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THE FATTY ACID COMPOSITION OF CELL LIPIDS IN WALNUT BACTERIAL PATHOGENS

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Walnut (Juglans regia) is the most economically important and widespread nut crop in Ukraine. As bacterial diseases of walnut can reduce the yield of this culture by up to 40%, the monitoring of pathogens in a given crop and their identification are extremely important. The fatty acid composition of cell lipids is used in the taxonomy of plant pathogenic bacteria. The objective of this study was to determine the fatty acid composition of cell lipids of Agrobacterium, Xanthomonas, and Pseudomonas collection strains that can actually infect walnut, and those isolated from affected walnut trees in different regions of Ukraine. Fatty acid methyl esters were obtained by two different methods of extraction, with the use of 5% acetyl chloride in methanol at 100°C for 4 h, or 1.5% sulfuric acid in methanol at 80°C for 1 hour. Fatty acid methyl esters were analyzed using gas chromatography–mass spectrometry system. According to the found similarity of the fatty acid composition, the strains isolated from the affected walnut were related to representative collection strains of A. tumefaciens, X. arboricola and P. syringae. It should be noted that during the isolation of fatty acids with the use of 1.5% solution of H_2SO_4 in methanol, the amount of individual saturated and unsaturated fatty acids in the studied strains decreased and almost all hydroxyl acids, identified as a key taxonomic markers, disappeared in comparison with the using of 5% solution of acetyl chloride in methanol at the hydrolysis stage.

K e y w o r d s: fatty acids, extraction, composition, phytopathogenic bacteria, Agrobacterium, Xanthomonas, Pseudomonas, walnut.

According to numerous studies, the fatty acid composition of bacterial cell lipids is a taxonomically significant feature for identifying bacteria. Over years of research, the fatty acid composition of cell lipids in most bacteria, including phytopathogenic ones, has been characterized in detail. Therefore, this characteristic is often used in the polyphasic taxonomy of plant pathogenic bacteria.

In some cases, fatty acid data were compared with those of DNA-DNA and DNA-rRNA homology studies, where a good correlation between these methods was found. Discrimination was possible below the species level [1].

At the same time, the fatty acid composition of cell lipids can depend on the environment, the time of cultivation, as well as the method of fatty acid extraction.

The most important factor behind the repeatable fatty acid composition is the extreme care required to standardize the growth conditions of the organisms under study, as acyl chain levels vary with cultivation temperature, cultivation time, and medium composition, among other cultivation conditions [2, 3].

Often, different levels of individual fatty acids of the same strain can be obtained in two different laboratories that appear to use or claim to use the same media and growth conditions. Therefore, it is now recommended to compare the fatty acid composition of new strains with that of closely related species previously described and grown under the same standardized culture conditions. Typically, cultivation conditions and the composition of the culture medium affect the fatty acid composition, leading to differences that can impact the accurate identification of microorganisms [4].

Occasionally, it's important to use the same batch of medium to cultivate organisms simultaneously in order to compare the fatty acid composition of closely related strains or species, as slight differences in its composition can lead to changes in their fatty acid qualitative and quantitative perfor-

mance. Microbiologists usually determine the fatty acid composition of bacteria of the same species at the same optimal cultivation temperature, since organisms of the same species usually have the same cultivation temperature ranges. However, sometimes two or more strains of the same genus may have different cultivation temperature optima, and it is necessary to compare the qualitative and quantitative composition of fatty acids at the same cultivation temperature. Cultivation of microorganisms at one general temperature will ensure relative standardization of microbial cultivation conditions and obtaining a conditionally standard fatty acid composition of cellular lipids used in bacterial taxonomy [5, 6].

A significant part of fatty acids is very tightly bound to various protein and carbohydrate complexes that are insoluble in organic solvents. It is evident that the extraction of bacterial cells with a lipid solvent will not yield a substantial proportion of the fatty acids. To obtain a greater quantity of contained fatty acids, it is imperative to subject the cells to hydrolysis, a process that releases the so–called "bound" fatty acids for subsequent extraction with a solvent [7].

Therefore, when trying to perform the most accurate analysis of cellular lipids, the researcher faces a dilemma. If the extraction conditions are too soft, they will not be able to release all the components present; on the other hand, if the conditions are too rigorous, they may damage or destroy some of the more labile components. Fortunately, fatty acids are relatively resistant to the hydrolytic treatments needed to fully extract them from the cell. Nevertheless, exposure to extreme conditions should be minimized to prevent changes in the more labile fatty acids [8].

