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ABSCISIC ACID AND ETHYLENE PRODUCTION BY BIOTECHNOLOGICAL STRAINS OF *Bradyrhizobium japonicum*

N. O. Leonova

Zabolotny Institute of Microbiology and Virology
of the National Academy of Sciences of Ukraine, Kyiv

E-mail: natikleo@online.ua

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The aim of the work was to research production of phytohormones that inhibit the plant growth and development — abscisic acid and ethylene by various nitrogen–fixing symbiotic efficiency biotechnological strains of *Bradyrhizobium japonicum* under in vitro conditions. The amounts of abscisic acid were determined by SDTLC-chromatography of high resolution and that of ethylene — by gas chromatography.

It was shown that symbiotic nitrogen–fixing bacteria of soybean were able to synthesize abscisic acid (56.5–72.0 μg/g of absolutely dry biomass) and ethylene (0.046–3.461 nmol/h·g of absolutely dry biomass). It was revealed that the differences in amounts of abscisic acid and ethylene in *B. japonicum* were strains properties and they were not related directly to conditions of cultivation, nitrogenase activity of the bacteria and their effectiveness in symbiosis with soybean plants. The ability of biotechnological strains of *B. japonicum* to synthesize the phytohormones inhibiting plant growth and development under *in vitro* conditions may be an additional factor that increases virulence of the bacteria and determines their potential activity under infection of plants. The obtained data are important for development of the technology of microbiological preparations–legume inoculants with combined properties.

Key words: *Bradyrhizobium japonicum*, abscisic acid, ethylene, symbiosis, soybean.

Due to the environmental impact caused by people, which threatens increasingly agriculture of Ukraine, there is a need to restore natural ecosystems, to maintain their biological diversity and to protect them from destruction. Therefore, it is important to create and apply modern biotechnologies based on the use of environmentally safe microbiological preparations of new generation with combined properties of means for increasing crop yields and their resistance to extreme environmental factors. In recent years efforts of scientists were focused on development of complex bacterial preparations (inoculants) based on living nitrogen-fixing soil microorganisms, which include phytohormones, vitamins, amino acids and other physiologically active substances.

At establishing symbiotic interactions between nodule bacteria and leguminous plants the highly specialized structures — nodules are formed which develop on host plants roots, and in which the nitrogen fixation takes place directly [1]. Mechanism of nodules formation (or nodulation) is highly specific. It includes cascade of chemical reactions with signaling molecules of different chemical nature. Among them the phytohormones of both the plants and bacterial origin are important (auxins, cytokinins, gibberellins, abscisic acid and ethylene).

The ability to production phytohormones is inherent in a wide range of soil microorganisms. Among them there are the symbiotic nitrogen–fixing bacteria (rhizobia, from families *Rhizobiaceae* and *Bradyrhizobiaceae*) [2–4]. It is known that the phytohormones synthesized by rhizobia, take part in regulation of such processes of symbiosis as: rhizogenes stimulation; increase of roots surface area; enhancement of exometabolites exchange efficiency in the plant–soil–organisms system; stimulation of plant roots cell proliferation (nodulation); control of nodules number and they mass; increase nitrogenase activity of bacteroides; enhancement of plant resistance to environmental stress factors [5–7].

Previously we have researched biosynthesis of the phytohormones auxins and cytokinins by free–living bacteria *Azotobacter chroococcum* and symbiotic strains of *Bradyrhizobium japonicum* with various effectiveness rates [8, 9]. However, the ability of these

microorganisms to production phytohormonal compounds that inhibit the of plant growth and development, which include abscisic acid (ABA) and gaseous hormone ethylene, and their role in symbiosis functioning of remain researched insufficiently. Such studies are essential for development of the microbiological preparations–legume inoculants technology with combined properties, because in the composition of these preparations should be the optimal ratio of living cultures of nitrogen–fixing bacteria and their metabolites for efficient formation and functioning of symbiosis.

