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Original Research

Response of Bacteria to Heavy Metals Measured as Changes in FAME Profiles

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Abstract

The effects of Cd, Ni, Cu, or Zn on the whole cell-derived fatty acid profiles of four bacterial strains isolated from heavy metal-polluted soils located in Upper Silesia was determined. Based on the fatty acid methyl ester (FAME) profiles, the strains were identified and named as *Enterobacter intermedius* AM15, *Enterobacter intermedius* MH8b, *Pseudomonas putida* MH1d, and *Klebsiella pneumoniae* AM12. The obtained results showed changes that were dependent both on tested strains and metal used. The most significant changes were observed for strains cultured in the Ni presence. In the FAME profiles of MH8b, AM15, and AM12 strains, a significant increase of cyclopropane fatty acids was observed. Moreover, exposure for Ni resulted in the appearance of a new fatty acid in the FAME profiles of AM15 and MH8b strains. In turn, Cd and Zn caused a decrease of the content of cyclopropane fatty acids as compared to control. For AM15 and AM12 strains cultured on media with heavy metals, the ratio of saturated to unsaturated fatty acids were higher than that in control. The same phenomenon was also observed for MH8b strain exposed only to the highest concentration of Ni and Cd.

Keywords: heavy metals, fatty acids, MIC, metal-tolerant strains

Introduction

Some metals, such as Ca, Co, Cu, Ni, and Zn, are essential, serving as micronutrients and taking part in redox-oxidation reactions. They stabilize molecules through electrostatic interactions, regulate osmotic pressure, and are components of various enzymes, form of charge and concentration gradients across cytoplasmatic membranes [1, 2]. On the other hand, many other metals, such as Hg, Pb, Cd, and Au, have no biological functions and are toxic to microorganisms even at minimal concentrations. Both nonessential and essential (at higher concentrations) metals can block functional groups of important molecules and transport channels for required nutrient ions, damaged cell membranes and DNA structure, and alter enzyme specificity leading to disruption of the cellular functions [1]. For example, mercury, cadmium, and silver bind SH groups, resulting in an inhibition of the sensitive enzymes [1, 3]. Hassen et al. [4] have reported the inhibition of protein biosynthesis in the presence of Hg, Cd, and Cu. In turn, nickel, copper and chromium generate reactive oxygen species (ROS), which catalyze the peroxidation of membrane lipids, causing the oxidative damage of proteins and nucleic acids [5-7]. Copper also accelerated the oxidation of hydroquinone and benzoquinone [8]. It has been proven that copper may influence the activity of some chemicals. For example, in the Ames *Salmonella* mutagenity test, this metal increased the mutagenity potential of adriomycin more than 700% in comparison with control [9].

As a consequence of the environmental exposure to heavy metals, microorganisms have evolved metal resistance strategies, including exclusion by permeability barrier,

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cellular sequestration, enzymatic transformation, reduction of metal to less toxic forms, and efflux of the metal ions from the cell. Genes responsible for heavy metal resistance are mainly located on plasmids; therefore, these determinants might easily transfer within an ecological system such as soil [10-12].

Stress conditions induce a variety of responses inside bacterial cells. One of these mechanisms is altering the fatty acid composition of their lipids to maintain membrane fluidity. In response to toxic substances, bacteria can modify their lipid acyl chain structure by changing the ratio of saturation to unsaturation, cis to trans unsaturation, branched to unbranched structure, type of branching and also acyl chain length [13-16]. Moreover, bacteria may respond to heavy metals stress, changing their biochemical activity and phenotypical markers [17].

Many studies have been carried out on the changes of microbial fatty acid profiles in response to factors like temperature, nutrients, pH, and aromatic compounds [13, 15, 18], while there is very little information available on the effects on heavy metals on microbial fatty acids. However, some authors have reported several changes in cellular lipid content for eukaryotic organisms. For example, Einicker-Lamas et al. [19] showed that cadmium caused an increase of the protein and lipid contents per cell in tested cell types of *Euglena gracilis*. Similarly, changes in lipids composition involving the alteration of whole-cell fatty acid profiles of two strains of *Euglena gracilis* were observed by Rocchetta et al. [7]. In turn, it has been shown that copper [20] and cadmium [21] may induce the changes in membrane permeability of *Saccharomyces cerevisiae*.

The aim of this study was to elucidate the changes in the whole cell-derived fatty acid composition of metal-tolerant strains under exposure to increasing concentrations of heavy metals.

