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PHYSIOLOGICAL CHARACTERIZATION OF CARBAZOLE DEGRADING BACTERIA ISOLATED FROM A FORMER GASWORKS SITE

Heterocyclic compounds could remain in the environment as hazardous and resistant pollutants. The aim of this study was to determine the physiological features of *Methylobacterium* sp. GPE1 strain being able to degrade heterocyclic compounds. Ten various substrates (sugars, alcohols and acids) were used in this assay, for metabolic purposes and eight ones were used as a sole source of carbon and energy. The comparison of the results with data taken from the literature indicated that GPE1 could be a new species of *Methylobacterium* sp.

1. INTRODUCTION

Coal is widely used since XIX century when the industrial revolution started. Although oil replaced it at XX century, coal is still one of the most important resources. Poland is one of the most coal-dependent countries, as its 94% of electricity is produced with coal. Moreover, as the oil reserves drastically decrease, coal thanks to its enormous reserves may come up to our next demands in energy. Nevertheless, coal, coal tar, their handling and applications may release big quantities of heterocyclic compounds.

Many of heterocyclic compounds are known to be toxic, carcinogenic or mutagenic. For instance, dibenzofuran, used as an insecticide or in PVC production, in short term exposures may cause skin, eye, nose and throat irritation, while long term exposure would cause rashes on the skin. Indole is another chemical compound highly toxic for aquatic organisms, a source of irritation and damages to the skin. Acridine is classified as a known human carcinogen. Its presence induces mutation through incor-

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poration into the DNA [1]. In this context, there is a need to develop techniques for prevention or removal of the heterocyclic compounds.

Biodegradation seems to be a promising way for neutralization of hazardous pollutants both *in situ* and *ex situ* [2–4]. Bacteria belonging to the genus *Methylobacterium* seems to have good properties for this purpose [5]. *Methylobacterium* sp. are methylotrophs, non motile, rod-shaped and obligatory aerobic. They oxidize one carbon compound with oxygen. Specimens of *Methylobacterium* sp. assimilate methanol with the serine pathway.

The aim of this paper was to evaluate the metabolic profile of *Methylobacterium* sp. GPE1 able to degrade carbazole for its characterization and identification.

2. EXPERIMENTAL

The strain used in this study was isolated from a former gasworks site by enrichement culture prepared with carbazole as a sole source of carbon and energy. The samples used for selection and isolation of microorganism were derived from gasworks contaminated site [6]. In our previous work, biodegradative potential of GPE1 strain was investigated with several heterocyclic compounds used as a sole source of carbon and energy. The results indicated GPE1 to have a wide catabolic features*.

To determine metabolic characteristics of the strain, 50 cm^3 Erlenmayer flasks were used containing 30 cm^3 of mineral salt medium (MSM) and various substrates. The MSM was prepared in the following proportions: KH_2PO_4 – 1.56 g/dm^3, Na_2HPO_4 – 2.13 g/dm^3, $(NH_4)_2SO_4$ – 0,50 g/dm^3, $MgSO_4 \cdot 7H_2O$ – 0.01 g/dm^3 and microelements – 1 cm^3 of solution ($FeCl_3 \cdot 6H_2O$ – 2.7 g/dm^3, H_3BO_3 – 0.1 g/dm^3, $Ca(NO_3)_2 \cdot 5H_2O$ – 0.05 g/dm^3, $ZnSO_4 \cdot 7H_2O$ – 0.1 g/dm^3, $CuSO_4 \cdot 5H_2O$ – 0.005 g/dm^3, $MnCl_2 \cdot H_2O$ – 0.05 g/dm^3). All of the substrates used in this study (glycerol, L-glutamic acid, ethanol, methanol, D-glucose, rhamnose, L-arabinose, D-fructose, D-xylose and D-galactose) were added to a final concentation of 0.15%.

Samples were inoculated with bacterial biomass and incubated at 30 °C on magnetic stirrer. The utilization of each compound was monitored by increase of turbidity for 18 days. The data obtained in the experiment were compared with those taken from the literature. The final pH of each sample was also measured.

