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Influence of lipo-polysaccharides of Pseudomonas syringae pv. atrofaciens on photosynthetic apparatus of wheat

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The purpose. Study influence of lipo-polysaccharides (LPS) of phytopathogenic bacteria *Pseudomonas syringae pv. atrofaciens (McCulloch* 1920) *Young, Dye and Wilkie* 1978 on physiological-biochemical processes in plants of wheat. **Methods.** Classical microbiologic, biochemical, statistical. Extraction of LPS is realized by 0,85% solution of chloride of sodium. Induction of fluorescence was measured by device «Floratest». **Results.** Stimulating influence of treatment with LPS of various strains of P. *syringae* pv. *atrofaciens* on pigment compound of leaves of plants of summer wheat of grade Pecherianka is determined. Essential lowering background fluorescence in leaves of summer wheat of grade Pecherianka is fixed. At treating with LPS of *P. syringae* pv. *atrofaciens 9417* and *P. syringae* pv. *atrofaciens 9780 — on 38,7* and *37,4%* accordingly, at treating with LPS of *P. syringae* pv. *atrofaciens* UKM V-1011 — on 16,6%, whereas in alternative with treating with LPS of *P. syringae* pv*. atrofaciens* 9400 background fluorescence authentically did not differ from the control. Lipo-polysaccharides of *P. syringae* pv. *atrofaciens* 9417 and LPS of *P. syringae* pv. *atrofaciens* 9780 oppressed dark phase of photosynthesis. **Conclusions.** Lipo-polysaccharides of *P. syringae* pv. atrofaciens cause increase of amount of photosynthetic pigments — chlorophylls *a* and *b* at simultaneous lowering in content of carotenoids (for LPS of *P. syringae* pv. *atrofaciens* 9417 and LPS of *P. syringae* pv. *atrofaciens* 9780) which is accompanied by lowering of the functional activity of photosynthetic apparatus. That spread not only to «light» phase of photosynthesis, but also on efficiency of «dark» responses of cycle of Calvin, reducing their efficiency.

Key words: *phytopathogenic bacteria, exometabolites, induction, photosynthetic pigments, fluorescence, peroxidase, catalase, photosystem.*

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Phytopathogenic gram-negative bacteria are characterized by the presence of highly active biopolymer in the outer membrane of the cell wall – lipopolysaccharide (LPS). Last one, due to its surface location, plays a significant role in the interaction of prokaryotes with macroorganisms. In particular, after phytopathogenic bacteria have entered the plant tissues LPS induces the synthesis of some bioactive mediators: the products of defense genes and antimicrobial metabolites, and causes the changes of normal physiological and biochemical processes in plant cells [1]. Treatment the plants with the LPS of *Burkholderia cepacia*, *Pseudomonas aeruginosa* and *Pectobacterium carotovorum* activates hormone-dependent NO synthase and induces the production of nitric oxide and reactive oxygen species (ROS) [2, 3], *Ralstonia solanacearum* LPS activates the soluble peroxidase, the LPS of *P. syringae* pv. *atrofaciens* 9400 and *P. syringae* pv. *atrofaciens* 9417 cause the increase of peroxidase activity in *Allium cepa* seedlings [5].

It is known that one of the first stages of the plant response to biotic stress is the formation of ROS, in particular hydrogen peroxide and superoxide anion radical [6]. The induction of oxidative stress and the synthesis of ROS occurs both due to the influence of intact bacteria cells on plants and their individual components, for example, LPS [6]. ROS have been found to affect the genetic material of organisms, for

instance, the significant increase of these compounds content can provoke DNA damage. The mutagenic activity of LPS for mammalian cells is thought to be mediated by ROS [7]. We have previously found that the LPS of the causal agent of wheat basal bacteriosis is characterized by genomodulating activity in *A. cepa*-test that may be mediated by ROS formation [5].

Thus, LPSs play an important role in pathogenesis and are able to induce protective responses in plants. However, the endotoxins interaction with macroorganisms is complex and has not been extensively studied.

Aim. The aim of the work was to study the effect of the LPS of the causal agent of wheat basal bacteriosis *Pseudomonas syringae* pv. *atrofaciens* strains (McCulloch 1920) Young, Dye & Wilkie 1978 on the physiological and biochemical processes in the host plant cells.

Research methodology. The lipopolysaccharides of *P. syringae* pv *atrofaciens* strains were investigated. These strains were isolated during long-term monitoring of the pathogen in cereal crops and are stored in the collection of the Department of Phytopathogenic Bacteria of D.K. Zabolotny Institute of Microbiology and Virology of NASU (Table 1).