It's an accepted fact that walnut (Juglans regia) is the most economically important and widespread nut crop in Ukraine. Despite the widespread occurrence of bacterial diseases of walnuts, this problem has not been sufficiently studied in Ukraine. Nevertheless, there was an ongoing study on walnut cultivation in Ukraine, and insignificant plantation areas were identified [9].

According to the literature, bacterial diseases of walnuts are quite damaging and can reduce the yield of this culture by up to 40% [10].

In view of the increasing number of pathogens, monitoring crops for these pathogens and their timely, accurate diagnosis and identification is critically important [11-13].

The objective of this study was to examine how the extraction method affects the fatty acid composition of cell lipids in walnut bacterial pathogens.

Materials and Methods

In the research, we used a collection of strains of species that can potentially and actually affect walnut: *A. tumefaciens* – 8460, 8463, 8467, 8469, 8466, 8465, 8464, 8461, UCM B–1000, UCM B–1002, UCM B–1006, UCM B–1003T; *X. arboricola* pv. *juglandis* – 8865, 8665, 8866; *P. syringae* pv. *syringae* – UCM B–1027T. The strains were kindly provided by the Department of Phytopathogenic Bacteria of the D. K. Zabolotny Institute of Microbiology and Virology of the National Academy of Sciences of Ukraine (IMV NASU) and the Ukrainian Collection of Microorganisms (UCM). The study also used strains isolated from affected walnut trees in regions of Ukraine covering 4 climatic zones: *Agrobacterium* sp. – 1or, 3or, 8or; *Xanthomonas* sp. – 1op, 3op, 1nut, 2nut, 3nut, 4nut, 6nut, 7nut, 8nut, 11nut, 12nut, 13nut, 14nut, 15nut, 17nut, 18nut; *Pseudomonas* sp. – 4n, 5n, 5nut, 9nut, 10nut, 16nut.

To determine the fatty acid composition of the bacteria, they were converted to methyl esters through direct transesterification [14]. The fatty acid composition of cellular lipids was determined using two different protocols that varied in their hydrolysis conditions. Bacteria, regardless of the isolation method, were grown on potato dextrose agar for 24 h. The one–day culture was washed with physiological solution, then precipitated by centrifugation for 40–60 min at 1500 g. For the study, 10 mg of cells were used in terms of the dry weight of the bacteria. In the next step, fatty acid methyl esters were obtained using two different hydrolysis conditions of the cells: (1) 5% acetyl chloride in methanol at 100 °C for 4 h, and (2) 1.5% sulfuric acid in methanol at 80 °C for 1 h. In both cases, the esters were extracted using an ether–hexane mixture (1:1). The separation of methyl esters of fatty acids was carried out using a gas chromatography-mass spectrometry system (Agilent 6800N/5973 Inert). Methyl esters were identified automatically by comparison with standards based on their retention times. The fatty acid composition was determined using Agilent ChemStation software and expressed as a percentage of the total peak area. The obtained data were processed using the program Excel. The results of the research were processed statistically using the

arithmetic mean and standard error ($M \pm m$) and the credible interval for assessing the degree of probability (P) using Student's t-test. Differences were considered statistically significant at $P < 0.05$.

Results and Discussion

Cellular lipids of the collection *A. tumefaciens* and isolated strains are characterized by the presence of fatty acids with a carbon chain length of 14 to 19 atoms, namely: saturated – hexadecanoic (C16:0), octadecanoic (C18:0); unsaturated – hexa– (C16:1) and octadecenoic (C18:1); hydroxyl – 3–hydroxydecanoic (3–OH–C10:0), 3–hydroxytetradecanoic (3–OH–C14:0), 3–hydrohexadecanoic (3–OH–C16:0) and cyclopropane acids with 17 (C17:0 cyclo) and 19 (C19:0 cyclo) carbon atoms.

In the largest amounts in the cellular lipids of the *A. tumefaciens* collection and isolated strains of *Agrobacterium* sp. were present: cis-9 octadecenoic (C16:1 cis9) – from 47.58% to 79.05%. As minor components, Cis-9,10-Methylene hexadecanoic (C17:0 cyclo) and octadecanoic (C18:0) acids were detected (their content ranged from 0.5 to 1%).

