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M.I. DIMOVA*, G.O. IUTYNSKA

Zabolotnyi Institute of Microbiology and Virology, NAS of Ukraine,
154 Akademika Zabolotnoho Str., Kyiv, 03143, Ukraine

* Author for correspondence; e-mail: mdildiv@gmail.com

FATTY ACID COMPOSITION OF *COMAMONAS TESTOSTERONI* UNDER HEXACHLOROBENZENE LOADING CONDITIONS

Changes in the lipid composition in bacterial membranes are considered to be the most important adaptation mechanisms to adverse chemical factors. ***The aim*** *of the study was to compare the hexachlorobenzene effects on the fatty acid composition of total lipids Comamonas (C.) testosteroni.* ***Methods.*** *The study was performed with C. testosteroni UCM B-400 and B-401, B-213 strains. Bacteria were grown in the Luria-Bertrani (LB) liquid medium containing 10 and 20 mg/L of hexachlorobenzene (HCB). After cultivation, the biomass was separated by centrifugation and the fatty acid composition of total lipids was determined through analyzing its methyl esters. To assess the cell membrane properties, such parameters as the lipid unsaturation index, the average carbon chain length of fatty acids, and the membrane viscosity index were determined.* ***Results.*** *In the fatty acids spectrum of C. testosteroni B-400 after cultivation in a medium containing 20 mg/L of HCB, the contents of unsaturated hexadecenoic (C16:1) and octadecenoic (C18:1) acids were lower by 10.6 and 5.5 %, respectively, and that of saturated hexadecanoic (C16:0) acid was higher by 8.4 %, compared to the control. The fatty acid composition of C. testosteroni B-401 was more stable compared to strain B-400. Collection strain C. testosteroni B-213 compared to strains isolated from soil with high HCB load, in the presence of 10 and 20 mg/L of HCB had the highest relative content of saturated hexadecanoic acid (C16:0) up to 38.33—40.7%. Unsaturated octadecenoic acid decreased at the doses 10 and 20 mg/L to 1.5—2 % compared to the control. In all strains under the HCB impact, there was an increase in the relative content of C17-cyclopropanoic acid compared to control variants.* ***Conclusions.*** *C. testosteroni UCM B-400, B-401, and B-213 bacteria under cultivation conditions in HCB-containing medium, decreasing the degree of lipid unsaturation and increasing the relative content of C17-cyclopropanoic acid can be considered as the main mechanisms regulating the cytoplasmic membrane fluidity; the displaying of these protective reactions had a strain trait and did not depend on the adaptation in natural isolating places.*

Keywords: *Comamonas testosteroni, fatty acid composition, lipid unsaturation, membrane fluidity, hexachlorobenzene.*

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Soil pollution is a global anthropogenic origin problem. In recent years, increased food demand has been due to the using chemicals to protect crops from phytopathogens and pests. Over a long time period, this has led to the dispersion and accumulation of pollutants in the environment, primarily in the soil. Organochlorine pesticides are one of the common contaminants present in the soil [1]. Hexachlorobenzene (HCB) is included in this group of persistent toxicants. Like other aromatic compounds, it has a high capacity to adsorb on soil particles, organic matter, and clay minerals [2].

Metabolic adaptive reactions occur in subjected soil bacteria to constant physical and chemical influences of the environment [3, 4]. Fatty acid profile modification is also one of the adapted cell mechanisms to counteract the negative effect of toxic compounds including phenol and aliphatic hydrocarbons. Changes in the cell membrane fluidity associated with an increase in unsaturated, branched, cyclic, and hydroxy fatty acids may be a cell reaction to the presence of aromatic compounds in its surrounding [5, 6]. Change in the fluidity of cell membranes by regulating their lipid composition, in particular, the ratio between iso and anti-iso branched fatty acids and isomerization of *cis*-unsaturated fatty acids into the corresponding *trans*-isomers is one of the effective adaptive mechanisms [2]. The cytoplasmic membrane fluidity is the most important parameter to determine cell survival under stress conditions. Fluidity is provided by different types of combinations of membrane components' mobility and is determined by the ratio between unsaturated and saturated fatty acids in membrane lipids and the permanent movement of fatty acidic tails [7, 8].