Materials and Methods

Isolation and Identification of Metal-Tolerant Strains

Bacterial strains were isolated from heavy metal-polluted soils located near the Huta Katowice steelworks in Dąbrowa Górnicza or the zinc-lead heap in Brzeziny in Upper Silesia, a highly industrial region in southern Poland. Soil samples (5 g) were placed in a flask containing 45 ml of 0.85% (w/v) sterile NaCl medium and shaken at 130 rpm for 30 min. Then, the serial 10-fold dilutions of soil suspensions were plated onto Tryptic Soy Agar (TSA, Difco) supplemented with 3 mM Cu or 5 mM Zn and incubated at 28ºC for 5 days prior to colony selection. Based on the tolerance level of isolates to heavy metal and their ability to grow under laboratory conditions, four out of 35 isolated bacterial strains were selected for further experiments. Identification of these strains was carried out on the basis of their cellular fatty acid profiles using the standard MIDI-MIS method [22].

Determination of Metal Tolerance Pattern

The metal-tolerance pattern of each bacterial strain was determined by the minimum inhibitory concentration (MIC) approach. For this purpose, the strains were cultured on medium amended with increasing concentrations of metals: 3-12 mM for Cu, Ni or Zn, and 0.5-4.5 mM for Cd. The metals were added to the medium in a form of water solution prepared from the analytical-grade salts of $CuCl_2$, $NiCl_2$, $ZnCl_2$, or $CdCl_2$, and sterilized by using membrane technique (0.2 μm pore size sterile filters). The inoculated plates were incubated at 28ºC for 3 days. The MIC was defined as the lowest concentration of the metal at which bacterial growth was not observed.

Analysis of Fatty Acid Methyl Ester (FAMEs) Profiles

To establish the impact of heavy metals on the FAME profiles, all strains were cultivated on TSA medium amended with different concentrations of Cu, Cd, Ni, or Zn. Bacterial strains cultured on medium without metals were used as controls. For the experiments the cells from exponential growth phase were used. The whole-cell cellular fatty acids were isolated and identified according to the standard procedure of MIDI-MIS consisting of four steps [22]. In the first step, 40 mg of bacterial biomass was saponified so that lysis of cells and liberation of fatty acids from cellular lipids occurred. Then, fatty acids were methylated and in the next step FAMEs were extracted from aqueous to an organic phase. The final step was base wash of the organic extracts prior to chromatographic analysis. Than the extracted fatty acids were analyzed by gas chromatograph Helwet-Packard 6890, equipped with an Ultra 2-HP (crosslinked pheny methyl siloxane) capillary column and hydrogen as carrier gas. Fatty acid methyl esters were detected by a flame ionization detector (FID) and identified by Microbial Identification Software (Sherlock version 3.0) using MIDI microbial calibration standards and TSBA 5.0 library. Each experiment was performed in triplicate. FAMEs are named by standard nomenclature: the total number of carbon atoms, followed by colon and the number of double bonds. The position of the first double bond is indicated by ω, followed by the number of carbon atoms from the aliphatic end. Methyl branching at the iso and anteiso positions and at the 10^{th} carbon atom from the carboxy end is designated by the prefixes i, a, and 10Me, respectively. The prefix cy denotes cyclopropane fatty acids.

Results and Discussion

Bacterial Identification and Tolerance to Heavy Metals

Bacterial strains isolated from heavy metal-contaminated soils around the steel works were selected for study. The FAME analysis allowed us to identify the strains named

Table 1. Minimum inhibitory concentrations (MIC) of various metals to the isolates.

Bacterial strain	MIC (mM)			
	Cd	Ni	Zn	Cu
Pseudomonas putida MH1d	3	10	11	9
Enterobacter intermedius MH8b	3	9	9	9
Enterobacter intermedius AM15	3	10	11	11
Klebsiella pneumoniae AM12	3	7	9	8

MH1d, MH8b, AM12, and AM15 as *Pseudomonas putida, Enterobacter intermedius, Klebsiella pneumoniae,* and *Enterobacter intermedius*, respectively. The similarity indices (SIM) calculated by the MIDI system on the level of 0.894, 0.633, 0.754, and 0.782 for MH1d, MH8b, AM12, and AM15, respectively, showed very good matches. SIM is an indication of the confidence with which the isolate is identified. Tested strains differed in their metal tolerance-pattern as indicated by data of MIC values (Table 1). The most tolerant species was *Enterobacter intermedius* AM15, with MICs of 3, 10, 11, and 11 mM for Cd, Ni, Zn, and Cu, respectively. The order of toxicity of the metals was similar for all strains and was found to be Cd>Ni>Cu>Zn. The high tolerance of individual strains and bacterial group isolated from contaminated sites to the same metals has been reported in other studies. The authors noticed higher sensitivity of bacteria to Cd and Ni in comparison with Zn and Cu [4, 11, 23, 24].