In this connection, the aim of the work was to research the production of phytohormones that inhibit the plants growth and development — ABA and ethylene by various nitrogen–fixing symbiotic efficiency biotechnological strains of *B. japonicum* under the conditions *in vitro*.

Materials and Methods

The objects of research were the nodule soybean bacteria strains with different nitrogen fixation activity under symbiosis conditions: highly effective biotechnological strains of *B. japonicum* UCM B-6018, UCM B-6023, UCM B-6035 and UCM B-6036, which were high virulent, formed active nitrogen–fixing apparatus on soybean roots under symbiosis conditions and increase in the crop yield and the protein content in soybean seeds (strains are from the collection of Department of general and soil microbiology, Zabolotny Institute of Microbiology and Virology of the National Academy of Sciences of Ukraine, deposited in the Ukrainian collection of microorganisms, UCM). These strains are industrial and they used as producents to bacterial preparations (inoculants) for crop plants in the factory for the production of biological products in Ukraine.

In the research, in order to compare the biosynthesis ability of symbiotic bacteria of soybean, were used nonindustrial rhizobia such as: *B. japonicum* 21110 strain with small efficiency, which had low virulence, formed nodules with low nitrogen–fixing activity on soybean roots and had little effect on the crop yield and its quality (strain received in previous years from the collection of the All-Russian Research Institute of Agricultural Microbiology RAAS, St. Petersburg, Pushkin); and ineffective strain of *B. japonicum* 604k (the control strain), which had high virulence but formed nodules without nitrogen–fixing activity on soybean roots (strain granted in previous years, the Institute of Agriculture Crimea, NAAS).

Characteristics of the studied *B. japonicum* strains under conditions of establishment and functioning of symbiosis with soybean plants, which included such indicators as: the average number and mass of nodules and their nitrogenase activity per plant, were obtained in previous years by the Department of general and soil microbiology, Zabolotny Institute of Microbiology and Virology, NAS of Ukraine (Table).

The cultivation of bacteria was performed in flasks with volume of 750 ml rocking (220/min) at 26–28 °C during 72–96 hours in the liquid nutrient medium of Iswaran with such composition (g/l): mannitol — 10.0; east extract — 2.0; calcium gluconate — 1.5; K_2HPO_4 — 0.5; $MgSO_4 \cdot 7H_2O$ — 0.2; NaCl — 0.1; $FeCl_3 \cdot 6H_2O$ — 0.01; pH 7.2. The cultures of *B. japonicum* in the exponential growth phase (92–96 h) grown on the Iswaran medium were used as inoculums. The amount of inoculums was 5% of the medium volume.

Characteristics of nitrogen–fixing strains of *B. japonicum* in the formation and functioning of symbiosis with soybean plants

***B. japonicum* strains**	**Average number of nodules per plant, pcs.**	**The weight of nodules per plant, mg**	**Nitrogenase activity of nodules, μmol C_2H_4 per plant for 1 hour**
604k (control, ineffective)	565 ± 29*	880.0 ± 70.0*	0.009 ± 0.001*
21110 (small efficiency)	16 ± 2	970.5 ± 17.8	0.70 ± 0.02
UCM B-6018 (highly effective)	30 ± 3	1370.0 ± 33.7	1.09 ± 0.12
UCM B-6023 (highly effective)	36 ± 3	803.0 ± 11.2	1.21 ± 0.09
UCM B-6035 (highly effective)	38 ± 5	1275.0 ± 34.5	1.63 ± 0.14
UCM B-6036 (highly effective)	40 ± 6	1080.0 ± 23.4	1.05 ± 0.08

* Data are accordance with the work [10].

For separating the biomass the bacteria culture broth was centrifuged for 20 min at 9000 rev/min and 4 °C. Bacterial cells were washed with saline three times in order to clean from exopolymers residues, each time they were centrifuged under the same conditions. The supernatants were used for further studies for the purpose of extraction of ABA, and the residue of cells was suspended in distilled water and then dried at 103–105 °C in an oven until the constant weight was reached. The amount of absolutely dry biomass (ADB) of microorganisms was determined gravimetrically.