3. RESULTS

The GPE1 strain isolated from a former gasworks site is rod shaped, gram negative, slow growing bacteria, producing red or pink pigment insoluble in water. It was

*The results of investigation have not been published so far.

not able to grow on nutrient agar and formed small red colonies on mineral agar plates. The GPE1 was identified by 16S rRNA sequencing similarly to described in our previous studies [4]. DNA sequences alignment assigned this strain to be a member of *Methylobacterium* sp.

Table 1

Utilization of sugars, alcohols and acids by (GPE_1)[a]

Substrate	Day of incubation								Final pH
	1	4	5	7	8	12	15	18	
Ethanol	–	+ P	+ P	+ P	+ P	+ P	+ P	+ P	6.83
D-Fructose	–	W	W, B	+ B	+ B	+ B	+ B	+ B	6.66
D-Glucose	–	+	+ P	+ P	+ P	+ P	+ P	+ P	6.73
D-Xylose	–	–	– B	W, B	+ B	+ B	+ B	+ B	6.80
Galactose	–	–	–	–	–	–	–	–	6.80
Glycerol	–	+ P	+ P	+ P	+ P	+ P	+ P	+ P	6.71
L-Arabinose	–	vW	vW	vW	vW	vW	vW	vW	6.73
L-Glutamic acid	–	+ P	+ P	+ P	+ P	+ P	+ P	+ P	7.30
L-Rhamnose	–	–	–	–	–	–	–	–	6.83
Methanol	–	+ P	+ P	+ P	+ P	+ P	+ P	+ P	6.75

[a]+ growth, – no observable growth, W – weak, B – brown colour in solution, P – pink colour, vW – very weak.

The results presented in this paper describe the research under characterization and identification of *Methylobacterium* sp. GPE1 strain. The first observable effects were noticed in the 4th day of incubation when methanol, ethanol and glutamic acid were metabolized (Table 1). Weak growth was also observed for fructose and it turned strong in furher days of incubation. In 5th day, most of the substrates were utilized and the cultures were showing high turbidity excluding xylose, where strong growth was observed in 8th day. The only substrates which were not metabolized (according to turbidity assessement) where galactose and rhamnose. Depending on substrate used, the cultures where the growth was observed were pink or brown. The metabolic profile of GPE1 strain was compared with other *Methylobacterium* species presented in the literature and this comparison is presented in Table 2.

pH of samples after 18 days of incubation were also measured. Significant pH changes were not observed. The values ranged from 6.7 for fructose to 7.3 for glutamic acid; its initial value was approximately 7.0.

3. DISCUSSION

Bacteria belonging to *Methylbacterium* sp. is known to have a positive influence on environemnt (due to metabolism of methane or phytohormones production). The

strain investigated in this study has a catabolic potential for carbazole degradation and its identification and characterization is an important task. The comparison of GPE1 strain with other strains previously described in the literature suggests that two strains utilize the same substrates as GPE1, e.g.: *M. thiocyantum* and *M. phyllosphaerae* [7, 17].

Table 2

Physiological features of GPE_1 compared with data taken from literature[a]

