,00	2	40.00
sum	190	66	34.74	54	28.42

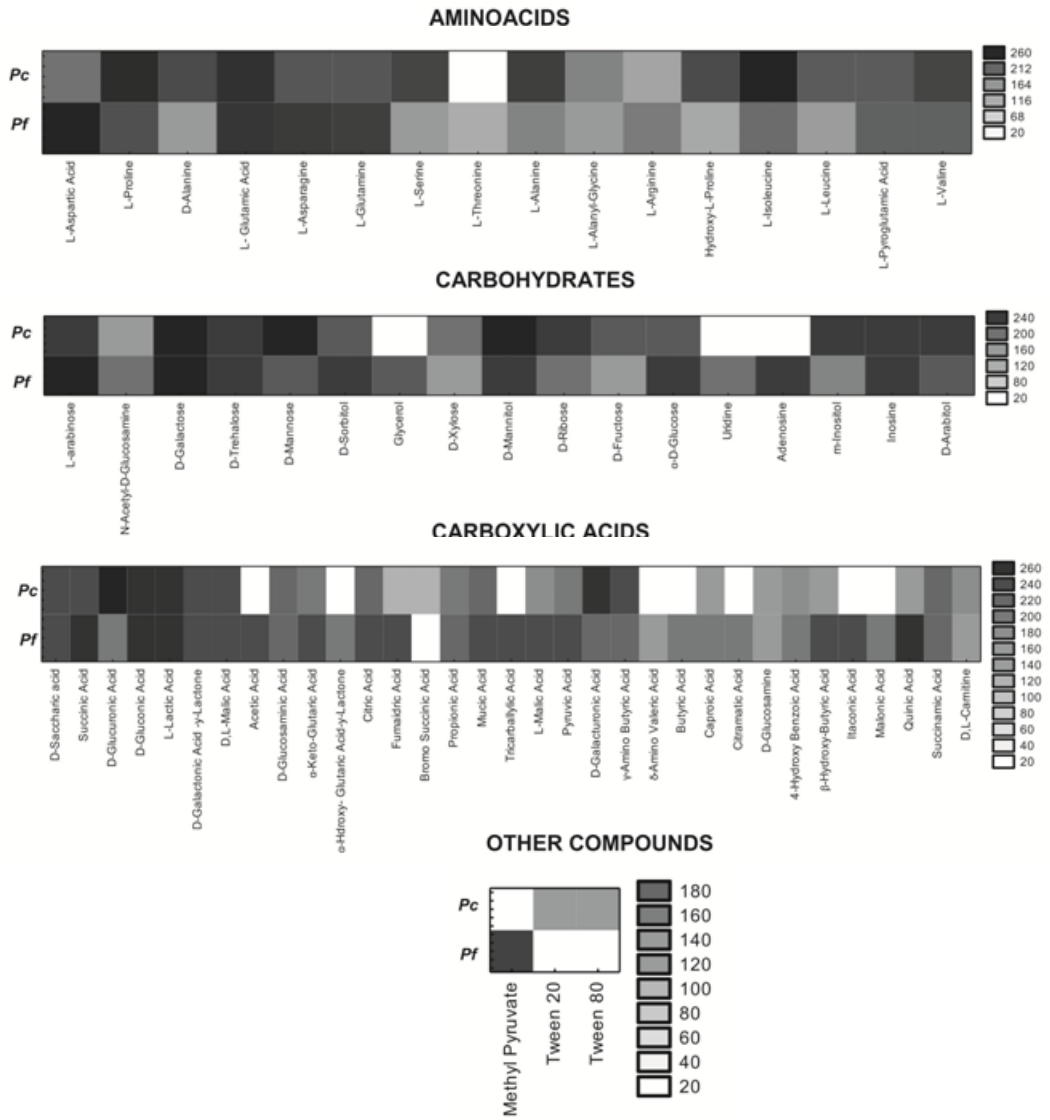

Fig. 3.Carbon sources (PM 1and PM 2) utilized by *Paenibacterium castaneae* and *Pseudomonas fluorescens* isolated from phenolic-contaminated water. The response of each compound was presented as a gray scale ranging from white (0 OUs) to black (300 OUs). Pc - *Paenibacterium castaneae*, Pf - *Pseudomons fluorescen*