The extracellular ABA was isolated from the supernatant of *B. japonicum* by extraction of phytohormones with ethyl acetate at pH 3.0 [11]. The obtained extracts were evaporated in vacuum at 40–45 °C. The dry residue was dissolved in 5 ml of ethanol and transferred to micro test tubes. Ethanol extracts from the supernatant of the studied soybean rhizobia were used for accumulative thin-layer chromatography. The previous purification and the concentration of ABA were performed on plates with silica gel, mark Silufol UV_{254} (Chemapol, Czech Republic), in the mixture of solvents applied subsequently: chloroform; 12.5% aqueous ammonia; ethyl acetate: acetic acid (20:1). The purified in that way extracts were separated on plates with silicon oxide Merck, №5554, F_{254} (Germany) in the mixture of solvents: chloroform: ethyl acetate: acetic acid (100:100:1) [12]. The quantitative detection was performed with a scanning spectrodensitometer ("Sorbfil", Russia). The amount of extracellular ABA was calculated in μg per 1 g ADB of the producer. The standard was the synthetic ABA by Sigma-Aldrich (Germany).

The amount of ethylene produced by symbiotic nitrogen-fixing soybean bacteria was measured by gas chromatography. The bacteria were cultivated in flasks of 25 ml on solid synthetic medium slant of Iswaran, in which L-methionine (Met) was added with concentration of 0.1 g/l [5]. The flasks were tightly closed with rubber stoppers and special metal clamps in order to prevent escape of the synthesized ethylene. The further cultivation of *B. japonicum* strains was performed for 72 hours at 28 °C. After the cultivating of bacteria the gas mixture from the flasks was analyzed with a gas chromatograph ("Chrome-5", Czech Republic) with a flame ionization detector (a column with β-β′oxide propionitrile). Recalculation was performed on the calibration schedule built according to ethylene dilutions.

All experiments were performed in 6 iterations. The results obtained were processed statistically using computer program Excel from licensed Microsoft Office 2010. In the Table and the Figures the average values and standard errors ($M \pm m$) are presented. Values of $P < 0.05$ were considered to be significant.

Results and Discussion

The study results revealed that the ABA production by symbiotic soybean bacteria under the conditions *in vitro* did not correlate with nitrogenase activity of the strains in symbiosis. It is noted that the ability to form large amount of this phytohormone — plant growth inhibitor is a strain property of high virulent biotechnological rhizobia. Thus, significant amounts of ABA (56.5–72.0 μg/g ADB) are capable to synthesize both the highly effective microsymbionts of soybean *B. japonicum* UCM B-6035 and UCM B-6036 and the ineffective strain of *B. japonicum* 604k (Fig. 1). The ABA synthesis was almost 20 times higher in the effective industrial strains of *B. japonicum* UCM B-6035 and UCM B-6036 than in the other active industrial strains of *B. japonicum* UCM B-6018 and UCM B-6023.

According to the literature, this fact may indicate that in various strains of symbiotic soybean bacteria synthesized ABA can both increase and reduce activity of nitrogenase enzyme, which is directly involved in converting air nitrogen into amine nitrogen. Therefore, processing of legumes with biological preparations–inoculants, which contain large amount of bacterial ABA specifically, can negatively affect the formation of nodules and reduce the efficiency of symbiotic nitrogen fixation [6]. At the same time, the ability of *B. japonicum* to production ABA is an additional factor that increases potential activity of these strains in infection of plants. Also, it is known that ABA can reduce high concentrations of growth stimulating substances [13].

