

EFFICACY OF THE ASSOCIATION “SPRING WHEAT – *AZOSPIRILLUM BRAZILENSE* B-7318”

Yu. O. Vorobei, O. O. Shakhovkina, O. V. Lohosha, T. O. Usmanova

Institute of Agricultural Microbiology and Agroindustrial manufacture, NAAS
97 Shevchenka Str., Chernihiv, 14030, Ukraine; e-mail: yu.a.vorobey@gmail.com

Objective. To evaluate the ability of *Azospirillum brasilense* B-7318, a bacterium that intensively fixes molecular nitrogen, to form stable effective associations with spring wheat plants. **Methods.** Microbiological, application (to determine the intensity of azospirilla development in the root spheres of plants), acetylene (to study nitrogenase activity), phosphate (to determine the activity of glutamine synthetase in plant leaves), field experiment, statistical. **Results.** A new strain of *A. brasilense* B-7318, capable of intensively fixing molecular nitrogen, was obtained by the method of analytical selection. It was shown that inoculation of spring wheat seeds results in effective colonization of the surface of roots and the rhizosphere soil by diazotrophs during inoculation of spring wheat seeds (the highest density of bacteria was registered up to 2 mm from the root surface). Potential nitrogenase activity in the rhizosphere soil when *A. brasilense* B-7318 was used for inoculation didn't significantly differ from the values of the control variant (without inoculation). At the same time, potential nitrogenase activity on the washed roots of inoculated plants significantly (by 4.9 times) exceeded this parameter in the rhizosphere of plants of the control variant. *A. brasilense* B-7318 also contributes to a significant increase in glutamine synthetase activity in the leaves of wheat plants (by 57%). When wheat seeds were inoculated with *A. brasilense* B-7318 bacterial suspension, the protein content in plant leaves increased by 9.7%. The results of determining the structure of the crop in the field experiment proved that azospirilla provided a significant increase in the length of the ear (by 8.1%), the number of grains in the ear (by 4.1%), the mass of grains from the ear (by 14.8%) and the weight of 1,000 grains (by 6.1%), as well as an increase in yield by 15.7% compared to the control. **Conclusion.** *A. brasilense* B-7318, which is characterized by high nitrogenase activity in pure culture, is able to actively colonize the root spheres of spring wheat plants, increase glutamine synthetase activity and protein content in leaves, as well as contribute to the increase of potential nitrogenase activity on plant roots and improvement of the wheat yield

Key words: *Azospirillum brasilense*, PGPR, spring wheat, nitrogenase activity.

Introduction. The study of the interaction of plants with associative nitrogen-fixing bacteria of the genus *Azospirillum*, started by J. Döbereiner in the early 70s of the last century, continues even now in many countries. Azospirilla are of great interest among scientists due to their ability to stimulate the growth of a wide range of plant species, which allows them to be considered typical growth-regulatory microorganisms (plant-growth-promoting rhizobacteria or PGPR) [1]. Due to the widespread distribution of bacteria of the genus *Azospirillum*, a comprehensive study of their biological features and

mechanisms of influence on plants, the interaction of azospirilla with non-leguminous plants is considered by the scientific community as a general model of plant-microbial interactions [2].

Forming associations with plants, bacteria of the genus *Azospirillum* supply assimilated nitrogen to the root zone, synthesize and secrete phytohormones, vitamins, substances of antibacterial and antifungal nature. Instead, the plant supplies diazotrophs with available sources of carbon and phosphorus, which are components of root exudates, and also creates condi-

tions favourable for the nitrogen fixation process. The functioning of such an association ensures mutually beneficial coexistence of micro- and macro-partners.

Analysis of recent studies and publications. It is known that the effect of azospirilla on plants is multi-vector. Azospirilla intensify the process of biological nitrogen fixation in close interaction with plant roots of many agricultural crops [3–6]. Representatives of the genus *Azospirillum* are able to produce phytohormones of auxin, gibberellin, cytokinin nature, which regulate various aspects of plant development, namely: they activate seed germination, responsible for the morphogenesis of roots and shoots, play an important role during the formation of fruits and seeds, delay the aging of leaves, etc. [7–10].