A comparative analysis of the spectra revealed that the qualitative and, especially, the quantitative composition of fatty acids depends on the extraction method. In particular, it was found that the 1st method, which involves the use of a 5% solution of acetyl chloride in methanol during hydrolysis, has less effect on the content of some fatty acids compared to the 2nd method, which uses a 1.5% solution of H_2SO_4 in methanol. The greatest effect was on the amount of hydroxy acids. Thus, the content of 3–hydroxytetradecanoic (3–OH–C14:0) in the fatty acid spectra of both isolated and collection strains obtained by the 1st method is 1.4–1.7 times higher than that obtained by the 2nd method.

The amount of 3–hydroxyhexadecanoic (3–OH–C16:0) acid obtained by the 1st method is 1.9–2 times higher than that obtained by the 2nd method. The fatty acid spectra obtained by the 2nd method did not contain 3–hydrodecanoic (3–OH–C10:0) acid, which was detected in some strains under the conditions of isolation by the 1st method. Also, the amount of hexadecenoic (C16:1) acid decreases by 1.2–1.4 times and hexadecanoic (C16:0) acid by 1.3 times (collection strains) when extraction by the 2nd method. The other fatty acids detected in the spectra, regardless of the method of their isolation, remain at nearly the same levels or do not change significantly.

It should also be emphasized that the fatty acid profiles of *Agrobacterium* sp. strains collected and isolated from walnuts are similar. This fact indicates that the isolated strains are related to the representatives of the *A. tumefaciens* species.

The fatty acid spectra of isolated strains of *Xanthomonas* sp. and collection *X. arboricola* pv. *juglandis* revealed fatty acids with carbon chain lengths from 12 to 18, namely: saturated – decanoic (C10:0), undecanoic (C11:0), tetradecanoic (C14:0), pentadecanoic (C15:0), hexadecanoic (C16:0), octadecanoic (C18:0); unsaturated acids – hexa– (C16:1) and octadecenoic (C18:1); hydroxy – 3–hydroxydecanoic (3–OH–C10:0), 2–hydroxydecanoic (2–OH–C10:0),

Table 1. Fatty acid composition of cell lipids of isolated A. tumefaciens strains

Fatty acid	Isolated *A. tumefaciens* strains	
	Percentage of the total peak area	
	5% acetyl chloride in methanol	1.5% sulfuric acid in methanol
C14:0 3OH	9.28 ± 0.50	5.44 ± 1.92
C16:1 cis 9	3.41 ± 1.45	2.82 ± 0.66
C16:0	10.06 ± 4.17	7.73 ± 1.15
C16:0 3OH	3.21 ± 0.54	1.59 ± 1.82
C17:0 cyclo	0.90 ± 0.97	0.88 ± 0.38
C18:1 cis 9	65.74 ± 12.49	65.93 ± 4.61
C18:0	0.65 ± 0.24	0.58 ± 0.24
C19:0 cyclo	2.30 ± 1.32	2.33 ± 0.42

Table 2. Fatty acid composition of cell lipids of A. tumefaciens collection strains

Fatty acid	*A. tumefaciens* collection strains	
	Percentage of the total peak area	
	5% acetyl chloride in methanol	1.5% sulfuric acid in methanol
C14:0 3OH	8.17 ± 1.01	5.73 ± 0.8
C16:1 cis 9	4.45 ± 2.61	3.08 ± 0.56
C16:0	10.04 ± 0.85	8.08 ± 0.77
C16:0 3OH	2.85 ± 0.38	1.54 ± 0.4
C17:0 cyclo	0.75 ± 0.27	1.16 ± 0.72
C18:1 cis 9	65.18 ± 5.61	72.49 ± 2.93
C18:0	0.56 ± 0.14	0.66 ± 0.1
C19:0 cyclo	2.57 ± 0.76	3.47 ± 0.93

3–hydroxydodecanoic (3–OH–C12:0), 2–hydroxydodecanoic (2–OH–C12:0), 3–hydroxytetradecanoic (3–OH–C14:0), and 3–hydroxyhexadecanoic (3–OH–C16:0). In the fatty acid spectra of isolated *Xanthomonas* sp. and collection strains of *X. arboricola* pv. *juglandis*, a wide range of iso and anteiso forms of fatty acids was also detected, in particular: 13–methyl tetradecanoic (C15:0 iso), 12–methyl tetradecanoic (C15:0 anteiso), 14–methyl pentadecanoic (C16:0 iso), 15–methyl hexadecanoic (C17:0 iso), 14–methyl hexadecanoic (C17:0 anteiso). In addition, cyclopropane fatty acid with 17 carbon atoms was detected in the fatty acid spectra of cellular lipids.