Fatty Acid Composition Analysis

To assess the influence of heavy metals on the FAME profiles of tested strains, the whole cell-derived fatty acid compositions of bacteria growing on media amended with or without metals were compared. The predominant fatty acids for all investigated strains cultured on control medium were C16:0, C16:1 ω7c, and C18:1 ω7c. These three components accounted for almost 70% of all isolated fatty acids. The content of cyclopropane fatty acids in all strains ranged from 6.2 to 8.5% of the total fatty acids. In turn, the percentage of hydroxylated fatty acids was about 6.4%, 7.7%, 8%, and 12.4% of the total FAMEs for *E. intermedius* MH8b, *K. pneumoniae* AM12, *E. intermedius* AM15 and *P. putida* MH1d, respectively (Tables 2-5).

The whole-cell fatty acid profiles derived from strains cultivated on media amended with heavy metals revealed a marked shift in their composition as compared with control. For the interpretation of heavy metal impact on bacteria, the identified fatty acids were grouped into the following classes: saturated odd-numbered, even-numbered, branched, hydroxy, cyclopropane, and unsaturated fatty acids (Figs. 1-4). The addition of Cd, Cu, Ni, or Zn to growth medium resulted in a different response of the bacterial strains. Metal treatments caused changes in the distribution of saturated straight chain fatty acids with odd carbon number. In the presence of the highest metal concentrations, in both *Enterobacter* strains as well as in *K. pneumoniae* AM12, the amount of these fatty acids decreased, while for *P. putida* such a reaction was observed only when bacteria were cultivated on media with Ni addition.

Important changes in the FAME profiles were observed for the percentage of cyclopropane fatty acids. In the presence of the highest nickel concentrations, the amount of cy17:0 increased by 73 and 146% in *E. intermedius* MH8b and AM15 strains as compared with control, whereas for *K. pneumoniae* AM12 the highest increase (86%) of this fatty acid was found for culture of strain with 4 mM Ni. A similar trend was found for AM15 and AM12 strains growing on media with the highest concentrations of Cd, Zn, or Cu, as well as for the MH8b strain cultured on medium amended with the highest Cd amount. Interestingly, both *E. intermedius* strains growing on medium with the addition of nickel formed a fatty acid cy19:0 ω8c that was not detected in the control samples. Similar effect have been reported previously for bacterial cells growing on media supplemented with toxic organic compounds, e.g. naphthalene or phenol used as a carbon and energy source [25]. It seems that the formation of a new fatty acid may be a general mechanism responsible for adaptation to toxic compounds. In contrast, in the FAME profiles obtained for *P. putida* growing on media with the highest metal concentrations, a decrease of the amount of cyclopropane fatty acids was detected. The same results were observed for MH8b strain under exposure to 8 mM Zn. The functions of cyclopropane fatty acids are not obvious, and neither complete absence nor their overproduction in all phases of growth have been observed to affect growth rate or survival under laboratory conditions. However, strains lacking these acids are highly sensitive to acid shock [14, 26]. The increasing level of cyclopropane fatty acids may be explained by the conversion of monounsaturated fatty acids, which was observed in our studies for AM15 (Cu, Ni), AM12 (Cd, Ni, Zn, Cu), and MH8b (Cd, Ni). This conversion resulted in an increase of the saturation ratio. The same effect is obtained by the conversion of monounsaturated to saturated fatty acid with an even carbon number.

It was recognized that the increase of lipid saturation is a well-known post-synthetic modification caused by the addition of the methyl group across cis double bound, and usually occurs at the late exponential or early stationary phase of growth [14]. Pepi et al. [27], who studied the simultaneous effect of toluene and arsenic on the fatty acid profile in *Pseudomonas* sp. ORAs5 and *Bacillus* sp. ORAs2 observed an increase in the degree of fatty acid saturation. This effect was especially noticed for *Bacillus* sp. ORAs2 exposed to constant toluene and increasing arsenic concentrations. In turn, for *Pseudomonas* sp. ORAs5 such changes were observed in the presence of varying toluene and constant arsenic concentrations, while only minor changes occurred with increasing arsenic and constant toluene concentrations. The high level of saturation achieved by increasing the amounts of cyclopropane and saturated fatty acids led to decreased fluidity and permeability of cell membranes. Fatty acids with a cyclopropane

Table 2. Percentage of total fatty acids from *E. intermedius* AM15 grown on medium amended with metals.

