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ORIGINAL PAPER

Some Properties of Methylotrophic Bacteria Isolated from Sewage Sludges Derived from Mechanical and Biological Sewage Treatment Plants

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Abstract

The presented studies were carried out to get more information about physiological properties of methylotrophic bacteria selected from sewage sludges derived from mechanical and biological sewage treatment plants. All the isolated bacterial strains belonged to facultative methylotrophs. The majority of them utilized glucose, starch and lipids. Moreover, most of them were also found to possess proteolytic properties and were able to hydrolyze urea but they were incapable of asparagine ammonification.

Key words: glucose, starch, lipids, proteins and urea utilization; methylotrophic bacteria; sewage sludges

Introduction

Sewage sludges, as by-products of sewage treatment plants, require utilization. They differ from one another regarding both the quantity and quality of chemical composition, as well as their microbial populations.

Because of a high content of mineral and organic substances found in sewage sludges, their agrotechnical utilization is quite obvious. According to literature data (Rosik-Dulewska, 2002), the content of nitrogen in sewage sludge dry matter can range from 0.7 to 7.6%, while the C:N ratio frequently oscillates between 10 and 13. The content of phosphorus is similar or even slightly higher than in natural organic fertilizers and it ranges from 0.6 to 9.2%, while the proportion of potassium is from 0.1 to 0.6% and it is always lower than in natural organic fertilizers. The amount of calcium ranges from 1.0 to 10%, depending on the type of the sewage sludge.

All basic groups of microorganisms can be found in sewage sludges (Kutera, 1978), including also methylotrophic bacteria which are characterized by the capacity for utilizing single-carbon compounds (Durska, 2006). Methylotrophic bacteria oxidize methane developed during the process of methanogenesis, released during methane fermentation, as well as methanol formed during processes of demethylation of compounds containing esters and methyl ethers (Pivetau *et al.*, 2001). Methylotrophic bacteria exhibit a capability for decomposing such toxic compounds as: vinyl chloride, ethylene trichloride, dichloromethane (Doronina *et al.*, 2000), trichlorobenzene (Sullivan and Chase, 1996), trinitrotoluene (van Aken *et al.*, 2004a), as well as cyanides (Wood *et al.*, 1998).

The aim of our experiments was to get more information about methylotrophic bacteria selected from sewage sludges derived from mechanical and biological sewage treatment plants. Capabilities of sewage sludges for metabolizing some carbon and nitrogen compounds have been determined.

Experimental

Material and Methods

Material used. The experimental material included sewage sludges, derived from communal sewages, obtained from two sewage treatment plants: mechanical sewage treatment plant in Poznań (sludge 1) and biological sewage treatment plant in Szamotuły (sludge 2).

Table I presents chemical properties of the examined sewage sludges. The following parameters were determined in the experimental sewage sludges: organic carbon – by Tiurin method, organic nitrogen

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Table I
Chemical properties of sewage sludges

Properties	Sludge 1	Sludge 2
Organic matter %	30.6	65.83
Organic carbon %	11.83	34.17
Organic nitrogen %	0.93	5.40
Ash %	69.4	34.17
C/N	12.64	6.33
pH	6.8	7.1

– by Kjeldahl method, organic matter and ash – according to Lityński e*t al*. (1976), as well as pH in H_2O using the potentiometric method.

Microbiological procedures. Microbiological analyses were carried out in sewage sludges after shaking for 30 min in 2% sodium pyrophosphate solution, using laboratory shaker – type 358S (Elpan).

The strains of methylotrophic bacteria were isolated from the analysed sludges, using medium prepared according to Urakami and Komagata (1979) supplemented with methanol. Morphological characteristics, as well as the dimensions of cells were obtained using the Gram stained preparations and micrometric methods.

Physiological properties were determined with the use of selected media in laboratory conditions.