Table 3. Fatty acid composition of cell lipids of isolated strains of Xanthomonas sp.

Fatty acid	Isolated strains of *Xanthomonas* sp.	
	Percentage of the total peak area	
	5% acetyl chloride in methanol	1.5% sulfuric acid in methanol
C10:0 3OH	0.32±0.04	–
C10:0	–	–
C11:0	–	–
C10:02OH	0.62 ± 0.03	–
C12:0 2OH	0.25 ± 0.01	–
C12:0 3OH	1.70 ± 10.87	–
C14:0 3 OH	0.33 ± 0.49	–
C16:0 3OH	–	–
C14:0	1.79 ± 0.31	2.05 ± 0.63
C14:0 iso	4.57 ± 6.56	3.16 ± 3.10
C15:0 anteiso	18.30 ± 34.37	12.41 ± 3.55
C15:0 iso	9.41 ± 0.54	9.30 ± 10.78
C15:0	2.17 ± 0.13	3.64 ± 1.26
C16:0 iso	2.28 ± 1.84	6.35 ± 1.35
C16:1 cis 9	5.70 ± 10.06	1.75 ± 0.45
C16:0	19.39 ± 9.52	32.65 ± 4.94
C16:1	26.54 ± 10.15	20.30 ± 1.80
C17:0 iso	0.95 ± 0.67	1.85 ± 0.45
C17:0 anteiso	0.61 ± 2.83	1.30 ± 0.90
C17:0 cyclo	0.67 ± 0.63	–
C18:1 cis 9	0.64 ± 1.8	0.99 ± 0.09
C18:1 cis 11	–	0.96 ± 0.49

Note. "–" No detected

It was found that the highest amounts of hexadecadecyl fatty acid in the cell lipids of collection *X. arboricola* pv. *juglandis* and isolated strains of *Xanthomonas* sp. contain hexadecanoic (C16:0) from 32.1% to 34.35%, hexadecenoic (C16:1) from 19,66% to 23.3%, and 12–methyl tetradecanoic (C15:0 anteiso) acids from 12.27% to 13.41% of the total peak area, which is a characteristic feature of the genus *Xanthomonas* [15].

The comparative analysis of fatty acid profiles obtained by the two methods mentioned above differs in terms of qualitative and quantitative indicators. In particular, during fatty acid isolation using the 2nd method, almost all hydroxy acids, except 3–hydroxydodecanoic acid (3–OH–C12:0), disappear compared to the first method. In the extraction of fatty acid esters by the 2nd method, the content of this acid remains either at the same level or slightly decreases compared to the first method. The second method also affects the amounts of 13–methyl tetradecanoic (C15:0 iso) and 12–methyl tetradecanoic (C15:0 anteiso) acids, reducing their quantities by 1.6–2.6 times. The amount of hexadecenoic (C16:1) acid also decreases by 1.1–1.3 times when extracted by the 2nd method.

The fatty acid profiles of *Pseudomonas* sp. strains isolated from walnut and the type strain *P. syringae* pv. *syringae* UCM B–1027T revealed fatty acids with carbon chain lengths from C10 to C19, including unsaturated – hexa– (C16:1) and octadecenoic (C18:1); saturated – decanoic (C10:0), dodecanoic (C12:0), tetradecanoic (C14:0), hexadecanoic (C16:0), and octadecanoic (C18:0) acids; hydroxy – 3–hydroxydecanoic (3–OH–C10:0), 2–hydroxydodecanoic (2–OH–C12:0), 3–hydroxydodecanoic (3–OH–C12:0); and cyclopropane acids with 17 and 19 carbon atoms.

The highest amounts in cell lipids of *P. syringae* pv. *syringae* UCM B–1027T and isolated strains of *Pseudomonas* sp. were: cis–9 hexadecenoic (C16:1 cis9) – from 34.23% to 35.4%; cis–9 octadecenoic acid (C18:1 cis9) – from 22.5% to 23.35%; and trans–hexadecenoic acid (C16:1 trans) – from 29.68% to 30.54%.