Fatty acid	Control	Metals (mM)											
		Cd 0.5	Cd 1	Cd 2	Ni 2	Ni 4	Ni 6	Zn 3	Zn 5	Zn 8	Cu 3	Cu 5	Cu 7
12:00	3.7	3.6	3.5	3.3	4.0	4.6	3.5	3.1	3.2	3.2	3.6	3.8	3.9
13:00	0.6	0.5	0.0	0.0	0.3	0.2	0.0	0.4	0.2	0.0	0.6	0.4	0.6
14:00	6.7	6.9	7.5	7.2	7.1	7.6	7.5	6.3	6.7	7.3	6.8	6.9	7.0
14:0 3OH	7.2	7.7	7.2	8.2	8.4	8.2	8.6	7.4	8.1	8.1	8.7	7.6	8.8
15:00	3.3	3.4	0.9	0.0	3.4	2.8	1.2	4.0	2.2	1.3	3.9	3.1	3.0
15:1 iso	0.3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
16:00	26.9	26.8	33.8	35.2	27.4	29.2	32.0	29.3	31.5	34.4	25.8	27.7	27.7
16:1 ω7c	27.0	29.0	29.2	28.1	24.0	19.5	19.2	29.9	29.7	28.9	24.5	24.8	24.8
17:1 ω8c	0.2	0.0	0.0	0.0	0.0	0.0	0.0	0.4	0.0	0.0	0.2	0.0	0.0
cy 17:0	6.4	6.5	5.0	7.4	10.0	14.7	15.9	6.7	6.8	7.0	8.9	7.6	8.8
17:00	2.7	2.5	0.2	0.0	2.6	1.8	1.0	2.4	1.3	0.8	2.9	2.2	1.7
18:00	2.5	0.6	2.4	0.0	1.2	1.3	1.0	0.6	0.7	1.1	1.0	2.4	1.1
18:1 2OH	0.8	0.9	0.0	0.0	0.0	0.0	0.0	0.1	0.0	0.0	0.0	0.0	0.0
18:1 ω9c/ ω12t/ω7c	11.0	10.6	9.4	10.6	10.7	9.1	9.0	8.3	8.6	7.1	12.0	12.6	10.9
cy19:0 ω8c	0.0	0.0	0.0	0.0	0.0	0.3	0.5	0.0	0.0	0.0	0.0	0.0	0.0
Sat.	59.9	58.5	60.4	61.3	64.3	70.5	71.0	60.2	60.6	63.2	62.0	61.6	62.6
Unsat.	39.4	40.4	38.6	38.7	34.6	28.5	28.2	38.7	38.3	36.0	36.7	37.4	35.6
Sat./Unsat. ratio	1.5	1.4	1.6	1.6	1.9	2.5	2.5	1.6	1.6	1.8	1.7	1.6	1.8

The values are the means of three replicates (standard error < 5%).

Table 3. Percentage of total fatty acids from *K. pneumoniae* AM12 grown on medium amended with metals.