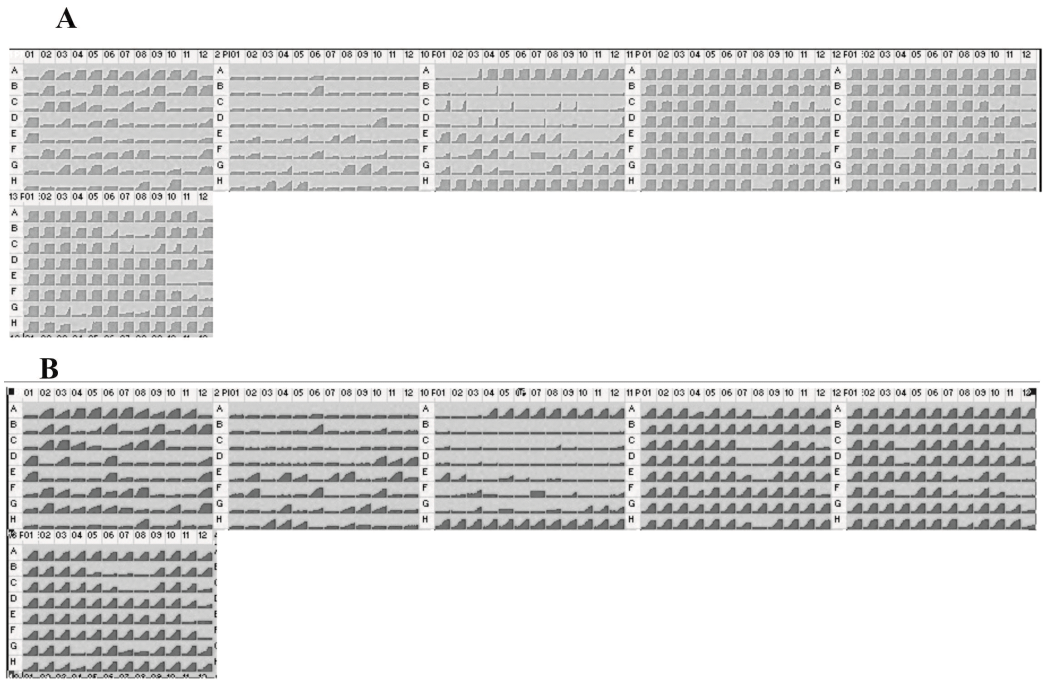

Fig. 4.PM analysis for sensitivity to C-sources (PM1, PM2), various chemicals (PM11, PM12, PM13) and pH effects (PM10). Growth kinetic of two strains: *Paenibacterium castaneae* (A) and *Pseudomons fluorescens* (B)

Table 4. Sensitivity of *Pseudomonas fluorescens* and *Paenibacterium castaneae* to various compounds (antibiotics and toxic ions) (analysis of PM 11-13)

PMs	Compounds	Effects/chemical structure	*Pseudomons fluorescens*	*Paenibacterium castaneae*
PM11	Amikacin	wall, lactam	R	R
	Chlortetracycline	protein synthesis, 30S ribosomal subunit, tetracycline	S	R
	Lincomycin	protein synthesis, 50S ribosomal subunit, lincosamide	R	R
	Amoxicillin	wall, lactam	R	R
	Cloxacillin	wall, lactam	R	R
	Lomefloxacin	DNA topoisomerase	R	R
	Bleomycin	DNA damage, oxidation	R	R
	Colistin	membrane, cyclic peptide	S	S
	Minocycline	protein synthesis, 30S ribosomal subunit, tetracycline	S	S
	Capreomycin	protein synthesis	R	R
	Demeclocyline	protein synthesis, 30S ribosomal subunit, tetracycline	S	S
	Nafcillin	wall, lactam	R	R
	Cefazolin	wall, cephalosporin	R	R
	Enoxacin	DNA topoisomerase	S	S
	Nalidixic acid	DNA topoisomerase	R	R
	Chloramphenicol	protein synthesis, amphenicol	R	R
	Erythromycin	protein synthesis, 50S ribosomal subunit, macrolide	R	R
	Neomycin	protein synthesis, 30S ribosomal subunit, aminoglycoside	S	R
	Ceftriaxone	wall, cephalosporin	R	R
	Gentamicin	protein synthesis, 30S ribosomal subunit, aminoglycoside	R	R
	Potassium tellurite	toxic anion	R	R
	Cephalothin	wall, cephalosporin	R	R
	Kanamycin	protein synthesis, 30S ribosomal subunit, aminoglycoside	S	S
	Ofloxacin	DNA topoisomerase	R	R