- Growth at the temperature of 4°C and 56°C in a selective medium for methylotrophs, according to Urakami and Komagata (1979) – after 21 days of incubation.
- Glucose decomposition in liquid medium with glucose according to Rodina (1968). Degradation of glucose was assessed on the basis of the growth of bacteria and their capability for the formation acid products – after 21 days of incubation.
- Starch decomposition in the medium with starch according to Rodina (1968), on the basis of Lugol liquid staining reaction, after 5 days of incubation,
- Decomposition of lipids in Tween 80 medium according to Kreisel and Schraner (1987), after 10 days of incubation, on the basis of turbidity appearing around the growing bacteria,
- Proteins decomposition in the medium with gelatine according to Rodina (1968), with Frazier reagent, after 21 days of incubation,
- Asparagine ammonification in the medium with asparagine according to Rodina (1968), on the basis of staining reaction of liberated ammonia with Nessler reagent, after 21 days of incubation,
- Urea hydrolysis in Tidwell, Heather and Merkle medium according to Rodina (1968), on the basis of staining reaction of liberated ammonia with Nessler reagent, after 21 days of incubation.

Results

The examined sewage sludges differed regarding to the total organic matter, carbon and organic nitrogen as well as ash contents (Table I). Sewage sludge derived from the biological sewage treatment plant contained twice more organic matter, three times more organic carbon and six times more organic nitrogen in comparison with the sludge derived from the mechanical sewage treatment plant. Concentrations of hydrogen ions did not differ significantly between the examined sludges.

Table II
Morphological properties of methylotrophic bacteria isolated from the sewage sludges derived from mechanical and biological sewage treatment plants

Mechanical sewage treatment plant				Biological sewage treatment plant			
Morphology	Staining by Gram method	Cell dimensions µm	Number of strains	Morphology	Staining by Gram method	Cell dimensions µm	Number of strains
Cocci	Gram-positive	Diameter 0.8	17	Cocci	Gram-positive	Diameter 0.8–1.0	10
Cocci	Gram-negative	Length 0.8–1.0 Width 0.7–0.9	3	Cocci	Gram-negative	Diameter 0.8–0.9	5
Bacilli	Gram-positive	Length 1.0–3.0 Width 0.8–1.2	15	Bacilli	Gram-positive	Length 4 Width 1	2
Bacilli	Gram-negative	Length 1.0–1.1 Width 0.7–0.8	4	Bacilli	Gram-negative	–	0
Coccobacilli	Gram-positive	Length 0.8–0.9 Width 0.7–0.8	4	Coccobacilli	Gram-positive	Length 0.8–1.0 Width 0.7–0.9	6
Coccobacilli	Gram-negative	Length 0.8–1.2 Width 0.7–1	5	Coccobacilli	Gram-negative	Length 0.8–1.0 Width 0.7–0.9	6
Spore-forming bacilli	Gram-positive	Length 5 Width 1	2	Spore forming bacilli	Gram-positive	Length 2.0–7.0 Width 1.0–1.2	21

Fifty methylotrophic strains of bacteria isolated from each of the sewage sludges were morphologically differentiated into gram-positive and gram-negative: cocci, bacilli, coccobacilli and spore forming bacilli (Table II).

Seven groups of bacteria were isolated from the sludge derived from the mechanical sewage treatment plant. Among 50 strains of methylotrophic bacteria, 38 strains were Gram-positive bacteria, cocci and bacilli bacteria were dominating. In the case of sludge derived from biological sewage treatment plant, 6 groups were identified. Also in this case, among 50 isolated strains, 39 strains were Gram-positive bacteria, with spore forming bacteria constituting the dominant group.

Investigations were carried out using selective media supplemented with different C and N compounds allowing to get information about biochemical properties of the isolated methylotrophic bacterial strains derived from sludges obtained from mechanical and biological sewage treatment plants (Figs. 1 and 2). The obtained results showed that methylotrophic bacteria strains isolated from both types of sewage sludges can be metabolically active not only at the temperature of 28°C, which is optimal for this group of bacteria, but also at lower or higher temperatures