According to the literature, the most important factor for the taxonomy of *P. syringae* bacteria is the presence of hydroxyl fatty acids. 3–hydroxydecanoic (3–OH–C10:0), 2–hydroxydodecanoic (2–OH–C12:0), and 3–hydroxydodecanoic (3–OH–C12:0) were detected in the fatty acid profiles. These fatty acids were present at levels ranging from 0.97 to 1.22%.

Table 4. Fatty acid composition of cell lipids of collection strains of Xanthomonas arboricola pv. juglandis

Fatty acid	Collection strains of *Xanthomonas arboricola* pv. *juglandis*	
	Percentage of the total peak area	
	5% acetyl chloride in methanol	1.5% sulfuric acid in methanol
C10:0 3OH	0.44 ± 0.81	–
C10:0	1.46 ± 10.56	–
C11:0	0.61 ± 3.10	–
C10:02OH	0.76 ± 1.62	–
C12:0 2OH	0.46 ± 0.27	–
C12:0 3OH	1.56 ± 1.74	–
C14:0 3 OH	0.35 ± 0.23	–
C16:0 3OH	3.24 ± 5.35	–
C14:0	1.42 ± 0.83	1.87 ± 0.73
C14:0 iso	5.72 ± 3.27	3.22 ± 0.79
C15:0 anteiso	12.48 ± 18.03	12.52 ± 1.14
C15:0 iso	5.99 ± 10.14	9.58 ± 2.94
C15:0	3.44 ± 4.54	3.38 ± 0.51
C16:0 iso	3.99 ± 5.79	6.89 ± 1.42
C16:1 cis 9	2.90 ± 1.75	1.80 ± 0.53
C16:0	18.24 ± 1.8	32.12 ± 2.52
C16:1	32.19 ± 14.7	20.49 ± 3.99
C17:0 iso	1.20 ± 1.47	1.81 ± 1.62
C17:0 anteiso	0.84 ± 0.73	1.15 ± 1.40
C17:0 cyclo	0.60 ± 0.4	–
C18:1 cis 9	0.54 ± 0.76	1.10 ± 2.41
C18:1 cis 11	–	1.15 ± 1.56

Note. "–" No detected

Our results on the fatty acid composition of cellular lipids from isolated *P. syringae* are consistent with the literature. For D. Stead, the distribution of certain hydroxyl fatty acids was one of the key factors used to classify species of the genus *Pseudomonas.* According to this characteristic, *P. syringae* pathovars are classified as group 1, subgroup 1a [16].

The characterization of the fatty acid composition of cell lipids allowed us to establish the quantitative and qualitative affinity of the isolated strains

Table 5. Fatty acid composition of cell lipids of isolated strains of Pseudomonas sp.

Fatty acid	Isolated strains of *Pseudomonas* sp.	
	Percentage of the total peak area	
	5% acetyl chloride in methanol	1.5% sulfuric acid in methanol
C10:0 3OH	1.02 ± 0.31	0.14 ± 0.09
C12:0	5.07 ± 0.76	3.05 ± 0.04
C12:0 2OH	1.10 ± 0.18	1.09 ± 0.13
C12:0 3OH	1.16 ± 0.31	0.47 ± 0.31
C14:0	0.79 ± 0.76	0.57 ± 0.09
C16:1 cis 9	35.14 ± 5.03	33.68 ± 2.96
C16:1 trans	30.04 ± 0.54	29.00 ± 0.13
C18:1 cis 9	22.90 ± 1.03	22.63 ± 0.40
C18:0	1.15 ± 0.54	1.06 ± 0.36
C19:0 cyclo	0.41 ± 0.18	0.45 ± 0.45

of *Pseudomonas* sp. from walnut and the type strain of *P. syringae* pv. *syringae* – UCM B–1027T.

Thus, when isolating fatty acid esters using the second method, compared to the first, the content of 3–hydroxydecanoic (3–OH–C10:0) acid decreases by 8.5 times; 3–hydroxydodecanoic (3–OH–C12:0) acid by 2.3 times; and 2–hydroxydodecanoic (2–OH–C12:0) acid by 1.1 times. It should be noted that the amounts of individual saturated and unsaturated fatty acids also decrease. The other fatty acids detected in the spectra of *Pseudomonas* species were not affected by the extraction method and remain at almost the same levels or do not change significantly.