Also, the membrane functions are influenced by other local factors, besides the membrane fluidity. The bacterial ability to compensate for changes in membrane fluidity caused by toxic compounds is named "homeovascular adaptation" and is mainly achieved by changing the fatty acid composition. Variations in fatty acid length, saturation, and cis/trans configuration of unsaturated fatty acids are major factors influencing membrane fluidity [9]. One of the homeophasic adaptation mechanisms of bacteria to the influencing negative environmental factors is the change in the lipid unsaturation degree [8, 10]. The lipid unsaturation degree is an important indicator for maintaining the required level of cytoplasmic membrane fluidity and, accordingly, the microbial adaptation to adverse environmental factors. This mechanism is characteristic for gram-negative bacteria [4, 11].

Such mechanisms as shortening or lengthening the fatty acid chain (longer fatty acid chains reduce membrane fluidity), changes in the content of branched-chain fatty acids or fatty acids containing cyclopropane ring, and isomerization of the double bond of fatty acids with cis- into the trans-configuration are studied and established to be other membrane fluidity regulation mechanisms [3, 10]. Such changes in the cytoplasmic membrane prevent penetration of toxic substances into the cell.

It should be noted that the lipid composition changes in the membranes might be different for members of the same genus or even species. The effects of various toxic compounds with similar toxicity and hydrophobicity on the surface properties and changes in bacterial membrane lipopolysaccharides are not general, as demonstrated in *P. putida* by comparing the reaction to n-alkanols and chlorophenols [9, 12]. Thus, studies of the phenol tolerance mechanisms of the genus *Comamonas* representatives have shown that in some strains of this genus one mechanism may be predominant, while in others — several, for example, changes in *cis*-isomers to *trans*-isomers of hexadecenoic acid and the presence or absence of fatty acids with a cyclopropane ring [13]. However, changes in the lipid composition of representatives in C. *testosteroni* caused by various toxic compounds remain studied insufficiently. The aim of this study was to compare

the hexachlorobenzene (HCB) effects on the fatty acid compositions of *C. testosteroni* strains UCM B-400 and UCM B-401, as capable to HCB destroying, and the *C. testosteroni* UCM B-213 collection strain, not adapted to this pollutant.

Materials and methods. The study was performed with *C. testosteroni* UCM B-400 and B-401 strains, which were isolated from the Kalush organochlorine pesticides landfill (Ivano-Frankivsk, Ukraine). The strains have been shown to be resistant to high HCB doses (50—100 mg/L) and capable of its decomposition [14]. *C. testosteroni* UCM B-213 from the Ukrainian Collection of Microorganisms as one not adapted to high HCB doses was studied for comparison.

Bacteria were grown in a liquid nutrient Luria-Bertrani (LB) medium of the following content (in g/L): 5.00 $(NH_4)_2SO_4$ — 2.93 KH_2PO_4 — 5.87 K_2HPO_4 — 0.30 $MgSO_4{*}7H_2O$ — 2.00 NaCl — 0.01 $CaCl_2$ — 0.01 $FeSO_4$ [15]. Sodium succinate (4 g/L) was added as a carbon source. The HCB effect on the lipid membranes properties of the studied bacteria was determined in a laboratory experiment according to the following scheme: a) control l: strain growing in LB medium (pure control), b) control 2: strain growing in LB medium with the addition of 10 mL of acetone required to dissolve 10 mg/L HCB, c) control 3: strain growing in LB medium with the addition of 20 mL of acetone required to dissolve 20 mg/L HCB, d) strain growing in LB medium with the addition of 10 mg/L HCB (dissolved in acetone), e) strain growing in LB medium with the addition of 20 mg/L HCB (dissolved in acetone). Research of each strain was performed in three repetitions. Bacteria were cultivated in LB medium on shakers (121 rpm) for 7 days at 28 °C.

To separate cell biomass after culturing, the culture liquid was centrifuged at 5000 rpm for 30 min(Eppendorf centrifuge, rotor 5415R).