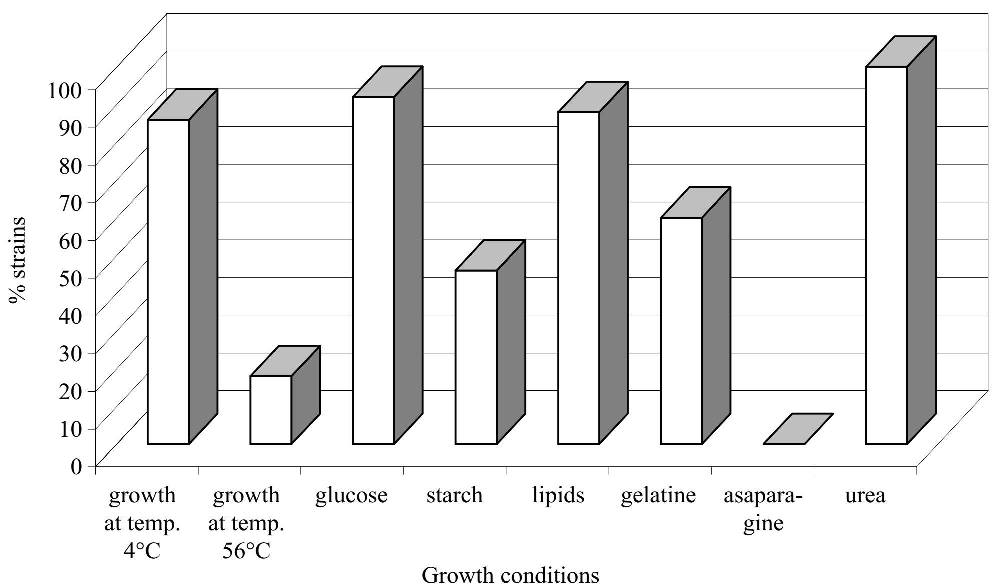

Fig. 1. Capability of methylotrophic bacteria isolated from sludge of mechanical sewage treatment plant for growing in different temperatures and utilizing different C and N compounds

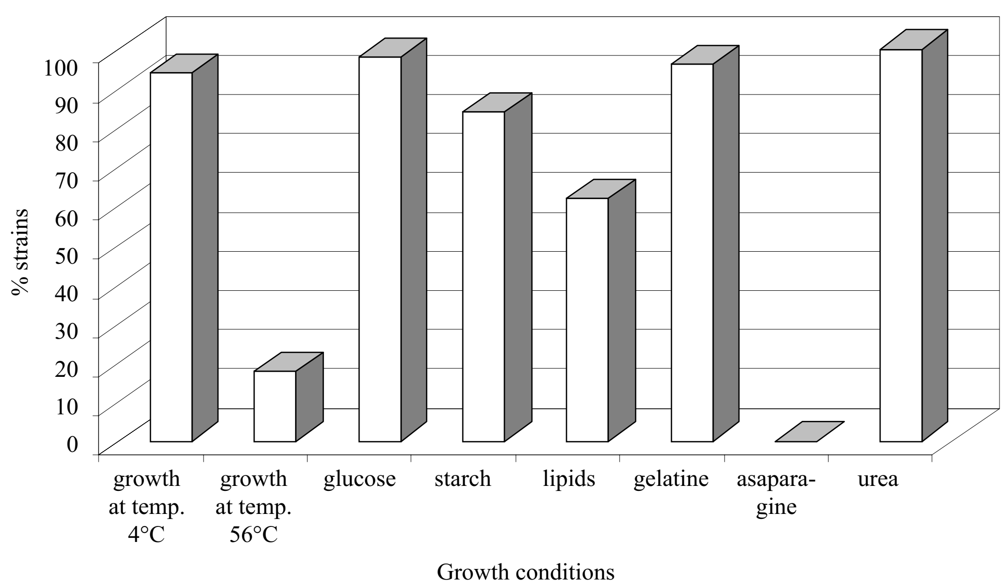

Fig. 2. Capability of metylotrophic bacteria isolated from sludge of biological sewage treatment plant for growing in different temperatures and utilizing different C and N compounds

than the optimal one. The majority of bacteria continued to grow at the temperature of 4°C (86% and 92%, respectively) and the absence of growth was recorded only in the case of 7 strains isolated from the mechanical sewage treatment plant and 3 strains from the biological plant. On the other hand, in the case of high temperature (56°C), only 9 strains (18%) of bacteria isolated from the sludge of each of the two sewage treatment plants were able to grow.

The isolated bacterial strains were also examined regarding to their capability for using different carbon and nitrogen sources. Almost all the examined strains isolated from the mechanical sewage treatment plant (Fig. 1), apart from methanol, utilized glucose as the only source of carbon**.** It was shown that 46 strains (92%) grew well in the presence of glucose. The majority of strains produced acid products. Less than one half of the strains (46%) exhibited a capability for degrading starch. Almost all the isolated strains (88%) decomposed lipids and only 6 strains failed to show this process.