It is known that ABA, on the one hand, is a phytohormonal adaptogen, therefore, it enhances the plant resistance to environmental stress factors. On the other hand, it stimulates nitrogenase activity by inhibiting production of nitric oxide in nodules [14]. At the same time, ABA is a chemical factor that determines the pathogenicity of microorganisms in infection of plants [2, 15]. This phytohormone inhibits the plant growth and development and accelerates defoliation and fruit ripening [16].

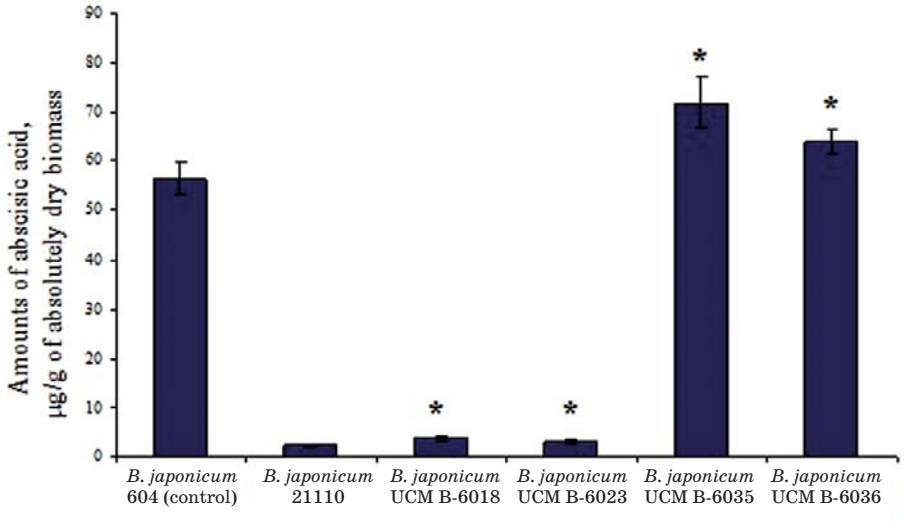

Fig. 1. **Production of extracellular abscisic acid by various nitrogen–fixing symbiotic efficiency biotechnological strains of *B. japonicum***
Here and after: $M \pm m$, $n = 6$; */** — $P < 0{,}05$ vs. control.

The role of ABA synthesis as the hormonal exometabolites for microorganisms is not studied enough yet. In the literature the data on the role of this compound in plant–microbe interactions are presented mainly.

In particular, the role of ABA in legume–rhizobia symbiosis also is not fully clear. The depressing effect of the hormone in formation of nodules was shown [17, 18] that was associated with the decrease in content of flavonoids (daidzein, genistein, kumestrol) in soybean roots. There are the data that ABA can interact with phytohormones cytokinins at process of root cortical cell division inhibiting it [19].

After inoculation with rhizobia the formation of nodules on a soybean plant slows down under the influence of exogenous ABA and, on the contrary, the number of nodules increases abruptly at reducing concentration of the hormone under action of the specific inhibitor (9-*cis*-epoxycarotenoid dioxygenase). The observation on the root hair deformation revealed that ABA blocked the stage between the root hair swelling and its curling. It is assumed that in this way ABA controls the number of nodules on the plants roots [17]. However, it was shown that ABA was not directly involved in system self–regulation of nodules number and inhibited their formation only locally [20].

We have obtained the data on small amounts of gaseous phytohormone–inhibitor of the plant growth ethylene that were produced by symbiotic soybean bacteria *B. japonicum* (Fig. 2). As it is evident from the results obtained, the level of ethylene synthesis in diverse biotechnological strains varies widely. Thus, highly effective industrial rhizobia strains of *B. japonicum* UCM B-6023, UCM B-6018 and UCM B-6035 have low rates of synthesis of this phytohormone. Instead, the ineffective strain of *B. japonicum* 21110 differs significantly in amount of synthesized ethylene, which is 75.2 times higher than the lowest value of *B. japonicum* UCM B-6035. The direct correlation between level of synthesis of the hormone and nitrogenase activity of the strains in symbiosis was not observed. According to the literature, the relatively low pool of ethylene in plant is important to the symbiotic bacteria, because this hormone significantly inhibits formation of nodules on the plants roots [21, 22]. Probably, this explains the relatively low level of ethylene synthesis by all studied strains of *B. japonicum*.