Fatty acid	Control	Metals (mM)											
		Cd 0.5	Cd 1	Cd 2	Ni 2	Ni 4	Ni 6	Zn 3	Zn 5	Zn 8	Cu 3	Cu 5	Cu 7
12:00	0.9	0.7	0.6	0.7	0.9	0.9	1.7	1.1	0.4	0.5	0.5	0.3	0.9
14:00	8.8	9.8	10.4	12.5	9.6	11.8	11.2	9.5	10.6	11.1	10.2	10.5	11.1
14:0 3OH	7.7	7.5	7.7	7.6	7.2	8.4	7.4	6.9	7.8	7.7	7.9	7.7	8.2
15:00	2.1	2.2	1.4	0.8	2.9	2.1	0.4	1.5	1.8	1.1	2.6	2.9	2.3
15:1 iso	0.3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
16:00	27.4	29.8	31.8	32.6	27.5	29.7	32.7	29.7	31.6	32.5	28.8	30.2	31.8
16:1 ω7c	19.5	17.8	23.7	22.7	16.5	10.0	15.7	22.1	20.0	18.4	16.8	15.9	18.0
17:1 ω8c	0.3	0.4	0.0	0.0	0.7	0.3	0.0	0.0	0.0	0.0	0.6	0.6	0.0
17:1 ω9c	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
cy 17:0	7.6	7.5	5.8	9.5	10.2	14.2	9.2	4.8	9.3	9.2	10.7	12.7	10.1
17:00	1.0	0.9	0.3	0.0	1.2	0.9	0.0	0.8	0.3	0.3	1.0	0.8	0.0
18:00	1.5	2.9	0.8	0.7	0.9	1.7	4.2	1.7	0.7	3.6	1.0	0.9	1.9
18:1 ω9c/ ω12t/ω7c	21.5	18.8	16.6	12.6	20.5	17.6	16.2	21.0	16.0	14.2	18.0	15.5	14.6
cy19:0ω8c	0.8	0.9	0.3	0.0	1.2	1.8	0.8	0.3	0.8	0.7	1.2	1.3	0.5
Sat.	57.8	62.1	59.0	64.2	61.5	71.4	67.4	56.2	63.1	66.7	63.7	67.3	66.7
Unsat.	41.6	37.1	40.2	35.4	37.7	27.8	31.9	43.1	36.1	32.6	35.4	31.9	32.6
Sat/unsat. ratio	1.4	1.7	1.5	1.8	1.6	2.6	2.1	1.3	1.8	2.0	1.8	2.1	2.0

The values are the means of three replicates (standard error < 5%).

Table 4. Percentage of total fatty acids from *E. intermedius* MH8b grown on medium amended with metals.

Fatty acid	Control	Metals (mM)											
		Cd 0.5	Cd 1	Cd 2	Ni 4	Ni 6	Ni 8	Zn 4	Zn 6	Zn 8	Cu 4	Cu 6	Cu 8
12:00	3.2	3.4	2.9	3.1	3.8	3.6	3.5	3.3	3.5	3.5	3.6	3.9	3.5
13:00	0.9	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
14:00	6.4	6.8	7.2	7.1	7.2	5.4	7.5	7.0	7.4	7.7	6.9	6.4	7.1
15:00	4.6	1.4	0.3	0.0	2.2	1.2	1.4	0.7	0.6	0.3	2.7	1.9	1.7
14:0 3OH	6.4	7.9	6.9	7.1	8.6	8.5	8.5	7.7	8.3	7.9	8.3	8.1	7.8
16:1 ω7c	23.3	28.9	26.4	20.8	22.6	20.6	17.2	26.2	26.6	27.8	25.8	25.4	24.2
16:00	24.1	27.6	33.7	35.8	28.1	30.3	30.9	31.0	31.7	32.4	25.1	26.4	28.4
cy 17:0	8.6	6.2	8.8	11.5	8.2	12.1	14.9	8.7	8.6	6.6	6.2	7.2	8.4
17:00	0.0	1.2	0.0	0.0	2.0	1.4	1.4	0.6	0.3	0.2	0.0	1.6	1.4
18:1 ω7c/ ω9t/ ω12t	10.8	12.0	9.7	10.4	12.5	11.0	10.5	11.3	10.1	9.7	14.3	14.3	12.5
18:00	2.9	1.6	2.1	3.4	3.6	1.7	1.9	1.8	1.5	2.3	2.5	2.2	2.5
18:1 2OH	0.0	2.0	0.4	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
cy 19:0 ω8c	0.0	0.0	0.0	0.0	0.0	0.6	0.8	0.0	0.0	0.0	0.0	0.0	0.0
Sat.	56.9	55.9	61.8	68.0	63.5	64.8	70.7	60.7	61.8	60.9	55.4	57.7	60.6
Unsat.	34.1	42.8	36.5	31.1	35.1	31.5	27.7	37.4	36.7	37.5	40.1	39.7	36.7
Sat./unsat. ratio	1.7	1.3	1.7	2.2	1.8	2.1	2.5	1.6	1.7	1.6	1.4	1.5	1.7

The values are the means of three replicates (standard error < 5%).

Table 5. Percentage of total fatty acids from *P. putida* MH1d grown on medium amended with metals.