The fatty acids spectrum of total lipids was determined by the analysis method of its methyl esters. To do this, the cells were hydrolyzed in a 5 % solution of acetyl chloride in methanol for 1 hr at 100 °C, followed by extraction with ether-hexane (1 : 1). Methyl esters identification was performed using the chromato-mass spectrometric system Agilent 6800N/5973 inert (Agilent, USA) in the temperature range of 150—250 °C [16]. Methyl esters were identified automatically by retention time using the NIST data library compared to the standards. The fatty acid content was determined using the AgilentChemStation software and displayed as a percentage of the total peak area.

The lipid unsaturation index was calculated by the formula [17]:

$$UI = (A+ (2*B) + (3*C))/100,$$

where UI is the index of unsaturation; A is the content of monounsaturated fatty acids, %; B is the content of binasaturated (biunsaturated) fatty acids, %; C is the content of trinasaturated (triunsaturated) fatty acids, %.

The index of the membrane viscosity was calculated using the formula [18]:

$$I_{VM} = (A + B_{trans}/B_{cis} + C),$$

where I_{VM} is the index of the membrane viscosity; A is the content of saturated fatty acid, %; B_{trans} is the content of *trans*-unsaturated fatty acids, %; B_{cis} is the content of *cis*-unsaturated fatty acids, %; C is the content of fatty acids with a cyclopropane ring, %.

The average carbon chain length of the fatty acids was calculated using the formula [10]:

$$L = \Sigma (FA*C)/100,$$

where L is the average carbon chain length; FA is the fatty acid content in cells, %; C is the number of carbon atoms in the direct chain of fatty acids.

Statistical analyses were performed using the MS Excel 2010 and Graph Pad Prism 8.0.1 statistical software, and differences were considered significant at $p < 0.05$. All results are reported as mean values ± standard deviation of the means (SDs).

Results. In the fatty acids, spectra of strains *C. testosteroni* B-400, B-401, and B-213 under the conditions of cultivation in a medium containing HCB, no qualitative changes were detected, and all marker acids characteristic of this species were present. However, there was a difference in the amount of acid compared to the pure control culture (control 1), as well as in variants with the acetone addition used to dissolve HCB doses: 10 and 20 mg/L (control 2 and control 3, respectively).

In the fatty acids spectrum of *C. testosteroni* UCM B-400 after 7 days of cultivation in a medium containing 10 mg/L HCB compared to control variants, no significant changes in the quantitative composition were observed, except a statistically significant decrease in unsaturated octadecenoic acid (C18:1) and increase in the relative content of saturated heptadecanoic (C17:0) acid. More noticeable changes occurred in the fatty acids composition cultivated in a medium with 20 mg/L HCB, where the content of unsaturated acids (hexadecenoic acid (C16:1) and octadecenoic acid (C18:1)) was lower (17.12 and 14.02%) than in the other experiments, but the content of saturated hexadecanoic acid (C16:0) was higher (38.61%) relative to the control (Table 1). Under the HCB presence cultivation conditions, the content of C-17 cyclopropanoic (2-hexyl-cyclopropaneoctanoic or 9,10-methylenehexadecanoic) acid increased. This fatty acid is one of the protective mechanisms against toxic stress. However, the content of saturated acids: dodecanoic and octadecanoic, under the cultivation conditions with HCB and in its absence almost did not differ.

The contentof *C. testosteroni* UCM B-401 in the fatty acid composition under the HCB action was more stable compared to strain B-400. Under cultivattion in a medium with 20 mg/L HCB, the content of saturated hexadecanoic (C16:0) acid increased by 34.55 ± 0.27% compared to the control, where its corresponding relative content was 30.54 ± 0.84%. The content of octadecenoic acid decreased by almost 2.5% compared to the control. It should be noted that the content of C17-cyclopropanoic acid after the HCB addition decreased to almost by 0.5% compared to the pure control and acetone controls (Table 2).