Fatty acids are a type constituent of almost all bacterial species. When determining the fatty acid composition of microorganisms, the cultivation conditions of strains before fatty acid extraction should be standardized as much as possible. In some cases, there may be exceptions when it is impossible to grow organisms under the same conditions, and these should be carefully documented. The fatty acid report includes all components that amount to 1% or more of the total peak area. In cases where major peaks are not identified, they will not be included in the peak name table. In such cases, it is necessary to report their presence and indicate the equivalent chain length. This will allow any future structure determination work to be linked to the equivalent chain

Table 6. Fatty acid composition of cell lipids of P. syringae pv. syringae collection strains

Fatty acid	*P. syringae* pv. *syringae* collection strains	
	Percentage of the total peak area	
	5% acetyl chloride in methanol	1.5% sulfuric acid in methanol
C10:0 3OH	1.02 ± 0.15	0.12 ± 0.03
C12:0	5.25 ± 0.79	3.07 ± 0.42
C12:0 2OH	1.25 ± 0.19	1.09 ± 0.16
C12:0 3OH	1.12 ± 0.17	0.49 ± 0.03
C14:0	0.70 ± 0.11	0.49 ± 0.05
C16:1 cis 9	35.79 ± 5.37	35.44 ± 4.96
C16:1 trans	30.85 ± 4.61	29.70 ± 4.08
C18:1 cis 9	23.59 ± 3.51	22.68 ± 3.11
C18:0	1.12 ± 0.12	1.03 ± 0.15
C19:0 cyclo	0.40 ± 0.12	0.50 ± 0.14

length given in publications. When comparing the structures of fatty acids, it should be remembered that they usually do not have significant variations within the same taxonomic group. Therefore, one should be cautious about reports of branched–chain fatty acids in a group that otherwise synthesizes straight–chain fatty acids and unsaturated fatty acids, etc. Similarly, the presence or absence of hydroxy acids is usually a characteristic feature, and their unexpected presence or absence should also be treated with caution.

Several authors have shown that the content of fatty acids in the bacterial cell membrane can change in response to changes in environmental conditions, such as temperature, pH, time of cultivation, or the presence of certain compounds in the culture medium [17, 18].

The composition of fatty acids is now believed to affect the fluidity, plasticity, and permeability of the cell membrane. Zhang and Rock commented on this phenomenon in detail and demonstrated that saturated straight–chain fatty acids create a bilayer membrane with high stiffness and low permeability. In addition, it was found that the presence of cis–unsaturated fatty acids leads to higher cell membrane permeability [19].

Previous studies have demonstrated that H_2SO_4–methanol is a highly effective reagent for methylation, but it should be noted that this reagent also has the same disadvantages as other acid catalysts by changing the isomeric distribution of conjugated fatty acids. Additionally, improper use of the reagent can result in the destruction of polyunsaturated fatty acids. Among the most commonly mentioned are the rearrangement of cyclopropene and cyclopropane fatty acids and the dehydration of hydroxylated and conjugated dienoic fatty acids [7].

Interestingly, H_2SO_4–methanol is a relatively mild acid hydrolysis method. Nevertheless, there are cases in the literature when the use of acid catalysts, such as H_2SO_4, at high concentrations or high temperatures, led to the formation of certain compounds that are fatty acid derivatives [15].

An alternative method is the use of acetyl chloride in the hydrolysis step, which has several advantages, namely, lower cost, longer shelf life (no need for refrigeration), and complete removal of the catalyst at the final extraction step.

Alkaline–catalyzed transmethylation has been reported to contribute to higher extraction yields only for short–chain fatty acids. In addition, it cannot be used with samples that contain large amounts of free fatty acids.

It is known that species of *P. syringae* pv. *syringae* are characterized by the presence of 3–hydroxydecanoic (3–OH–C10:0), 2–hydroxydodecanoic (2–OH–C12:0), 3–hydroxydodecanoic (3–OH–C12:0) acids. D. E. Stead established a pattern for all phytopathogens belonging to the *P. syringae* species (2–OH–C12:0 less than 3% of the total peak area, C16:1–C18:1 more than 52%, C16:0 to C16:1 ratio less than 0.9), which is reliable for all fatty acid profiles of the isolated *Pseudomonas* sp. strains [20, 21]. It is also known that in the identification and study of fatty acid profiles of bacteria of the genus *Pseudomonas* sp., there is a characteristic difference between the strains, which suggests that the pathogenicity of primary plant pathogens is not only a phenotypic factor, but also reflected in bacterial membranes, where more fatty acids are contained.