Fatty acid	Control	Metals (mM)											
		Cd 0.5	Cd 1	Cd 2	Ni 2	Ni 4	Ni 6	Zn 4	Zn 6	Zn 8	Cu 4	Cu 6	Cu 8
10:0 3OH	3.6	3.2	4.1	3.9	2.9	2.8	2.9	4.7	4.7	4.5	3.8	4.0	4.0
12:00	3.4	3.1	3.1	3.2	9.1	7.5	8.1	3.2	3.0	2.4	3.3	3.1	3.4
12:0 2OH	4.2	4.6	5.5	5.1	1.3	0.4	0.0	5.2	5.1	5.6	5.2	5.2	4.7
12:0 3OH	4.6	4.7	5.4	4.8	4.9	3.5	4.2	5.1	5.0	5.1	4.9	4.9	4.5
14:00	0.8	0.6	0.7	0.7	0.5	1.0	0.9	0.7	0.6	1.0	0.7	0.7	0.9
15:00	0.7	0.6	0.6	0.5	0.5	0.0	0.0	0.6	0.5	0.5	0.8	0.7	0.3
16:1 ω7c	29.8	32.7	33.2	32.2	22.9	31.7	26.6	31.4	27.6	21.7	30.2	30.6	34.0
16:00	27.1	22.3	25.2	26.3	32.5	31.4	36.1	28.0	28.3	28.5	26.1	27.0	28.0
cy 17:0	6.2	8.2	4.6	2.8	11.5	0.8	5.1	4.1	4.1	5.1	6.9	6.6	2.4
17:00	0.9	0.9	0.8	0.8	0.7	0.5	0.0	0.8	0.7	0.7	1.0	0.9	0.7
18:0 ante	0.5	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.7	0.0	0.2	0.2	1.0
18:1 ω7c	14.8	13.3	14.9	15.7	11.4	15.4	11.7	15.1	17.4	20.6	14.9	13.7	14.0
18:00	2.6	1.7	1.4	3.0	1.3	3.6	4.6	1.3	2.5	3.4	2.1	2.3	2.2
Sat.	54.4	49.8	51.3	51.1	65.1	51.4	61.9	53.6	54.4	56.8	54.7	55.5	51.0
Unsat.	44.6	46.0	48.1	47.8	34.3	47.2	38.3	46.4	44.9	42.2	45.1	44.5	48.0
Sat./unsat. ratio	1.2	1.1	1.1	1.1	1.9	1.1	1.6	1.2	1.2	1.3	1.2	1.3	1.1

The values are the means of three replicates (standard error < 5%).

ring increase the stability and rigidity of membranes and decrease their permeability to protons [28, 29]. On the other hand, Law [30] speculated that the conversion of monounsaturated to cyclopropane fatty acids may protect cells from chemical destruction at the site of unsaturation. It is interesting to note that in our studies the presence of Cd or Cu in growth medium resulted in a decrease in the cyclopropane fatty acid amounts of FAME profiles of *P. putida,* while monosaturated content increased. Fozo [31], studying the impact of low pH on membrane fatty acids suggested that increasing proportions of monounsaturated fatty acids is an adaptation to acidification of the environment. However, this effect was not observed in our studies.

Based on the FAME profiles, for each sample we calculated the value of saturated to unsaturated fatty acids ratio (sat/unsat). Saturation indices for MH1d, AM12, AM15, and MH8b control strains were 1.2, 1.4, 1.5, and 1.7, respectively. For *P. putida* MH1d this index was on the same level in each sample, with the exception when cells were cultured on media with 2 or 6 mM Ni. The vales of sat/unsat ratios calculated for AM15 and AM12 strains after growth on media with heavy metals were higher in comparison with control. The highest changes in fatty acid saturation were observed for all strains grown on medium amended with nickel. Similar data were obtained by Paraszkiewicz et al. [32], who studied the influence of this metal on the fatty acid composition of fungi *Curvularia lunata*. In contrast, the lower content of unsaturated fatty acids was observed in *Saccharomyces cerevisiae* [21] and in three strains of *Aureobasidium pollulans* cultured under cadmium stress [33]. A high degree of unsaturation increases the susceptibility of fatty acid to free radicals [34]. The decrease of fatty acid unsaturation in cells exposed to heavy metals could be part of a defense aimed at reducing the ability of heavy metals to generate oxidative stress [21]. However, nickel produces relatively low levels of reactive oxygen species, but can induce depletion of enzymes that are responsible for free radical elimination [35].