Collection strain *C. testosteroni* UCM B-213 in cultivation conditions with 10 and 20 mg/L HCB compared to the strains isolated from HCB-polluted soil had the highest relative content of saturated hexadecanoic acid (C16:0) up to 38.33—40.7% and heptadecanoic (C17:0)

Table 1. **Fatty acid composition of total lipids for *C. testosteroni* UCM B-400 under different cultivation conditions, % of the total content**

Fatty acid	Control 1	Control 2	Control 3	10 mg/L HCB	20 mg/L HCB
C10:3OH	1.36 ± 0.18	1.38 ± 0.37	1.13 ± 0.44	1.53 ± 0.14	2.19 ± 0.1
C12:0	2.03 ± 0.66	2.30 ± 0.31	2.43 ± 0.69	2.54 ± 0.15	2.44 ± 0.49
C14:0	0.35 ± 0.01	0.35 ± 0.07	0.54 ± 0.12	0.56 ± 0.05	0.65 ± 0.03
C16:1	20.49 ± 0.26	21.90 ± 4.4	18.80 ± 1.88	19.14 ± 0.41	14.02 ± 0.50
C16:0	30.21 ± 1.45	29.48 ± 0.75	32.78 ± 1.10	32.52 ± 0.35	38.61 ± 0.77
C16:2OH	2.12 ± 0.45	3.32 ± 0.09	3.50 ± 0.06	2.66 ± 0.63	2.10 ± 0.69
C17cyclo	13.34 ± 0.32	13.03 ± 3.72	15.81 ± 1.44	16.53 ± 0.47	17.49 ± 0.86
C17:0	0.17 ± 0.04	0.92 ± 0.13	1.35 ± 0.07	1.92 ± 0.05	2.17 ± 0.10
C18:2	1.65 ± 0.27	2.30 ± 0.92	2.86 ± 0.03	2.50 ± 0.05	2.51 ± 0.10
C18:1	27.78 ± 0.33	24.59 ± 0.66	20.32 ± 1.53	19.05 ± 0.36	17.12 ± 0.67
C18:0	0.50 ± 0.04	0.44 ± 0.04	0.50 ± 0.09	0.49 ± 0.01	0.40 ± 0.11

Differences between data groups are significant, $p < 0.0001$.

up to 0.66—0.70%. At the same time, the content of unsaturated hexadecenoic (C16:1) acid was higher than that of B-400 (18.80—21.90%), but lower compared to B-401 (31.83—33.84%). The content of unsaturated octadecenoic acid (C18:1) decreased at 10 and 20 mg/L HCB doses to by 1.5—2% compared to the control (Table 3).

The lipid unsaturation index, membrane fluidity index, and the average carbon chain length of fatty acids were determined to generalize characteristics of the HCB effect on the lipid composition of membranes (Table 4).

The lipid unsaturation index values for strain UCM B-400 at 10 and 20 mg/L HCB doses indicated adecrease compared to the controls (with the addition of solvent) by 9.8 and 20%, respectively, which was connected to an increase in the relative content of hexadecanoic (C16:0) and

Table 2. **Fatty acid composition of total lipids of *C. testosteroni* UCM B-401 under different cultivation conditions, % of the total content**

Fatty acid	Control 1	Control 2	Control 3	10 mg/L HCB	20 mg/L HCB
C10:3OH	1.39 ± 0.08	0.76 ± 0.19	0.73 ± 0.25	1.30 ± 0.35	1.49 ± 0.10
C12:0	3.43 ± 0.78	1.28 ± 0.22	1.91 ± 0.38	1.75 ± 0.39	1.55 ± 0.29
C14:0	0.35 ± 0.02	0.44 ± 0.10	0.61 ± 0.19	0.43 ± 0.10	0.39 ± 0.08
C16:1	31.83 ± 0.43	33.84 ± 0.95	32.75 ± 1.37	33.4 ± 0.06	32.14 ± 1.04
C16:0	30.54 ± 0.84	31.62 ± 1.7	32.17 ± 1.25	33.0 ± 0.71	34.55 ± 0.27
C16:2OH	1.60 ± 0.28	2.09 ± 0.89	2.90 ± 0.92	2.61 ± 0.38	2.43 ± 0.41
C17cyclo	3.64 ± 0.11	3.09 ± 0.70	3.86 ± 0.60	2.65 ± 0.44	2.92 ± 0.13
C17:0	0.33 ± 0.04	0.45 ± 0.18	0.48 ± 0.85	0.54 ± 0.06	0.78 ± 0.26
C18:2	0.45 ± 0.19	0.45 ± 0.23	1.01 ± 0.63	0.70 ± 0.07	0.71 ± 0.3
C18:1	26.04 ± 0.61	25.26 ± 0.42	21.39 ± 1.58	24.74 ± 1.70	23.61 ± 0.59
C18:0	0.41 ± 0.20	0.73 ± 0.16	0.79 ± 0.19	0.68 ± 0.11	0.68 ± 0.08

Differences between data groups are significant, $p < 0.0001$.