The ability of *Azospirillum* spp. to the synthesis of abscisic acid, proline, polyamines and trehalose ensures resistance of inoculated plants to adverse environmental conditions (moisture deficit, salt stress) and activation of protective mechanisms [11–15]. Cohen et al. [12] reported an increase in the abscisic acid level and drought resistance of plants upon corn inoculation. The results of Ilyas & Bano [13] indicate that azospirilla strains isolated under conditions of water deficit were characterized by increased production of abscisic acid. According to Casan et al. [14], inoculation of rice seedlings with *A. brasilense* promoted the development of the plant root system and at least partially alleviated osmotic stress due to the synthesis of the polyamine cadaverine by azospirilla. Corn plants treated with a modified strain of *A. brasilense* with an increased level of trehalose production were more resistant to drought and formed more biomass than plants inoculated with wild-type *A. brasilense* [15].

Along with the production of bacterial metabolites, azospirilla induce the biosynthesis of secondary metabolites with antimicrobial activity in the plant organism [16; 17]. According to Chamam et al. [17], changes in the profile of secondary metabolites of rice plants treated with azospirilla were primarily associated with flavonoids and hydroxycinnamic acid derivatives. Walker et al. [18] showed the difference between control and azospirilla inoculated corn plants via the production of benzoxazinoids — substances associated with resistance to pathogens.

Literature evidence also suggest that after inoculation with azospirilla, the water potential of plants improves, the water content in apoplasts and the elasticity of the cell wall increases, the content of chlorophyll and photoprotective pigments increases; the respiratory conductance [19], the availability of nutrients [20], the efficacy of nitrogen use [21] and its content in the obtained products increase [22], the yield of agricultural crops increases as an integral parameter of plant development [3–6; 23; 24].

The ability of nitrogen-fixing bacteria to colonize the root spheres of plants is one of the most important criteria for selecting an efficient inoculant. High survivability of associative nitrogen fixers in the root zone ensures a close and therefore potentially effective interaction with the plant and resistance to adverse environmental conditions. Underestimation of the importance of this factor often leads to the lack of a positive result from the use of diazotroph-based biological preparations [25].

According to Bashan et al. [3], the ability of bacteria of the genus *Azospirillum* to colonize roots is related to their ability to fix atmospheric nitrogen. Thus, the authors showed that the factors that inhibit nitrogen fixation (nitrates) also inhibit the spread of bacteria, and the factor that stimulates nitrogen fixation (microaerophilic conditions) increases the ability of azospirilla to colonize roots [26].

Objective of this work was to identify the ability of a selected strain of *Azospirillum brasilense* with a high potential of nitrogen-fixing activity to colonize the root spheres of plants, which is a necessary condition for the creation of efficient “diazotroph-plant” associations, as well as to contribute to the optimization of the production process of spring wheat.

Materials and methods. Morphological, cultural and physiological properties of nitrogen-fixing bacteria were studied using the generally accepted methods [27].

In the conditions of laboratory aseptic experiments, the development of *A. brasilense* B-7318 in the root zone of spring wheat plants was studied using a modification of the application method — by analysing the microbial landscape of the rhizosphere using polyethylene terephthalate films (PET films) of fouling made of a biocompatible hydrophobic transparent polymer, which makes it possible to investigate cenosis in its native architecture [28]. The number

of bacterial cells (fifty in average) was counted in the rhizoplane (on the surface of the roots) and in the rhizosphere (in the root zone (0.5–2 mm from the root surface) and in the zone 2–4 mm away from the root). The reference strain *A. brasilense* Sp7 was used as a positive control.

Bacterial nitrogenase activity in pure culture and potential nitrogenase activity (PNA) in the root zone of wheat plants were determined by the acetylene method [29]. PNA in the rhizosphere and on the washed roots of plants was studied under the conditions of a field experiment. Weights of rhizosphere soil and crushed roots of wheat plants were placed in 40 cm³ vials, 10 mL of pre-melted and cooled to 45 °C Döbereiner semi-liquid medium (g/L) were added: KH₂PO₄ — 0.4; K₂HPO₄ — 0.1; MgSO₄ × 7H₂O — 0.2; CaCl₂ · 2H₂O — 0.02; FeCl₃ × 6H₂O — 0.01; NaMoO₄ · 2H₂O — 0.002; L-malic acid — 3.5. The vials were closed with cotton-gauze stoppers and incubated in a thermostat at 26 ± 2 °C for 72 hours. After incubation in the thermostat, the cotton-gauze stoppers were replaced with rubber ones and acetylene was introduced in the amount of 10 % of the gas phase of the vial. After exposure of the samples to acetylene, samples were taken and analysed on a Chrom-5 gas chromatograph with a flame ionization detector, measuring the amount of ethylene formed during incubation [30].