Ethylene plays the dual role in formation of nitrogen–fixing nodules: it can suppress their formation or under certain concentrations it can stimulate rhizobium infection [23, 24]. It is known that inhibition of ethylene synthesis contributes to an increase in number of nodules formed on the roots of peas, alfalfa and other legumes [7].

In order to study mechanisms that rhizobia use to control nodulation the gene of synthesis of enzyme 1-aminocyclopropane-1-carboxylic acid (ACA) deaminase, inhibiting the formation of ACA–ethylene synthesis precursor in higher plants, was isolated and characterized in *Rhizobium leguminosarum*

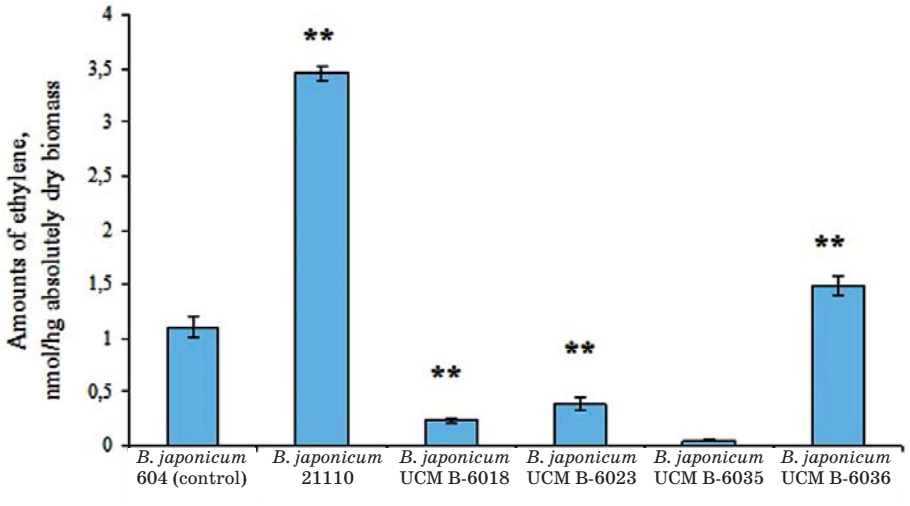

Fig. 2. **Production of ethylene by *B. japonicum***

bv. *viciae* 128C53K. Due to the action of ACA–deaminase, bacteria can reduce ethylene synthesis in plants [24].

There is an information that ethylene, as a component of signaling system, takes part in bacterial infection in legumes and it is a negative regulator of the *Nod*-factor signaling [23, 25]. Increase of endogenous ethylene level in cells of the host plant under NO_3 can lead to further restrictions of nodulation in addition to regulatory impact (autoregulation) on these processes regarding ethylene, synthesis of which is initiated by *Nod*-factor. The impact of nitrate on ethylene synthesis is associated with the activation of ACA oxidase [24]. However, the mechanism of ethylene influence on formation of legume–rhizobia symbiosis is not fully understandable. It was shown that ethylene inhibited all primary host plant responses to rhizobia infection including output of Ca^{2+} into the cytosol [22]. It is assumed that ethylene can affect the activation of *Nod*-factor signaling pathway. It can act as a secondary messenger, regulating nodulation in response to the nitrogen status of the host plant, and as a negative regulator of rhizobia infection [21, 25].

Production of ethylene by rhizobia, in particular *B. japonicum*, was studied by cultivating the bacteria on the medium with of L-methionine, which resulted in increased phytohormone synthesis under these conditions. In addition, if L-methionine is present in apoplast or root exudates, the association of *Bradyrhizobium* with the plant can increase level of endogenous ethylene in a plant and thereby inhibit the formation of nodules [5].