Table 3. **Fatty acid composition of total lipids of *C. testosteroni* UCM B-213 under different cultivation conditions, % of total content**

Fatty acid	Control 1	Control 2	Control 3	10 mg/L HCB	20 mg/L HCB
C10:3OH	3.04 ± 1.21	1.77 ± 0.70	4.06 ± 0.43	2.25 ± 0.36	2.60 ± 1.03
C12:0	3.30 ± 0.32	1.96 ± 0.23	3.68 ± 0.38	2.63 ± 0.51	3.06 ± 1.14
C14:0	0.75 ± 0.03	0.31 ± 0.02	0.37 ± 0.10	0.50 ± 0.19	0.53 ± 0.04
C16:1	27.64 ± 0.05	25.02 ± 0.55	29.82 ± 0.32	28.31 ± 0.11	28.0 ± 0.69
C16:0	35.46 ± 1.20	36.30 ± 1.83	37.86 ± 0.60	38.33 ± 0.69	40.70 ± 1.94
C16:2OH	2.98 ± 0.87	3.39 ± 0.51	3.14 ± 0.12	2.43 ± 0.50	2.56 ± 0.17
C17cyclo	1.83 ± 0.30	1.93 ± 0.27	1.98 ± 0.63	2.06 ± 0.05	2.50 ± 1.25
C17:0	0.32 ± 0.05	0.41 ± 0.27	0.59 ± 0.08	0.66 ± 0.03	0.70 ± 0.14
C18:2	1.51 ± 0.11	2.18 ± 0.01	1.33 ± 0.11	1.37 ± 0.16	1.49 ± 0.03
C18:1	21.65 ± 0.60	22.61 ± 0.75	19.94 ± 1.76	18.53 ± 0.54	17.34 ± 0.76
C18:0	0.59 ± 0.17	0.64 ± 0.09	0.59 ± 0.09	0.61 ± 0.10	0.64 ± 0.04

Differences between data groups are significant, $p < 0.0001$.

C17-cyclopropanoic acids. Fatty acid spectrum changes in lipid membranes were also characterized by a shortening of the average carbon chain length and an increase in the membrane viscosity index. Lipid unsaturation index for UCM B-401was stable under the action of 10 and 20 mg/L of HCB. There were also no significant changes in the membrane fluidity index and the average carbon chain length . Regarding UCM B-213, there was a tendency to decrease the saturation index and membrane viscosity index. Herewith, in the fatty acid composition of this strain, C17-cyclopropanoic acid was present, the content of which at the 20 mg/L dose of HCB was the highest. An increas e in the content of octadicadienoic (linoleic (C18:2)) fatty acid, relative to the control, also indicates adaptation to the toxic substance.

Discussion. Studies of adaptation mechanisms in soil bacteria are an important issue in view of the ever-increasing anthropogenic load on agricultural soils, in particular the increase in pesticide contamination levels. One of the important adaptation mechanisms to the adverse effects of chemical environmental factors is to reduce the lipid membrane permeability due to changes in its fatty acid composition [11]. It is found that when *Pseudomonas stutzeri* was cultivated to grow in a naphthalene medium, the ratio of saturated to unsaturated fatty acids increased from 1.1 to 2.1, while for another strain of *Pseudomonas* sp. JS150 it increased from 7.5 to 12.0 [19]. In our studies, a decrease in lipid unsaturation was also observed under HCB load. In particular, there was observed an increase in the relative content of saturated hexadecanoic acid in the lipids of *C. testosteroni* UCM B-400, B-401, and B-213. This can be considered as an important adaptation mechanism.