Strain	Ethanol	D-Fructose	D-Glucose	D-Xylose	Galactose	Glycerol	L-Arabinose	L-Glutamate	L-Rhamnose	Methanol
GPE1	+	+	+	+	–	+	W	+	–	+
M. aminovarans	NA	+ [7, 8]	– [7, 10]	– [7, 10]	– [8]	NA	– [8]	+ [7, 10]	NA	+
M. adhaesivum	NA	NA	– [13]	– [13]	NA	NA	NA	+ [13]	NA	NA
M. aquaticum	NA	+ [9]	+ [9]	– [9]	+ [9]	– [9]	– [9]	NA	– [9]	+
M. chloromethanicum	NA	– [7]	– [7, 10]	– [7, 10]	NA	NA	NA	+ [7, 10]	NA	+
M. dichloromethanicum	NA	+ [7]	– [7, 10]	– [7, 10]	NA	NA	NA	– [7] + [10]	NA	+
M. extorquens	+ [7]	– [7, 8]	– [7, 10]	– [7, 10]	– [8]	NA	– [7, 8]	V [10]	NA	+
M. fujisawaense	+ [7]	V [7], – [8]	+ [7, 10]	+ [7, 10]	+ [8]	NA	+ [7, 8]	+ [7, 10]	NA	+
M. hispanicum	NA	+ [9]	– [9, 12]	– [9, 12]	– [9]	+ [9]	– [9, 12]	+ [12]	– [9]	+
M. Iners	NA	NA	– [14]	NA	NA	NA	– [14]	NA	NA	NA
M. isbiliense	NA	NA	– [15]	– [15]	NA	NA	NA	+ [15]	NA	NA
M. jeotgali	NA	NA	– [7, 12]	+ [7, 12]	NA	NA	NA	NA	NA	NA
M. lusitanum	+ [7]	+ [7]	– [7, 10]	– [7, 10]	NA	NA	– [7]	– [7, 10]	NA	+
M. mesophilicum	+ [7]	– [7, 8]	+ [7, 10]	– [7] + [10, 12]	+ [8]	NA	+ [7, 8]	+ [7, 10]	NA	+
M. nodulans	+ [12]	NA	– [10, 12]	+ [10, 12]	NA	NA	+ [12]	+ [10, 12]	NA	+
M organophilum	+ [7]	+ [7, 8]	+ /– [10]	– [7, 10]	– [8]	NA	– [7, 8]	V [10]	NA	+
M. orphanophilum	+ [11]	NA	+ [11]	– [11]	NA	NA	– [11]	NA	NA	+
M. oryzae	W	NA	– [12]	– [12]	NA	NA	+ [12]	+ [12]	NA	NA
M. populi	NA	NA	– [12]	– [12]	NA	NA	– [12]	– [12]	NA	+ [12]
M. phyllosphaerae	W [17]	NA	W [17]	+ [17]	NA	NA	+ [17]	+ [17]	NA	NA
M. radiotolerans	V [7]	– [7, 8]	+ [7, 10]	+ [7, 10]	+ [8]	NA	+ [7, 8]	+ [7, 10]	NA	+
M. rhodesianum	+ [7]	+ [7, 8]	– [7, 10]	– [7, 10]	– [8]	NA	– [7, 8]	V [10]	NA	+
M. rhodinum	+ [7]	+ [7, 8]	W/+ [10]	– /+ [10]	– [8]	NA	– [7, 8]	+ [7, 10]	NA	+
M. salsuginis	+ [18]	+ [18]	+ [18]	– [18]	NA	NA	– [18]	NA	NA	NA
M. suomiense	+ [7]	+ [7]	+ [7, 10]	– [7, 10]	NA	NA	– [7]	– [7, 10]	NA	+
M. thiocyantum	NA	+ [7]	+ [7, 10]	NA	NA	NA	W [7]	+ [7, 10]	NA	+
M. variabile	NA	NA	+ [19]	– [19]	NA	NA	NA	+ [19]	NA	NA
M. zatmanii	+ [7]	+ [7, 8]	– [7, 10]	– [7, 10]	– [8]	NA	– [7]	– [7, 10]	NA	+

[a]+ growth, – no growth, V – variable, W – weak, vW – very weak, NA – not available.

M. phyllosphaerae presents small differences in the biodegration of ethanol, D-fructose and L-arabinose. GPE1 grows weakly on L-arabinose, whereas *M. phyllosphaerae* grows normally with it. The growth is also weak when *M. phyllosphaerae* use ethanol or D-fructose as a carbon source, whereas GPE1 displays strong growth with these carbon sources. However, lack of data for some of the substrates precludes exact assignment of GPE1 to any of those species. Another interesting species described by Ito et al. [20] is *M. radiotolerans* which profile seems to fit to profile achieved for GPE1. But in this case we have a difference in utilization of fructose and galactose. The other species presented in Table 2 also show differences. Nevertheless, we have to take into consideration that not all of the genus *Methylobacterium* representants are present in the table. No data were found for *M. aerolata* and *M. podarium*.

Differentation of bacterial species is a problematic issue, thus taking into consideration the results obtained in this study we cannot reject that GPE1 strain is a member of a new species. For a final assessement additional expermients and analyses will be made.

4. CONCLUSION

Methylobacterium sp. GPE1 strain, able to degrade heterocyclic compounds shows a wide metabolic potential. Its physiological characterization confirmed the results of 16S rRNA gene sequencing but was not sufficient to discriminate the species of GPE1 strain. Further studies, including DNA hybridization, GC-content and fatty acids analysis are needed in order to determine if *Methylobacterium* sp. GPE1 strain belongs to a new species.

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