Therefore, the synthesis of ABA and ethylene by symbiotic rhizobia of soybean under the conditions *in vitro* is a specific property of researched biotechnological strains. This process does not depend on their nitrogenase activity in nodules and further functioning of symbiosis. The ability to synthesize the phytohormones, which show the depressing effect on the plant growth and development, can be an additional factor that increases virulence of strains of *B. japonicum* and can influence the growth of their pathogenicity in infection of plants. We can assume that ABA and ethylene of bacterial origin take part in the formation of legume–rhizobia symbiosis, and their role is primarily to stimulate cell division of roots tissues, which start the formation of symbiotic relationship and the creation of the optimal number of nodules for a plant. Besides that, excessive concentration of these compounds in bacterial biological preparations obtained from cultivation of *B. japonicum* and used for legume seeds processing before sowing, can significantly reduce quality of the obtained preparation and, accordingly, reduce the effectiveness of functioning of legume–rhizobia systems.

In addition, the results obtained are of practical importance for development of new biotechnologies for creation of efficient microbial preparations and inoculants for plant cultivation and further forecasting of microorganisms activity in agricultural ecosystems.

REFERENCES

1. *Desbrosses G. J., Stougaard J.* Root nodulation: A paradigm for how plant-microbe symbiosis influences host developmental pathways. *Cell Host Microbe*. 2011, 10 (4), 348–358.
2. *Tsavkelova E. A., Klimova S. Yu., Cherdyntseva T. A., Netrusov A. I.* Hormones and hormone-like substances of microorganisms: a review. *Appl. Biochem. Microbiol.* 2006, 42 (3), 229–235.
3. *Spaepen S., Vanderleyden J., Remans R.* Indole-3-acetic acid in microbial and microorganism-plant signaling. *FEMS Microbiol. Rev*. 2007, V. 31, P. 425–448.
4. The rhizosphere: biochemistry and organic substances at the soil-plant interface. 2nd ed. *R. Pinton, Z. Varanini, P. Nannipieri* (Ed.). *Boca Raton, FL: CRC Press.* 2007, 472 p.
5. *Boiero L., Perrig D., Masciarelli O., Penna C., Cassan F., Luna V.* Phytohormone production by three strains of *Bradyrhizobium japonicum* and possible physiological and technological implications. *Appl. Microbiol. Biotechnol.* 2007, V. 74, P. 874–880.
6. *Ferguson B. J., Mathesius U.* Phytohormone regulation of legume-rhizobia interactions. *J. Chem. Ecol*. 2014, 40 (7), 770–790.
7. *Mohd-Radzman N. A., Djordjevic M. A., Imin N.* Nitrogen modulation of legume root architecture signaling pathways involves phytohormones and small regulatory molecules. *Front. Plant Sci.* 2013, V. 4, Art. 385. http://dx.doi.org/10.3389/fpls.2013.00385.
8. *Dragovoz I. V., Leonova N. O., Biliavska L. O., Yavorska V. K.* Phytohormone production by some free-living and symbiotic soil microorganisms. *Reports of the NASU*. 2010, V. 12, P. 154–159. (In Ukrainian).
9. *Dragovoz I. V., Leonova N. O., Iutynska G. O.* Phytohormones synthesis by *Bradyrhizobium japonicum* strains with different symbiotic effectiveness. *Mikrobiol. Zh.* 2011, 73 (4), 29–35. (In Russian).