The activity of glutamine synthetase in the leaves of spring wheat plants was assessed by the phosphate method under the conditions of a vegetation experiment [31]. Weights of leaves weighing 1 g were ground in a porcelain mortar with 10 mL Tris-HCl, left for 10 min. It was centrifuged for 20 minutes at 3,000 rpm, the centrifuge was taken, 0.2 mL for each analytical repetition. The incubation mixture (final volume 3.4 mL) contained 100 mM Tris-HCl (pH 7.2), 150 mM sodium monoglutamate; 7.5 mM NH₄Cl; 10 mM ATP; 10 mM MgCl₂. The reaction was stopped by adding 0.5 mL of 20 % trichloroacetic acid. Phosphorus content was determined in the solution clarified by centrifugation with a molybdenum reagent based on the formation of a phosphorus-molybdenum complex. The solution was photometered at 750 nm. Enzymatic activity was calculated in μmol P_i in 1 mg of protein per 1 min by the formula:

$$A = \frac{P_{\text{standard}} \cdot (D_{\text{experiment}} - D_{\text{control}}) \cdot V \cdot 1,000}{D_{\text{standard}} \cdot m_{\text{protein}} \cdot M(P)}$$

where A = glutamine synthetase activity, μmol P/mg protein per hour;

P_{standard} = phosphorus content in the soluble standard, mg/mL;

D_{experiment} = optical density of the test solutions;

D_{control} = optical density of the solutions, in which the reaction was stopped immediately after addition of trichloroacetic acid;

D_{standard} = optical density of the solution with known phosphorus concentration;

V = volume of the incubation mixture;

m_{protein} = protein content in the test sample, mg;

M(P) = molar weight of phosphorus, g/mol;

1,000 = mg to μg conversion factor.

Biological and analytical repetition of the analysis is triplicate.

Protein content in the leaves of wheat plants was measured according to [32].

To study the structural parameters of the spring wheat harvest, 100 typical plants were selected from each variant of the field experiment. The length of the ear, the number and weight of grains in the ear, the weight of 1,000 grains of the control and experimental variants were determined [33]. Harvesting and recording of the harvest was carried out by the direct method (weighing of products from the accounting plot).

Conditions of pot and field experiments.

The pot experiment was conducted in the greenhouse. Light loam leached chernozem on loess deposits was placed in 1 dm³ plastic vessels. It is characterized by the following agrochemical parameters: pH_{sal} — 5.3; humus content — 3.03 %; easily hydrolyzed nitrogen — 95 mg/kg of soil; mobile compounds of phosphorus (P₂O₅) — 150 (according to Kirsanov); content of exchangeable potassium (K₂O) (according to Kirsanov) — 108 mg/kg of soil. In the pot experiment, seeds of spring wheat variety Variah were used. Scheme of the experiment:

1. Control (without inoculation).

2. Inoculation of seeds with *A. brasilense* B-7318. The experiment was repeated six times.

Field experiments were conducted in 2017–2018 at the experimental field of the Institute of Agricultural Microbiology and Agroindustrial Manufacture (IAMAM), National Academy of Agrarian Sciences (Chernihiv, Ukraine). The type of soil and its agrochemical characteristics are the same as in the vegetation experiment.

Spring wheat seeds of Tera variety were used in the field experiments. The sowing rate is 5 million similar seeds per hectare. The method of sowing is narrow-row, with a row spacing of 15 cm. The area of the accounting plot is 10 m², the repetition of the experiments is four times, the plots were allocated randomly. Scheme of experiments:

1. Control (without inoculation).

2. Inoculation of seeds with *A. brasilense* B-7318.

A. brasilense B-7318 for pre-sowing bacterization in vegetation and field experiments were grown on a liquid nutrient medium with the following composition (g/L): molasses — 30.0; corn extract — 30.0; (NH₄)₂SO₄ — 0.5; K₂HPO₄ — 0.25; KH₂PO₄ — 0.25; MgSO₄ × 7H₂O — 0.2; CaCO₃ — 3.0. Seed inoculation was carried out with a two-day culture of azospirilla with a titre of 2 × 10⁹ at the rate of 3 × 10⁵ bacterial cells per seed.