1. Characteristics of *P. syringae* pv. *atrofaciens* strains used in the study

Strain	Isolation source	Aggressiveness towards wheat	Serological group
UCM B-1011	patotype strain	4	IV
9400	wheat, variety “Rannia 93”, Kyiv region	4	II
9417	wheat, variety “Rannia 93”, Kyiv region	0	IV
9780	wheat, variety “Podolianka”, Poltava region	1	II

LPS of *P. syringae* pv. *atrofaciens* was obtained by extraction with 0.85% sodium chloride solution, purified by ultracentrifugation and lyophilized [8]. For research LPS solutions were prepared at the concentration of 5 mg / ml in sterile tap water.

The seeds of the spring wheat variety “Pecherianka” were used in the work. Experiments repeated three times, in each experiment used at least 30 seeds. The seeds were first rinsed in running water and then in sterile water and spread on sterile filter paper in Petri dishes (10 seeds per dish). To every dish with the seeds 5 ml of LPS solutions were applied. Germination was performed at 21 °C. The dishes with the seeds were moistened with sterile water periodically. The measurement of the physiological and biochemical parameters of wheat sprouts was made on the 7^{th}-8^{th} days from the beginning of the experiment [8].

Peroxidase activity in wheat plants was determined according to the method of Boyarkin and expressed as conventional units per mg of wet tissue weight. The activity of catalase was determined using the titrimetric method and expressed as ml of O_2 * g^{-1} * min^{-1} [9]. The pigment composition of the leaves was determined in plants by the extraction with DMSO and subsequent spectrometry on the 7^{th} day after treatment the seeds with LPS. The LPS effect on the condition and activity of the photosynthetic apparatus of the spring wheat plants variety “Pecherianka” was assayed using the biophysical method of chlorophyll fluorescence induction (CFI) measurement. The data were determined with portable device of domestic production «Floratest» with certain parameters [8, 10]. CFI was measured on the 5^{th} day after treatment the

seeds with LPS. The received digital data array was presented in graphic form. The corresponding critical parameters of the CFI were calculated, which is a reflection of changes in the functional units of the photosynthetic system [11, 12]. Determined parameters: ground fluorescence (F_0); number of Q_B non-renewable complexes not involved in linear electron transport: $K_{pl} = (F_{pl} - F_0)/(F_m - F_0)$; the photochemical efficiency of photosystem (PS) II: $F_1 = (F_m - F_0)/F_m$; fluorescence quenching: $qF = (Fm - Ft)/Ft$; the parameter reflected the activity of ribulose bisphosphate carboxylase (the major enzyme of the Calvin cycle): $K_i = (F_m - F_i)/F_m$ [10]. The calculations were performed using Microsoft Excel spreadsheets. Data were statistically processed using Statistica 8.0.

Research results. Lipopolysaccharide (LPS) of phytopathogenic bacteria has a recognized status as pathogenicity factor [1, 4]. However, a comprehensive data analysis of the effect of these biopolymers on plants proves that they can exhibit both toxic activity and be the growth promoters for plants [13, 14]. LPS effect on plant cells depends on both the properties of LPS (origin, method of isolation, concentration) and the properties of a plant (developmental phase, variety). Therefore, the studies relating to the effects of LPS of phytopathogenic bacteria on plants are still relevant.

During the study of the influence of the LPS of causal agent of spring wheat basal bacteriosis on the plants variety “Pecherianka”, a significant stimulating effect on the pigment content in the wheat leaves after treatment with LPS of different *P. syringae* pv. *atrofaciens* strains was found. The most significant increase in the content of chlorophyll *a* and chlorophyll *b* (by 70.0 and 72.7% respectively) was observed in the case of the action of *P. syringae* pv. *atrofaciens* UCM B-1011 LPS (Table 2).