10. *Sytnikov D. M., Kots S. Y.* Symbiotic properties of the soybean nodule bacteria inactive strain 604k. *Plant Physiology: problems and prospects: in 2 is.* Ed. V. V. Morgun. *Kyiv: Logos*. 2009. Is. 1, P. 466–470. (In Russian).
11. Methodical recommendations by phytohormones definition. *Kyiv: Inst. of Botany of NASU*. 1988, 78 p. (In Russian).
12. *Savinskiy S. V., Kofman I. Sh., Kofanov V. I., Stasevskaya I. L.* Methodological approaches to the determination of plant hormones using spectrodensitometry thin layer chromatography. *Physiol. Biochem. Cult. Plants.* 1987, 19 (2), 210–215. (In Russian).
13. *Tominaga A., Nagata M., Futsuki K., Abe H., Uchiumi T., Abe M., Kucho K., Hashiguchi M., Akashi R., Hirsch A. M., Arima S., Suzuki A.* Effect of abscisic acid on symbiotic nitrogen fixation activity in the root nodules of *Lotus japonicus*. *Plant Signal Behav*. 2010, 5 (4), 440–443.
14. *Tominaga A., Nagata M., Futsuki K., Abe H., Uchiumi T., Abe M., Kucho K., Hashiguchi M., Akashi R., Hirsch A. M., Arima S., Suzuki A.* Enhanced nodulation and nitrogen fixation in the abscisic acid low-sensitive mutant enhanced nitrogen fixation of *Lotus japonicus*. *Plant Physiol.* 2009, 151 (4), 1965–1976. doi: 10.1104/pp.109.142638.
15. *Mauch-Mani B., Mauch F.* The role of abscisic acid in plant–pathogen interactions. *Cur. Opin. Plant Biol*. 2005, V. 8, P. 409–414.
16. *Nambara E., Marion-Poll A.* Abscisic acid biosynthesis and catabolism. *Annu. Rev. Plant Biol.* 2005, V. 56, P. 165–185.
17. *Suzuki A., Akune M., Kogiso M., Imagama K., Osuki K., Uchiumi T., Higashi S., Han S., Yoshida S., Asami T., Abe M.* Control of nodule number by the phytohormone abscisic acid in the roots of two leguminous species. *Plant Cell Physiol.* 2004, 45 (7), 914–922.
18. *Suzaki T., Kawaguchi M.* Root nodulation: a developmental program involving cell fate conversion triggered by symbiotic bacterial infection. *Current Opin. Plant Biol*. 2014, 21 (10), 16–22.
19. *Ding Y., Kalo P., Yendrek C., Sun J., Liang Y., Marsh J. F., Harris J. M., Oldroyda Giles E. D.* Abscisic acid coordinates Nod factor and cytokinin signaling during the regulation of nodulation in *Medicago truncatula*. *Plant Cell*. 2008, 20 (10), 2681–2695. doi: 10.1105/tpc.108.061739.
20. *Biswas B., Chan P. K., Gresshoff P. M.* A novel ABA insensitive mutant of *Lotus japonicus* with a wilty phenotype displays unaltered nodulation regulation. *Mol Plant*. 2009, 2 (3), 487–499. doi: 10.1093/mp/ssp009.
21. *Dilworth M. J., James E. K., Sprent J. I., Newton W. E.* Nitrogen-fixing leguminous symbioses. *Springer Science & Business Media*. 2008, 403 p.
22. *Khatabi B., Schäfer P.* Ethylene in mutualistic symbioses. *Plant Signal Behav*. 2012, 7 (12), 1634–1638. doi: 10.4161/psb.22471.
23. *Xu J., Zhang Sh.* Regulation of ethylene biosynthesis and signaling by protein kinases and phosphatases. *Mol. Plant*. 2014, 7 (6), 939–942. doi: http://dx.doi.org/10.1093/mp/ssu059.
24. *Arshad M., Khalid A., Shahzad Sh. M., Mahmood T.* Role of ethylene and bacterial ACC deaminase in nodulation of legumes *In*. Microbes for legume improvement. *Vienna: Springer*. 2010, P. 103–122. doi: 10.1007/978-3-211-99753-6_5.
25. *Adie B., Chico J. M., Rubio-Somoza I., Solano R.* Modulation of plant defenses by ethylene. *J. Plant Growth Regul.* 2007, V. 26, P. 160–77. http://dx.doi.org/10.1007/s00344-007-0012-6.