The conversion of cis-isomers into trans-isomers of unsaturated fatty acids is considered as a regulating mechanism of the cytoplasmic membrane fluidity under the impact of hydrocarbons such as toluene and other toxic compounds [9—21]. The increase in the content of trans-isomers of unsaturated fatty acids alongside with a decrease in the content of the same cis-isomers under the phenol influence has been studied and described. Under the toluene, nitrotoluene, and 4-chlorophenol impact on some species of the genus *Pseudomonas*, such an increase has been described for the same fatty acids in [22]. Contrary to those findings, we have not observed such biochemical changes in the strains studied.

The increase in the content of 2-hexyl-cyclopropaneoctanoic (C17-cyclopropane) acid has been considered by some researchers as an important adaptation mechanism to the action of adverse chemical factors [23, 24]. In our studies, the fatty acid composition of the total lip-

Table 4. **Indices characterizing the lipid membranes state for *C. testosteroni* UCM B-400, B-401, and B-213**

Experiment variant	UI*	I_{VM} **	L***
C. testosteroni UCM B-400			
Control 1	0.52	50.08	16.56
Control 2	0.51	51.20	16.51
Control 3	0.45	56.32	16.48
10 mg/L HCB	0.46	58.03	16.47
20 mg/L HCB	0.36	66.35	16.33
C. testosteroni UCM B-401			
Control 1	0.60	40.45	16.47
Control 2	0.60	41.13	16.46
Control 3	0.60	41.16	16.44
10 mg/L HCB	0.59	41.68	16.38
20 mg/L HCB	0.56	44.84	16.35
C. testosteroni UCM B-213			
Control 1	0.52	49.19	16.38
Control 2	0.52	50.18	16.19
Control 3	0.50	50.87	16.18
10 mg/L HCB	0.50	51.51	16.17
20 mg/L HCB	0.50	51.79	16.01

* UI — unsaturation index, **I_{VM} — membrane viscosity index, ***L — average carbon chain length in fatty acids.

ids from all three studied strains an increasing the C17-cyclopropanoic acid content according to increasing HCB dose were show n. Such changes in some phenol-tolerant *C. testosteroni* have been observed [13]. The conversion of unsaturated fatty acids to cyclopropane-contained is considered to be the main adaptive response of the bacterial cell to the stressful environmental conditions due to stabilizing the cytoplasmic membrane fluidity [25]. It has been shown that the increase in membrane rigidity in the presence of fatty acids with a cyclopropane ring is due to the higher melting point of the acids compared to unsaturated fatty acids [10]. Some researchers have reported that the primary function of cyclopropane ring fatty acids in bacterial cells is to change the chemical properties of membranes without significant changes in their physical properties [23, 26]. The study results for the reaction to toluene of wild-type strain and knockout mutant in the cyclopropane synthase (the enzyme responsible for the synthesis of cyclopropanoic acids) showed that a wild-type strain in the stationary phase is more resistant to toluene shock, which indicates that the cyclopropanoic fatty acids synthesis is an adaptive reaction to aromatic hydrocarbons [9]. The synthesis of C17-cyclo propanoic acid for all three studied strains was recorded, according to increasing the HCB dose, the content of this acid was increased.

Therefore, *C. testosteroni* UCM B-400, B-401, and B-213 bacteria under cultivating conditions in an HCB-containing medium showed a decrease in lipid unsaturation and an increase in the relative content of C17-cyclopropane (2-hexyl-cyclopropaneoctanoic or 9.10-methylenehexadecanoic) acid, which can be considered as important mechanisms for regulating the cytoplasmic membrane fluidity. The cytoplasmic membrane fluidity was decreased by both mechanisms to reduce the toxic HCB effect. For the first time, the greatest extent of adaptive reactions was shown to be expressed in *C. testosteroni* UCM B-400 isolated from HCB-contaminated soil. However, adaptive reactions in strains UCM B-401 (isolated from contaminated soil) and UCM B-213 (unadapted, collection) were also observed, although they were less pronounced, therefore, the displaying protective reactions had a strain feature.