2. The influence of the LPS of P. syringae pv. atrofaciens on the pigment content in the leaves of spring wheat variety “Pecherianka”

LPS of *P. syringae* pv. *atrofaciens* (strain)	Pigments							
	Chlorophyll *a*, mg/g	% of control	Chlorophyll *b*, mg/g	% of control	Chlorophyll *a*+*b*, mg/g	% of control	Carotenoids, mg/g	% of control
Control	0,20±0,01	100,0	0,11±0,01	100,0	0,31±0,01	100,0	0,07±0,003	100,0
UCM B-1011	0,34±0,02*	170,0	0,19±0,01*	172,7	0,53±0,01*	170,9	0,07±0,003	100,0
9400	0,22±0,01*	110,0	0,12±0,01	109,1	0,34±0,01	109,7	0,07±0,003	100,0
9417	0,22±0,01*	110,0	0,12±0,01	109,1	0,34±0,01	109,7	0,06±0,003*	85,7
9780	0,23±0,01*	115,0	0,13±0,01	118,2	0,36±0,01*	116,1	0,05±0,002*	71,4

* – statistically significant differences from control at $p < 0.05$

The content of chlorophyll *a* in the leaves of the spring wheat variety “Pecherianka” in the case of the action of *P. syringae* pv. *atrofaciens* 9400 LPS and *P. syringae* pv. *atrofaciens* 9417 LPS increased by 10.0%, and under the action of *P. syringae* pv. *atrofaciens* 9780 LPS – by 15.0% (Table 2). In the leaves of the sprouts of the spring wheat variety “Pecherianka” in the case of the action of *P. syringae* pv. *atrofaciens* 9400 LPS and *P. syringae* pv. *atrofaciens* 9417 LPS the concentration of chlorophyll *b* increased slightly – by 9.1%, while by the action of *P. syringae* pv. *atrofaciens* 9780 LPS the increase of chlorophyll *b* concentration was more significant – by 18.2%. The content of carotenoids (which is known to be a

protective pigment) in the leaves of the spring wheat sprouts variety “Pecherianka” when grown in *P. syringae* pv. *atrofaciens* UCM B-1011 LPS and *P. syringae* pv. *atrofaciens* 9400 LPS solutions remained at the level of control plants, whereas under the use of *P. syringae* pv. *atrofaciens* 9417 LPS decreased by 14.3%, and in the variant with the use of *P. syringae* pv. *atrofaciens* 9780 LPS decreased more significantly – by 28.6% (Table 2).

It is known that the antioxidant system, in particular, its components – catalase and peroxidase, is one of the mechanisms of systemic phytoresistance. These enzymes maintain plant phytoimmunity due to their ability to deactivate free radicals and their derivatives that destroy plant cells [6].

Under the action of *P. syringae* pv. *atrofaciens* UCM B-1011 LPS the catalase activity in the leaves of the spring wheat sprouts of the variety “Pecherianka” decreased by 22.9%, whereas in the case of *P. syringae* pv. *atrofaciens* 9417 LPS, *P. syringae* pv. *atrofaciens* 9780 LPS and *P. syringae* pv. *atrofaciens* 9400 LPS the catalase activity was not significantly different from the control (Table 3).

Table 3. The influence of the LPS of *P. syringae* pv. *atrofaciens* on the oxidoreductase activity in the plants of the spring wheat variety “Pecherianka”

LPS of *P. syringae* pv. *atrofaciens* (strain)	Catalase activity		Peroxidase activity	
	ml of O_2 * g^{-1} * min^{-1}	% of control	$\Delta D_{670} g^{-1} \cdot sec^{-1}$	% of control
Control (water)	189,6±9,4	100,0	4,45±0,22	100,0
UCM B-1011	146,2±7,3*	77,1	3,88±0,19*	87,2
9400	174,3±8,7	94,5	4,57±0,22	102,6
9417	184,5±9,2	97,3	4,67±0,23	104,9
9780	191,5±9,5	101,0	4,64±0,23	104,3