Statistical processing of experimental data was carried out using Microsoft Office Excel 2010.

Results and discussion. The largest collection of *Azospirillum* genus in Ukraine, selected over the past 40 years, is stored in the National Collection of Beneficial Soil Microorganisms at IAMAM [34–38].

A. brasilense B-7318, isolated by us from the surface of washed roots of spring wheat of the Variah variety, was characterized by the highest nitrogenase activity in pure culture (9.07 µg nitrogen/mL of medium daily) among 54 strains of the genus *Azospirillum* isolated from the rhizosphere and rhizoplane of spring wheat of 12 varieties [4].

The results of studies on the viability of *A. brasilense* B-7318 indicate that azospirilla cells were localized in the mucigel on the surface of the roots and in the rhizosphere of wheat plants (Table 1). No significant difference was reported between the number of cells of the two

studied strains in different topological zones of the roots, but a clear tendency to increase the density of diazotrophs in the root zone (up to 2 mm from the root surface) was noted. The number of *A. brasilense* B-7318 per 10⁴ µm² in different root spheres averaged from 24 to 52 bacterial cells.

Considering a correlation between the parameters of the ability of azospirilla to colonize roots with the level of nitrogen-fixing activity [24; 39–41], we studied PNA in the root zone of spring wheat plants in the flowering phase under the conditions of a field experiment (Fig. 1). It was shown that the values of PNA in the rhizosphere soil when used for inoculation with *A. brasilense* B-7318 did not differ significantly from the values of the nitrogenase activity of the rhizosphere in the control (Fig. 1 a). At the same time, nitrogenase activity on washed roots of inoculated plants significantly (by 4.9 times) exceeded this parameter in the rhizoplane of the control variant (Fig. 1 b).

Under the condition of efficient interaction between diazotrophs and plants, molecular nitrogen is reduced to ammonium and assimilated by a plant. It should be noted that the key enzymes of nitrogen metabolism in bacteria are nitrogenase and glutamine synthetase [42]; the latter also plays a decisive role in plant metabolism, ensuring the formation of the amino acid glutamine from L-glutamate with the participation of ATP [43].

Correlations are known between the activities of nitrogenase and glutamine synthetase enzymes of bacteria [44–46]. The authors proved this hypothesis using irreversible enzyme inhibitors as well as glutamine-dependent mutants of *Azospirillum brasilense*. The complex system of regulation of the activity of glutamine synthetase and nitrogenase enzymes in azospirilla cells, both at the level of biosynthesis and at the post-translational level, is also discussed by

Table 1. The number of azospirilla in the root spheres of spring wheat plants

Azospirilla localization zones	Number of bacterial cells per 10 ⁴ µm ²	
	<i>A. brasilense</i> Sp7	<i>A. brasilense</i> B-7318
Surface of roots	20 ± 4	24 ± 4
Rhizosphere		
Root zone (0.5–2 mm)	49 ± 9	52 ± 2
2–4 mm zone near a root	44 ± 4	48 ± 2