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М.І. Дімова, Г.О. Іутинська

Інститут мікробіології *і* вірусології ім. *Д. К. Заболотного*
вул. Академіка Заболотного, 154, Київ, *03143,* Україна

ЖИРНОКИСЛОТНИЙ СКЛАД *COMAMONAS TESTOSTERONI*
ЗА УМОВ ГЕКСАХЛОРОБЕНЗОЛЬНОГО НАВАНТАЖЕННЯ

Дослідження адаптаційних механізмів у грунтових бактерій є важливим завданням з огляду на постійно зростаюче пестицидне забруднення земель сільськогосподарського призначення. Хлорорганічні пестициди є одними з поширених забруднень у ґрунті. До вказаної групи персистуючих токсикантів входить гексахлорбензол (ГХБ). Зміни ліпідного складу мембран у бактерій вважаються одним із найбільш важливих механізмів пристосування до дії несприятливих хімічних факторів. Зміни плинності клітинної мембрани, пов'язані зі збільшенням вмісту ненасичених, розгалужених, циклічних і гідроксижирних кислот можуть бути реакцією клітин на присутність ароматичних сполук в їхньому оточенні. **Метою роботи** було порівняльне дослідження впливу ГХБ на жирнокислотні профілі загальних ліпідів *Comamonas testosteroni*. **Методи.** Дослідження проводили зі штамами *C. testosteroni* УКМ В-400 і В-401, виділеними із забрудненого грунту і здатними до розкладу ГХБ, а також колекційним штамом В-213. Бактерії вирощували глибинним способом у рідкому поживному Luria-Bertrani (LB) середовищі, що містило 10 і 20 мг/л ГХБ. Після вирощування біомасу відокремлювали центрифугуванням і визначали жирнокислотний спектр загальних ліпідів методом аналізу їхніх метилових ефірів. Для оцінки властивостей цитоплазматичної мембрани бактеріальних клітин розраховували індекс ненасиченості ліпідів, середню довжину карбонового ланцюга жирних кислот та індекс в'язкості мембран. **Результати.** У спектрах жирних кислот *C. testosteroni* В-400, В-401, В-213 за умов культивування у середовищі, що містило ГХБ, не було виявлено якісних змін, тобто були наявні всі маркерні кислоти, характерні для цього виду. У спектрі жирних кислот *C. testosteroni* В-400 після культивування у середовищі, що містило 20 мг/л ГХБ, вміст ненасичених гексадеценової (С16:1) і октадеценової (С18:1) кислот був нижчим відповідно на 10.6 і 5.5 %, а насиченої гексадеканової (С16:0) — вищим на 8.4 % відносно контролю. Кількісний склад жирнокислотного профілю *C. testosteroni* В-401 виявився більш стабільним порівняно зі штамом В-400. Вміст насиченої гексадеканової кислоти (С16:0) після культивування у LB середовищі з 20 мг/л ГХБ був вищим на 4 % порівняно з контролем. Проте вміст ненасиченої гексадеценової (С16:1) кислоти був майже незмінним, зміни концентрації коливалися в межах 1—2 % між усіма варіантами дослідження цього штаму. Вміст іншої ненасиченої кислоти — октадеценової (С18:1) був нижчим на 2.5 % відносно контролю. Колекційний штам *C. testosteroni* В-213, порівняно зі штамами, виділеними з грунту з високим ГХБ навантаженням, а саме 10 і 20 мг/л, відрізнявся найвищим відносним вмістом насиченої гексадеканової кислоти (С16:0) — до 38.33—40.7 %. При цьому вміст ненасиченої октадеценової кислоти зменшився на 1,5—2 % порівняно з контролем. У всіх штамів за впливу ГХБ відмічено підвищення відносного вмісту С17-циклопропанової (2-гексил-циклопропаноктанової) кислоти порівняно з контрольними варіантами. **Висновки.** У бактерій *C. testosteroni* UCM В-400, В-401 і В-213 за умов вирощування у середовищі, що містить ГХБ, зменшення ступеню ненасиченості ліпідів та підвищення відносного вмісту циклопропаноктанової кислоти можна вважати основними механізмами регуляції плинності цитоплазматичної мембрани. Прояв зазначених захисних реакцій має штамову ознаку і не залежить від адаптації у природних місцях виділення.

Ключові слова: *Comamonas testosteroni, спектр жирних кислот, ненасиченість ліпідів, плинність мембран, гексахлорбензол.*