Table 4. Sensitivity of *Pseudomonas fluorescens* and *Paenibacterium castaneae* to various compounds (antibiotics and toxic ions) (analysis of PM 11-13)

PMS	Compounds	Effects/chemical structure	*Pseudomons fluorescens*	*Paenibacterium castaneae*
PM12	Penicillin G	wall, lactam	R	R
	Tetracycline	protein synthesis, 30S ribosomal subunit, tetracycline	R	R
	Carbenicillin	wall, lactam	R	R
	Oxacillin	wall, lactam	R	R
	Penimepicycline	protein synthesis, 30S ribosomal subunit, tetracycline	R	R
	Polymyxin B	membrane, cyclic peptide	S	S
	Paromomycin	protein synthesis, 30S ribosomal subunit, aminoglycoside	S	R
	Vancomycin	wall	R	R
	D,L-Serine hydroxamate	tRNA synthetase	S	S
	Sisomicin	protein synthesis, 30S ribosomal subunit, aminoglycoside	S	R
	Sulfamethazine	folate antagonist, PABA analog	R	R
	Novobiocin	DNA topoisomerase	R	S
	2,4-Diamino-6,7-diisopropylpteridine	folate antagonist, vibriostatic agent	R	R
	Sulfadiazine	folate antagonist, PABA analog	S	R
	Benzethonium chloride	membrane, detergent, cationic	S	S
	Tobramycin	protein synthesis, 30S ribosomal subunit, aminoglycoside	S	S
	Sulfathiazole	folate antagonist, PABA analog	S	R
	5-Fluoroorotic acid	nucleic acid analog, pyrimidine	S	R
	Spectinomycin	protein synthesis, 30S ribosomal subunit, aminoglycoside	R	R
	Sulfamethoxazole	folate antagonist, PABA analog	R	R
	L-Aspartic acid b-hydroxamate	tRNA synthetase	S	S
	Spiramycin	protein synthesis, 50S ribosomal subunit, macrolide	R	R
	Rifampicin	RNA polymerase	R	S
	Dodecyltrimethyl ammonium bromide	membrane, detergent, cationic	R	S
PM13	Ampicillin	DNA intercalator, inhibits RNA synthesis	R	R
	Dequalinium chloride	ion channel inhibitor, K+	R	R
	Nickel chloride	toxic cation	R	S
	Azlocillin	wall, lactam	R	R
	2,2`-Dipyridyl	chelator, lipophilic	S	S
	Oxolinic acid	DNA topoisomerase	R	R
	6-Mercaptopurine	nucleic acid analog, purine	R	R
	Doxycycline	protein synthesis, 30S ribosomal subunit, tetracycline	S	S
	Potassium chromate	toxic anion	S	S
	Cefuroxime	wall, cephalosporin	R	R
	5-Fluorouracil	nucleic acid analog, pyrimidine	R	R
	Rolitetracycline	protein synthesis, 30S ribosomal subunit, tetracycline	S	R
	Cytosine arabinoside	nucleic acid analog, pyrimidine	R	R
	Geneticin (G418)	protein synthesis, 30S ribosomal subunit, aminoglycoside	R	R
	Ruthenium red	respiration, mitochondrial Ca++ porter	S	S
	Cesium chloride	toxic cation	R	R
	Glycine	wall	R	R
	Thallium(I) acetate	toxic cation	S	S
	Cobalt chloride	toxic cation	S	S
	Manganese(II) chloride	toxic cation	R	S
	Trifluoperazine	phenothiazine, anti-cholinergic, anti-psychotic, sedative	R	R
	Cupric chloride	toxic cation	R	S
	Moxalactam	wall, lactam	R	R
	Tylosin	protein synthesis, 50S ribosomal subunit, macrolide	R	R

R – resistance; S - sensitive

CONCLUSIONS

Phenotypic profiling is an essential step for understanding genotype differences, stress response, media composition, and changes in environmental conditions for microorganisms. PM technology should be adapted to wide variety of environmental microorganisms applied in remediation technologies, biofuels production and climate change. Phenotypic changes between microorganisms, strains of the same species and communities can be detected as strains and environmental conditions are varied PM technology allows phenotypic testing to become a simple analysis on gene expression and allows to directly observe the consequence of a genetic change. More research to be done of better understand the advantages/disadvantages of the microassays. Further work needs to be conducted to optimize the use of the Biolog technology specially for the characterization of environmental microorganisms.

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