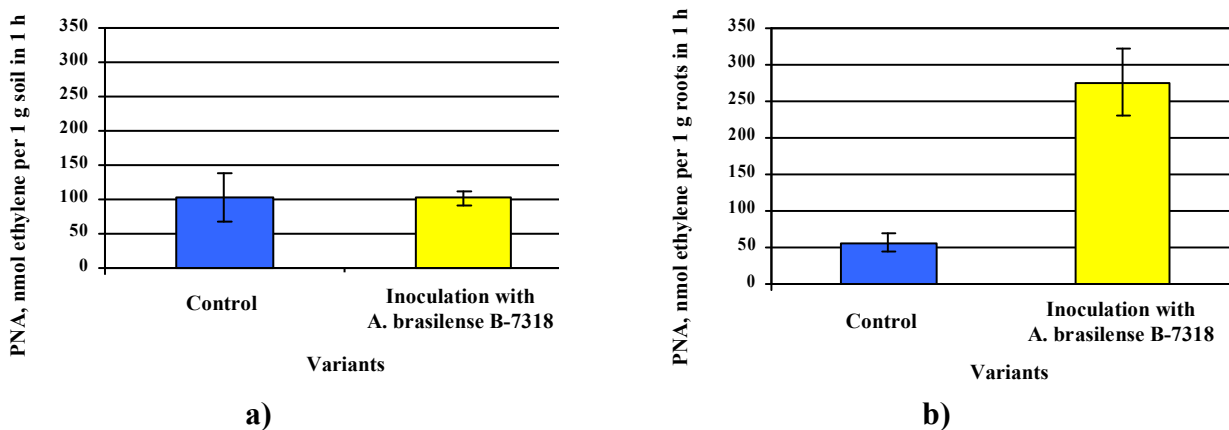


Fig. 1. Potential nitrogenase activity in the root zone of spring wheat plants upon inoculation with *A. brasilense* B-7318: a — in rhizosphere soil, b — on washed roots of plants (field experiment, 2018).

Zhang et al. [47]. At the same time, microorganisms can activate plant enzymes of nitrogen metabolism. For example, Komok [48] showed a reliable increase in the activity of glutamine synthetase in the leaves of soybean plants after pre-sowing treatment of seeds with microbial preparations Ryzobofit and Ryzohumin, the bioagents of which are nodule bacteria of soybean. The author noted that glutamine synthetase activity increased most significantly in the variant where seeds are inoculated with Ryzohumin, which, in addition to a highly efficient strain of soybean nodule bacteria, includes physiologically active substances, in particular, of cytokinin nature. Kopylov and Zhydenko [49] noted a significant increase in glutamine synthetase activity in the leaves of spring wheat plants after seed germination with the endophytic strain *A. brasilense* 102. The authors recorded the highest increase in enzymatic activity during joint treatment of wheat seeds with azospirilla and the soil saprotrophic fungus *Chaetomium cochliodes* 3250.

The results of our studies showed that *A. brasilense* B-7318, which is characterized by high nitrogenase activity, also contributes to a significant increase in glutamine synthetase activity in leaf tissues — by 57.0 % (Fig. 2 a).

Probably, high nitrogenase activity of azospirilla, as well as their ability to efficiently colonize the root surface, ensures the intensification of plant nitrogen metabolism due to the increase in nitrogenase and glutamine synthetase activity, which in turn influences protein synthesis in plant leaves. For example, we showed that upon inoculation of wheat seeds with *A. brasilense* B-7318 bacterial suspension,

the protein content in plant leaves increased by 9.7 % (Fig. 2 b). That is, a strain with high nitrogenase activity in a pure culture is characterized by the ability to actively colonize the roots of wheat plants, which has a positive effect on the parameters of nitrogenase activity in the rhizoplane, glutamine synthetase activity, as well as an increase in the content of water-soluble protein in the tissues of wheat leaves. Changes in enzymatic activity in plants can also be significantly influenced by the ability of azospirilla to synthesize phytohormones known from the literature [3; 7; 10].

The next stage of the work was to study the effect of seed inoculation with *A. brasilense* B-7318 bacterial suspension on the structural parameters of the spring wheat crop under the conditions of a field experiment (Table 2).

The ear length is a parameter that depends to the greatest extent on varietal characteristics of wheat, as compared with other structural parameters of the crop, and is not directly related to the size of the harvest, if we are talking about the comparison of different varieties based on this characteristic [50]. At the same time, according to our data, pre-sowing inoculation with azospirilla influences the ear length of spring wheat (Table 2). Thus, the length of the wheat ear of Tera variety in the variant using the inoculation of seeds with *A. brasilense* B-7318 significantly exceeded the corresponding parameter in the control by 8.1 %.