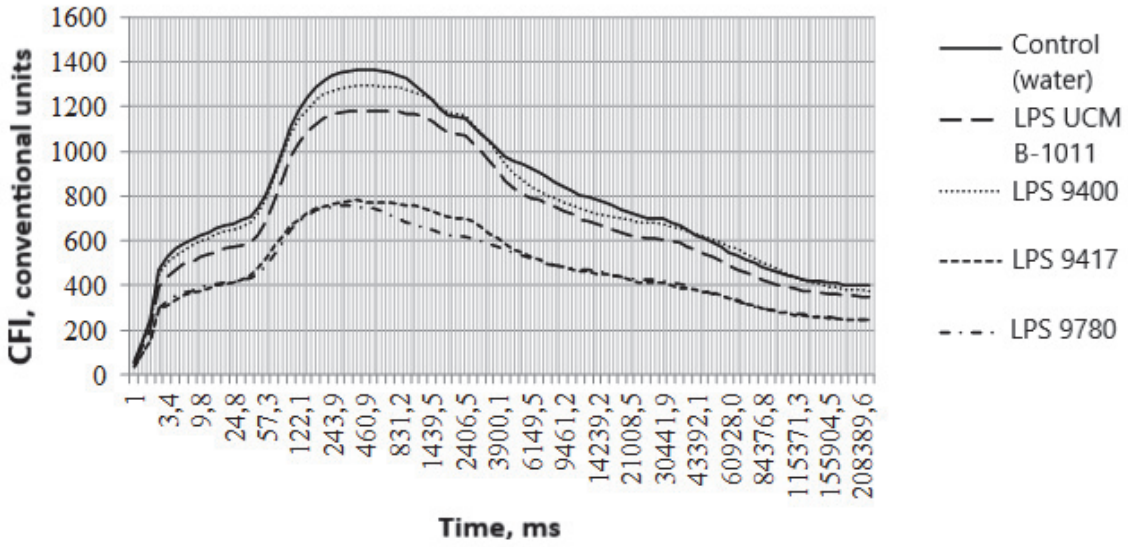
* – statistically significant differences from control at $p < 0.05$

The peroxidase activity in the leaves of sprouts of the spring wheat variety “Pecherianka” decreased with *P. syringae* pv. *atrofaciens* UCM B-1011 LPS solution and did not significantly differ from the control by the action of the LPS of *P. syringae* pv. *atrofaciens* strains 9417, 9780, and 9400 (Table 3).

It should be noted that during investigation of the effect of *P. syringae* pv. *atrofaciens* LPS on the onion plants of variety “Chalcedon” we found no reliable effect of the LPS on the catalase activity, while the peroxidase activity increased [5].

The next stage of the research was to determine the photochemical activity of the sprouts of the spring wheat variety “Pecherianka” by the biophysical method of measurement of chlorophyll fluorescence induction.

It is known that fluorescence is mainly emitted by chlorophyll *a*, located in Photosystem II (PSII). The change in its fluorescence reflects the changes in the redox state of the reaction centers (RCs) of this photosystem [15, 16, 17]. The obtained Kautsky induction curves are shown in Figure.



Chlorophyll fluorescence changes in the plant leaves of spring wheat variety "Pecherianka" induced by *P. syringae* pv. *atrofaciens* LPS

For the detailed analysis of the influence of *P. syringae* pv. *atrofaciens* LPS on the plant photosynthetic apparatus the critical parameters of these curves, which reflect the PS II functional state, were calculated.

It is known that the indicator such as ground fluorescence (F_0) is characterized by the emission of photons at the beginning of the fast fluorescence phase, when all the reaction centers of the chlorophyll molecules are opened and the absorbed energy migrates through the pigment matrix. Usually, such fluorescence is minimal – about 3%, and its growth indicates a breakage of the bonds between chlorophyll molecules, their degradation or the synthesis of new molecules [11]. Usually, the increase in F_0 is associated with the action of any stress factors – increase in temperature, nutritional deficiency, the action of phytopathogens, etc., and thus the fraction of absorbed excitation energy decreases [11, 12, 18]. We found a significant decrease in ground fluorescence in leaves of the wheat variety "Pecherianka", which occurred in the case of treatment with *P. syringae* pv. *atrofaciens* 9417 LPS and *P. syringae* pv. *atrofaciens* 9780 LPS – by 38.7% and 37.4%, respectively, under the action of *P. syringae* pv. *atrofaciens* UCM B-1011 LPS – by 16.6%, whereas in the case of *P. syringae* pv. *atrofaciens* 9400 LPS ground fluorescence was not significantly different from the control (Table 4). This decrease was correlated with the total content of chlorophyll increase in the leaves (Table 2).

4. The terminal parameters of chlorophyll fluorescence induction in the plants of the spring wheat variety "Pecherianka" underthe action of *P. syringae* pv. *atrofaciens* LPS

LPS of *P. syringae* pv. *atrofaciens* (strain)	Chlorophyll fluorescence parameters				
	F_0	$K_1=Fv/F_0$	K_{pl}	*Ki*	*qF*
Control (water)	470±23,5	0,64±0,03	0,220±0,01	0,77±0,03	2,44±0,12
UCM B-1011	392±19,6	0,65±0,03	0,213±0,01	0,77±0,03	2,39±0,12
9400	448±22,4	0,65±0,03	0,215±0,01	0,81±0,04*	2,45±0,12
9417	288±14,4	0,63±0,03	0,225±0,01	0,69±0,03	2,16±0,11
9780	294±14,7	0,60±0,03	0,239±0,01	0,74±0,04	2,09±0,10