The tested strains metabolized actively some nitrogen compounds. Over one half of the isolated strains (60%) grew in the presence of gelatine in the medium. On the other hand, none of the strains exhibited ammonification capabilities in the presence of asparagine in the medium. All strains hydrolyzed urea.

All the examined methylotrophic bacteria isolated from the sludge of the biological sewage treatment plant (Fig. 2), apart from methanol, could also utilize glucose as the only source of carbon as confirmed by the fact that 49 strains (98%) exhibited good growth in the presence of glucose. The majority of the bacterial strains obtained from the sludge of the biological treatment plant also produced acid products. Most of the isolated strains, namely 84%, were capable of metabolizing starch and that was two times more in comparison with the bacteria isolated from the sludge of the mechanical sewage treatment plant. However, lipids were decomposed by a smaller number of these strains than in the case of strains derived from the sludge of the mechanical plant, although they still constituted more than one half of them (62%).

Bacterial strains isolated from the sludge of the biological sewage treatment plant metabolized nitrogen compounds in a way similar to the strains from the mechanical plant. However, proteolytic properties were exhibited by almost all methylotrophs (96%). Only 2 strains failed to hydrolyze gelatine. Those strains responded to the presence of asparagine and urea in the medium in the same way as the strains from the mechanical plant, hence the isolated strains of methylotrophic bacteria were incapable of asparagine ammonification, but they hydrolyzed urea.

Discussion

From the ecological point of view, the process of sewage purification should be integrated with the disposal process of sludges which are the by-products of the former process. In the case of agricultural utilization of sludges in soil, they become incorporated in the topsoil alongside mineral and organic components ensuring the appropriate development of plants as well as of microorganisms. Investigations carried out on the utilization of sewage sludges allowed to isolate from them various physiological groups of bacteria (Wolna-Maruwka and Sawicka, 2006), including methylotrophic bacteria (Durska, 2006).

Properties of methylotrophic bacteria investigated by various researchers permitted to identify, apart from the obligatory methylotrophs, also the facultative methylotrophs which are capable, alongside the compounds of C1 type, for utilizing such multicarbon compounds as: arabinose, xylose, but more frequently glucose, fructose and organic acids-most frequently acetic acid, less often citric acid (van Aken *et al*., 2004b). Further it was demonstrated that methylotrophs take part in the transformation of nitrogen compounds. Some of them can utilize glutaminic and aspartic acids (Mc Donald *et al*., 2001), as well as methyl- dimethyl- and trimethylamines (Urakami *et al.*, 1993). Furthermore, methylotrophic bacteria are also characterized by the ability for fixing atmospheric nitrogen (Sy *et al*., 2001; Durska, 2004). In addition, some researchers emphasize the fact that some methylotrophs also take part in the transformations of sulphur compounds (de Zwart *et al*., 1997; Suylen *et al*., 2004).

There is not much information in the available literature about the occurrence of methylotrophs in sewage sludges and their capability for metabolizing substances found in them. Investigations carried out in the presented research project confirm the presence of facultative methylotrophs in the examined sewage sludges, the majority of which exhibited capabilities for metabolizing the examined carbon and nitrogen compounds, although they failed to ammonify asparagine. The results obtained in this study for the bacterial strains derived from two different types of sewage sludges are similar, except for some differences in the number of strains actively metabolizing a specific substrate. Results associated with nitrogen compounds are corroborated by the results of sewage sludge experiments carried out by Lalke-Porczyk and Donderski (2005) who showed that urea-hydrolyzing bacteria constituted a fairly numerous group. Some studies conducted on *Methylobacterium mesophilicum* confirm the capability of methylotrophic bacteria for producing urease and for hydrolizing urea. The results obtained in this study are also corroborated by investigations of other researchers who also reported the capability of methylotrophs for metabolizing simple sugars.

Summing up, the investigations on the metabolic capabilities of methylotrophic bacteria isolated from the experimental sewage sludges showed that the majority of the identified bacterial strains can exhibit metabolic activities at low temperatures and a certain group of these strains can also remain active at high temperatures. Investigations on their capability for metabolizing some C and N compounds confirmed that they are able to metabolize such carbon compounds as glucose, starch or lipids, as well as gelatine and urea. Furthermore, the obtained results indicate that methylotrophic bacteria and processes generated by them can play a significant role both during the process of sewage sludge stabilization and their natural management.

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