The number of grains per ear (graininess of an ear) is mostly determined by meteorological conditions, as well as the quality and timeliness of agrotechnical measures [50]. Thus, it has been established that the largest number of

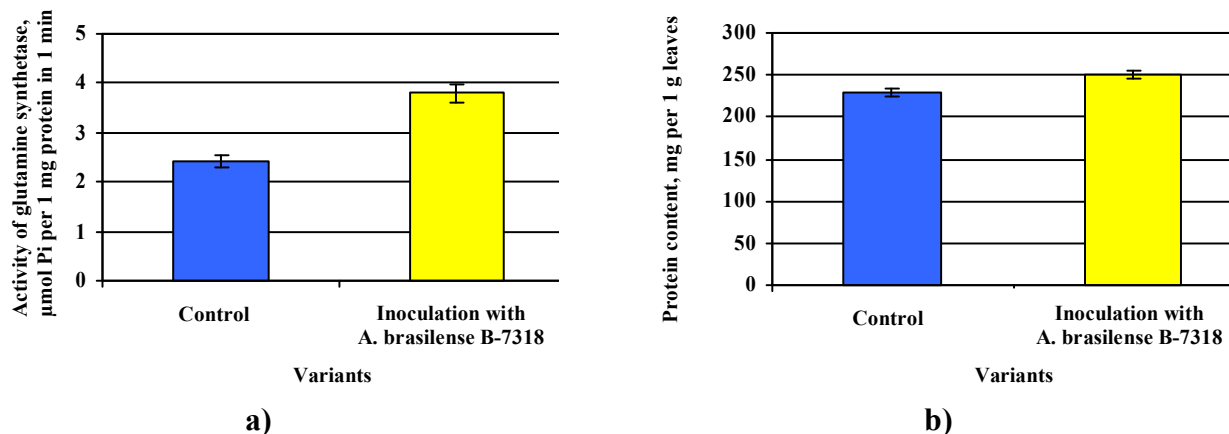


Fig. 2. Glutamine synthetase activity (a) and the content of soluble protein (b) in the leaves of spring wheat plants upon inoculation with *A. brasilense* B-7318 (pot experiment).

Table 2. Structural parameters of the yield of spring wheat of Tera variety after seeds inoculation with *A. brasilense* B-7318 (field experiment, 2018)

Variants of experiment	Ear length, cm	Number of grains per one ear	Weight of grains from one ear, g	Weight of 1,000 grains, g
Control	10.3	48.8	2.09	49.25
<i>A. brasilense</i> B-7318	11.1	50.8	2.40	52.25
LSD ₀₅	0.6	1.8	0.27	2.47

grains per ear is formed if the air temperature in late April — early May is about 12 °C. A lack of moisture in certain periods of vegetation reduces the grain size of the ear. The number of grains per ear predominantly depends on the level of supply of plants with mineral nutrients. According to Lykhochvor [51], when the rate of fertilizers was increased from N₈₀P₆₀K₆₀ to N₁₁₀P₈₀K₉₀, the grain size of an ear of winter wheat Myronivska 808 increased from 32–34 to 35–37 grains in variants with different rates of sowing seeds. A further increase in the fertilizer rate to N₁₄₀P₁₀₀K₁₂₀ on variants with sowing rates of 5.0 and 5.5 million/ha caused a decrease in the number of grains in the ear due to lodging. A feature of biological nitrogen, which is fixed by active diazotrophs and, in particular, *A. brasilense* B-7318, is its gradual entry into the root zone of plants throughout the growing season, which excludes excessive accumulation and ensures a positive effect on all stages of wheat plant development. Thus, we have shown an increase in the number of grains per one ear using pre-sowing inoculation by 4.1 % (Table 2).

Parameters of the weight of grains from one ear and weight of 1,000 grains depend on a number of factors that determine the weight of a grain; first of all, on the duration of its growth, the rate of accumulation of dry substances. Photosynthetic activity of the top three leaves of wheat plants has the greatest influence on grain size. The duration of single seed growth can be shortened due to high air temperature, lack of moisture, lack of nutrients in the soil, especially nitrogen. The highest weight of 1,000 grains is obtained by plants grown under favourable meteorological conditions during the period of forming and ripening of the grain. Grain size can be regulated by appropriate agrotechnical measures: precursors, fertilizer doses, sowing rates, sowing dates [50]. According to the results we obtained, bacterization of the seeds of spring wheat of Tera variety ensured a reliable increase in the weight of grains from one ear by 14.8 % and weight of 1,000 grains by 6.1 % (Table 2).