ПРОДУКУВАННЯ АБСЦИЗОВОЇ КИСЛОТИ ТА ЕТИЛЕНУ БІОТЕХНОЛОГІЧНИМИ ШТАМАМИ *Bradyrhizobium japonicum*

Н. О. Леонова

Інститут мікробіології і вірусології
ім. Д. К. Заболотного НАН України,
Київ

E-mail: natikleo@online.ua

Метою роботи було дослідження продукування фітогормонів, що пригнічують ріст і розвиток рослин — абсцизової кислоти та етилену, різними за ефективністю симбіотичними азотфіксувальними біотехнологічними штамами *Bradyrhizobium japonicum* в умовах *in vitro*. Кількість абсцизової кислоти визначали методом спектроденситометричної тонкошарової хроматографії, а кількість етилену — методом газової хроматографії.

Показано здатність симбіотичних азотфіксувальних бактерій сої продукувати позаклітинну абсцизову кислоту (56,5–72,0 мкг/г абсолютно сухої біомаси) та етилен (0,046–3,461 нмоль/год·г абсолютно сухої біомаси). Встановлено, що відмінності в кількості абсцизової кислоти й етилену у *B. japonicum* є штамовими особливостями і безпосередньо не пов'язані з умовами культивування, нітрогеназною активністю цих бактерій та їхньою ефективністю у симбіозі з рослинами сої. Здатність біотехнологічних штамів *B. japonicum* синтезувати фітогормони — інгібітори росту і розвитку рослин в умовах *in vitro* може бути додатковим чинником, що збільшує вірулентність бактерій і визначає їхню потенціальну активність під час інфікування рослин. Одержані дані мають важливе значення для розроблення технології виробництва мікробіологічних препаратів–інокулянтів для бобових рослин з комбінованими властивостями.

Ключові слова: *Bradyrhizobium japonicum*, абсцизова кислота, етилен, симбіоз, соя.

ПРОДУЦИРОВАНИЕ АБСЦИЗОВОЙ КИСЛОТЫ И ЭТИЛЕНА БИОТЕХНОЛОГИЧЕСКИМИ ШТАММАМИ *Bradyrhizobium japonicum*

Н. О. Леонова

Институт микробиологии и вирусологии
им. Д. К. Заболотного НАН Украины,
Киев

E-mail: natikleo@online.ua

Целью работы было исследование продуцирования фитогормонов, которые подавляют рост и развитие растений — абсцизовой кислоты и этилена, различными по эффективности симбиотическими азотфиксирующими биотехнологическими штаммами *Bradyrhizobium japonicum* в условиях *in vitro*. Количество абсцизовой кислоты определяли методом спектроденситометрической тонкослойной хроматографии, а количество этилена — методом газовой хроматографии.

Показана способность штаммов симбиотических азотфиксирующих бактерий сои продуцировать внеклеточную абсцизовую кислоту (56,5–72,0 мкг/г абсолютно сухой биомассы) и этилен (0,046–3,461 нмоль/ч·г абсолютно сухой биомассы). Установлено, что различия в количестве абсцизовой кислоты и этилена у *B. japonicum* являются штаммовыми особенностями и непосредственно не связаны с условиями культивирования, нитрогеназной активностью этих бактерий и их эффективностью в симбиозе с растениями сои. Способность биотехнологических штаммов *B. japonicum* синтезировать фитогормоны, ингибирующие рост и развитие растений, в условиях *in vitro* может быть дополнительным фактором, увеличивающим вирулентность бактерий и определяющим их потенциальную активность при инфицировании растений. Полученные данные имеют важное значение для разработки технологии производства микробиологических препаратов–инокулянтов для бобовых растений с комбинированными свойствами.

Ключевые слова: *Bradyrhizobium japonicum*, абсцизовая кислота, этилен, симбиоз, соя.