According to the literature, 99% of the studied strains of bacteria of the genus *Xanthomonas* contain a large amount of iso and anteiso fatty acids in their profiles. This feature is characteristic of all bacteria of this genus [22]. In particular, the content of C15:0 iso and C15:0 anteiso, C17:0 iso and C15:0 anteiso in the fatty acid spectra is a characteristic feature of many representatives of the genus *Xanthomonas*. In particular, according to the cluster analysis, bacteria of the species *X. albilineans*, *X. axonopodis*, *X. fra-*

gariae, *X. maltophilia*, and *X. populi* are related by their cell lipid fatty acid profiles. Several researchers reported that fatty acids C10:0, 2–OH–C10:0, 3–OH–C11:0, C12:0, C13:0 iso, 2–OH–C13:0, C17:1, and 3–OH–C17:0 accounted for less than 1.22% of the total sum peak area or were absent in all strains, which is consistent with our results.

Based on the literature, representatives of the genus *Agrobacterium* are characterized by the presence of the following fatty acids in their profiles: hexadecanoic (C16:0), 3–hydroxyhexadecanoic (3–OH–C16:0), and cyclopropane fatty acid with 19 carbon atoms (C19:0 cyclo). In addition, the presence of cyclopropane fatty acid with 17 carbon atoms (C17:0 cyclo) in cell lipids may indicate similarity to representatives of biovar I of this species. [23]

Conclusions. Thus, according to the similarity of the fatty acid composition of cellular lipids, the strains isolated from the affected walnut tissue are related to representative strains of *A. tumefaciens*, *X. arboricola* and *P. syringae*. Among the methods used in this study, method 1, using a 5% solution of acetyl chloride in methanol at the hydrolysis stage, had the least effect on the quantitative and qualitative indicators of the fatty acid composition of cellular lipids. It is also important to note that a 1.5% solution of H_2SO_4 in methanol used in the second method has a greater effect on both the qualitative and quantitative fatty acid composition of cell lipids of all studied strains of all phytopathogenic bacteria.

Conflict of interest. The authors have completed the Unified Conflicts of Interest form at http://ukrbiochemjournal.org/wp-content/uploads/2018/12/coi_disclosure.pdf and declare no conflict of interest.

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СКЛАД ЖИРНИХ КИСЛОТ КЛІТИННИХ ЛІПІДІВ У БАКТЕРІЯХ ПАТОГЕНІВ ГОРІХА ВОЛОСЬКОГО

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Волоський горіх (*Juglans regia*) є найбільш економічно важливою і поширеною горіховою культурою в Україні. Оскільки бактеріальні захворювання горіха можуть знизити врожайність цієї культури на 40%, моніторинг патогенів у даній культурі та їх ідентифікація є надзвичайно важливими. Склад жирних кислот клітинних ліпідів використовується в таксономії фітопатогенних бактерій. Метою цього дослідження було визначення складу жирних кислот клітинних ліпідів штамів *Agrobacterium*, *Xanthomonas* і *Pseudomonas*, які можуть інфікувати горіх, а також тих, що були виділені з уражених горіхових дерев у різних регіонах України. Метилові ефіри жирних кислот отримували двома різними методами екстракції: з використанням 5% ацетилхлориду в метанолі при 100°C протягом 4 год або 1,5% сірчаної кислоти в метанолі при 80°C протягом 1 год. Метилові ефіри жирних кислот аналізували за допомогою газової хромато-мас-спектрометрії. Відповідно до виявленої подібності жирнокислотного складу штама, ізольовані з ураженого волоського горіха, були споріднені з репрезентативними колекційними штамами *A. tumefaciens*, *X. arboricola* та *P. syringae*. Слід зазначити, що під час виділення жирних кислот із використанням 1,5% розчину H_2SO_4 у метанолі кількість окремих насичених і ненасичених жирних кислот у досліджуваних штамах зменшувалася, а майже всі гідроксильні кислоти, ідентифіковані як ключові таксономічні маркери, зникали порівняно з використанням 5% розчину ацетилхлориду в метанолі на стадії гідролізу.

Ключові слова: жирні кислоти, виділення, склад, фітопатогенні бактерії, *Agrobacterium*, *Xanthomonas*, *Pseudomonas*, волоський горіх.

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