* – statistically significant differences from control at $p < 0,1$

Another calculated index is rate coefficient K_1 that reflects the potential efficiency of PS II photochemistry and enrichment of leaves with photochemically active reaction centers, and represent the efficiency of light energy storage at the initial stages of photosynthesis. K_1 was equal to the control of the sprouts of the spring wheat variety "Pecherianka" under the action of *P. syringae* pv. *atrofaciens* UCM B-1011 LPS and *P. syringae* pv. *atrofaciens* 9400 LPS. In the case with the treatment of *P. syringae* pv. *atrofaciens* 9417 LPS this index decreased slightly and in the variant with the treatment of *P. syringae* pv. *atrofaciens* 9780 LPS it decreased significantly. In the last case the significant inhibition of carotenoid content was also observed (Table 2). Such decrease of K_1 indicates the destructive effect of the investigated LPS of *P. syringae* pv. *atrofaciens* 9417 and *P. syringae* pv. *atrofaciens* 9780 on the functional activity of primary photosynthesis processes in wheat leaves. Therefore, although in the case of the action of *P. syringae* pv. *atrofaciens* 9417 LPS and *P. syringae* pv. *atrofaciens* 9780 LPS the content of the chlorophyll in leaves increased, but its functional activity was inhibited.

According to K_{pl}, which growth indicates the slowdown in the linear transport of electrons to the PS II reaction centers (this is an indicator of stress), we found the most significant increase in its value due to the action of *P. syringae* pv. *atrofaciens* 9780 LPS, whereas the use of *P. syringae* pv. *atrofaciens* 9400 LPS and *P. syringae* pv. *atrofaciens* 9417 LPS did not affect this index (Table 4).

The coefficient induction value, that correlates with the activity of the ribulose bisphosphate carboxylase (key Calvin cycle enzyme) and indirectly corresponds to the efficiency of the dark phase of carbon fixation, increased by 5.2% only in the case of the action of *P. syringae* pv. *atrofaciens* 9400 LPS. In the variant of the action of *P. syringae* pv. *atrofaciens* UCM B-1011 LPS, *P. syringae* pv. *atrofaciens* 9780 LPS and *P. syringae* pv. *atrofaciens* 9417 LPS the value of the induction coefficient (Ki) was at the level of control (table 4).

The value of the fluorescence quenching parameter (*qF*) in the case of using *P. syringae* pv. *atrofaciens* UCM B-1011 LPS and *P. syringae* pv. *atrofaciens* 9400 LPS reached the control level (Table 4). This parameter was reducedunder the action of *P. syringae* pv. *atrofaciens* 9417 LPS by 11.5% and *P. syringae* pv. *atrofaciens* 9780 LPS – by 14.3%. It should be noted that the changes in the fluorescence quenching parameter reflect the changes in the redox state of the electron transport chain components and the primary electron carrier Q_A. The increase in *qF* relates to the activation of reactions that occur with the use of ATP and $NADPH_2$. This accelerates the oxidation of the electron transport chain components, the electron outflow

from Q_A and their involvement in the Calvin cycle reactions [11, 12]. According to the obtained data *P. syringae* pv. *atrofaciens* 9417 LPS and *P. syringae* pv. *atrofaciens* 9780 LPS suppressed these processes, slowing down the dark phase of photosynthesis.

Summarizing, it can be said that under the action of LPS of all the strains of *P. syringae* pv. *atrofaciens* the chlorophylls *a* + *b* content increased in the leaves of the 7-day-old plants of the wheat variety “Pecherianka”, which was accompanied by the decrease in the functional activity of the photosynthetic apparatus. This decreased the effectiveness not only of the “light” phase of photosynthesis, but also of their "dark" reactions of the Calvin cycle.

Conclusions

Therefore, the investigated LPS of *P. syringae* pv. *atrofaciens* caused the increase of the photosynthetic pigments content (chlorophylls *a* and *b*) while the content of carotenoids reduced (in the case of the treatment the wheat seeds with *P. syringae* pv. *atrofaciens* 9417 LPS and *P. syringae* pv. *atrofaciens* 9780 LPS). The tendency to slow down both “dark” and “light” photosynthesis phases was also observed. It should be noted that the rate of the inhibitory effect of *P. syringae* pv. *atrofaciens* LPS on the photosynthesis processes depended on the strain from which the biopolymer was isolated.

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