A significant proportion of saturated fatty acids with odd-carbon number were also converted to saturated fatty acids with even-carbon number (Figs. 1-4). Such membrane modification was reported earlier for psychrophilic strains during increasing temperature. The shift between odd- and even-chain fatty acids occurred mainly at the sub- and supra-optimal extremes of growth temperatures [36]. They suggested that such alternations resulted from changes in specificity for prime molecules (acetyl-CoA and propionyl-CoA for even- and odd-carbon straight chain fatty acids, respectively) by the fatty acid synthetase. It is possible that heavy metals act in a similar way. Russell [37]

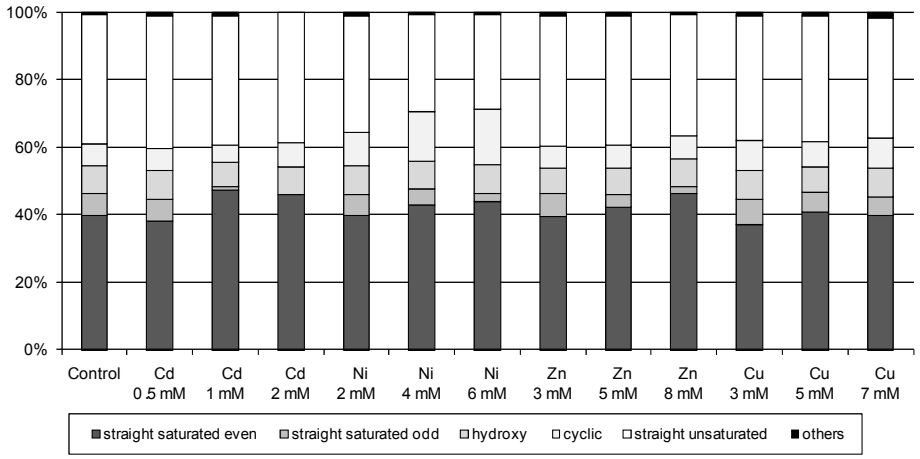

Fig. 1. Fatty acid composition of *Enterobacter intermedius* AM15.

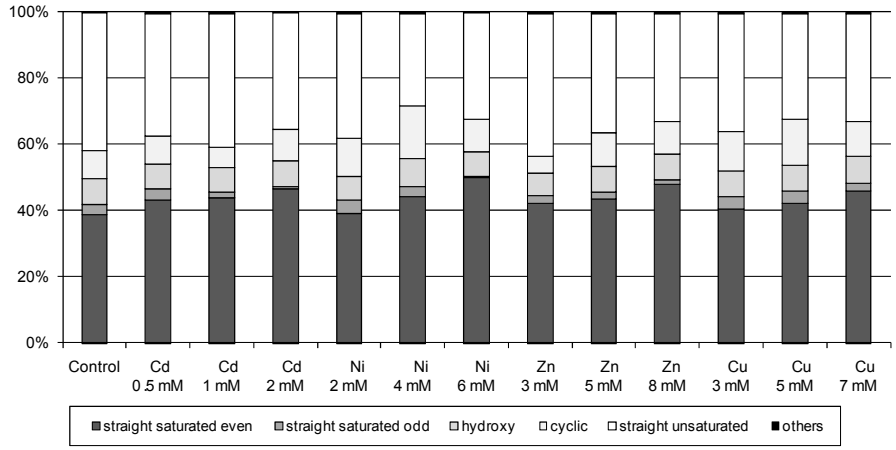

Fig. 2. Fatty acid composition of *Klebsiella pneumoniae* AM12.

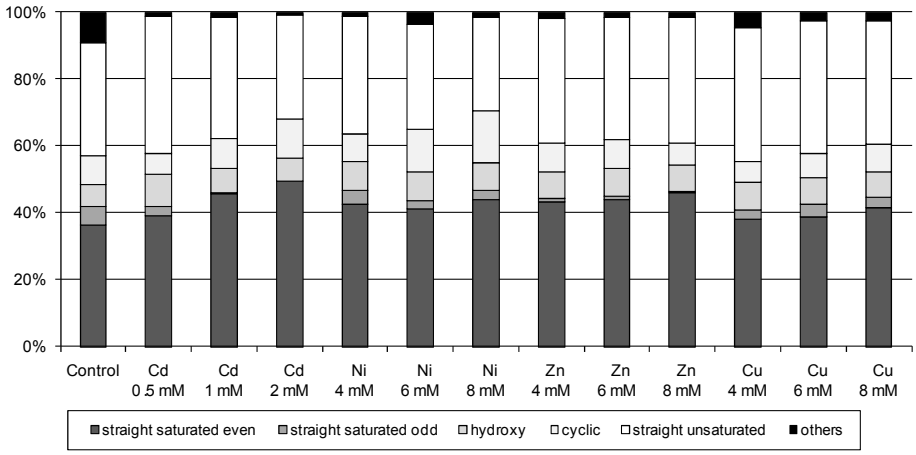

Fig. 3. Fatty acid composition of *Enterobacter intermedius* MH8b.

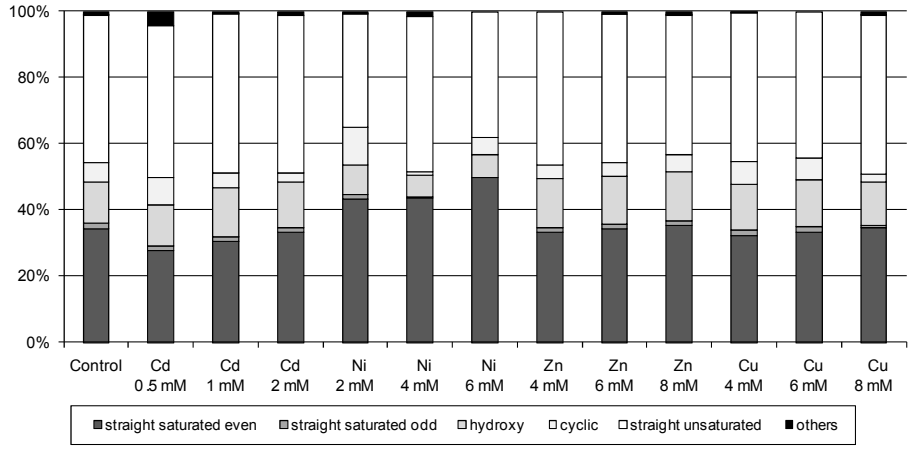

Fig. 4. Fatty acid composition of *Pseudomonas putida* MH1d.

has shown that the balance of even- and odd-chain length fatty acids can be modified by growth on different carbon sources.

The percentages of hydroxy fatty acids in the FAME profiles of bacterial strains cultured on media with metals were approximately at the same level as in control strains (Figs. 1-4). The only exception was observed for *P. putida,* where the percentages of these fatty acids were lower in the presence of Ni, and higher in the presence of Zn as compared with control. The 2- and 3-hydroxy fatty acids in Gram-negative bacteria are generally constituents of lipopolysaccharides.

In conclusion, our results demonstrated that heavy metals affected the composition of fatty acids in bacteria and nickel caused the most remarkable changes. It is known that heavy metal stress influences several aspects of lipid biochemistry. These include qualitative and quantitative alternation in lipids, inhibition of biosynthetic pathways and lipid peroxidation [38]. Nickel and, much more, copper exhibit the ability to produce reactive oxygen species, and catalyze peroxidation of membrane lipids. Cadmium does not appear to generate free radicals, but lipid peroxidation is a consequence of exposure to this metal, which is probably due to the inhibition of superoxide dismutase (SOD). Zinc does not induce free radical production at all, but does form stress protein [39, 40]. Although heavy metal toxicity toward bacteria is well documented, the precise molecular mechanism has not been clearly elucidated. As reported elsewhere, reactive oxygen species may play a role in heavy metal toxicity. The dependence of metal toxicity on the degree of fatty acid unsaturation supported the hypothesis [20].

In our study, each examined strain had its specific physiological traits for environmental adaptation. However, we observed similar results for closely related members of the family Enterobacteriaceae, e.g. *Enterobacter intermedius* AM15, MH8b, and *Klebsiella pneumoniae* AM12. Different variations were observed for *P. putida* that belongs to the *Pseudomonadaceae* family. For all tested bacteria, the modifications of fatty acids contents could be part of a defense or/and repair mechanism aimed at reducing the damage caused by heavy metal stress. Understanding the response of bacteria to heavy metals may be useful in bioremediation of metal-contaminated sites. For example, such bacteria may be useful in several biotechnological approaches involving biosorption, bioprecipitation, and bioaccumulation to decrease the amount of bioavailable toxic forms of metals. Moreover, it has been

shown that heavy-metal-resistant bacteria belonging to plant growth promoting microorganisms significantly increase heavy metal accumulation by plants [41, 42].

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