Literature has references that inoculation with *Azospirillum* is an efficient agricultural measure for increasing the yield of many agri-

cultural crops, in particular, winter rye, spring wheat, spring triticale, rice, corn, potatoes [5; 6; 21–24]. Our two-year results showed that the average yield of spring wheat of Tera variety after seed inoculation with *A. brasilense* B-7318 bacterial suspension increased by 15.7 % compared to the control (Table 3).

Conclusion. Therefore, PGPR bacterium *A. brasilense* B-7318, which is characterized by high nitrogenase activity in a pure culture, is able to settle down in the root zone of spring wheat plants (the number of bacterial cells per $10^4 \mu\text{m}^2$ in different root spheres ranged from 24 to 52 on average). The ability of *A. brasilense* B-7318 to actively colonize wheat plant roots contributed to a 4.9-fold increase in potential nitrogenase activity in the rhizoplane. Glutamine synthetase activity and soluble protein content in the leaves of spring wheat plants increased after inoculation by 57.0 % and 9.7 %, respectively. The inoculation provided a reliable increase in the structural parameters of the harvest (the ear length — by 8.1 %, number of grains per one ear — by 4.1 %, weight of grains in the ear — by 14.8 %, weight of 1,000 grains — by 6.1 %) and yield of spring wheat — by 15.7 %.

REFERENCES

1. Kloepper, J., Leong, J., Teintze, M., & Schroth, M. N. (1980). Enhanced plant growth by siderophores produced by plant growth-promoting rhizobacteria. *Nature*, 286, 885–886. <https://doi.org/10.1038/286885a0>
2. Pereg, L., de-Bashan, L. E., & Bashan, Y. (2016). Assessment of affinity and specificity of *Azospirillum* for plants. *Plant Soil*, 399, 389–414. <https://doi.org/10.1007/s11104-015-2778-9>
3. Bashan, Y., Holguin, G., & de-Bashan, L. E. (2004). *Azospirillum* – plant relationships: physiological, molecular, agricultural, and environmental

advances (1997–2003). *Can. J. Microbiol.*, 50(8), 521–577. <https://doi.org/10.1139/w04-035>

4. Vorobei, Yu. O., Nadkernychna, O. V., Shakhovna, O. O., Ushakova, M. A., & Leonov, O. Yu. (2008). Stvorennia efektyvnykh asotsiatsii “pshenytsia yara – diazotrofy rodu *Azospirillum*” [Creation of effective associations “spring wheat – diazotrophs of the genus *Azospirillum*”]. *Silskohospodarska mikrobiologhiia — Agricultural Microbiology*, 8, 71–81. [in Ukrainian]. <https://doi.org/10.35868/1997-3004.8.71-81>

5. Shakhovna, O. O. (2020). Efektyvnist vykorystannia asotsiatyvnykh diazotrofov dlia pidvyshchennia urozhainosti trytykale yarohto [The effectiveness of the use of associative diazotrophs to increase the yield of spring triticale]. *Visnyk ahrarnoi nauky — Bulletin of Agrarian Science*, 98(7), 25–30. [in Ukrainian]. <https://doi.org/10.31073/agrovisnyk202007-03>

6. Volkogon, V. V., Dimova, S. B., Volkogon, K. I., Sydorenko, V. P., & Volkogon, M. V. (2021). Biological nitrogen fixation and denitrification in rhizosphere of potato plants in response to the fertilization and inoculation. *Front. Sustain. Food Syst.*, 5. <https://doi.org/10.3389/fsufs.2021.606379>

7. Cassán, F., Vanderleyden, J., & Spaepen, S. (2014). Physiological and agronomical aspects of phytohormone production by model Plant-Growth-Promoting Rhizobacteria (PGPR) belonging to the genus *Azospirillum*. *J Plant Growth Regul.*, 33, 440–459. <https://doi.org/10.1007/s00344-013-9362-4>

8. Malhotra, M., Srivastava, S. (2009). Stress-responsive indole-3-acetic acid biosynthesis by *Azospirillum brasilense* SM and its ability to modulate plant growth. *Eur. J. Soil Biol.*, 45(1), 73–80. <https://doi.org/10.1016/j.ejsobi.2008.05.006>

9. Esquivel-Cote, R., Ramírez-Gama, R. M., Tsuzuki-Reyes, G., Orozco-Segovia, A., & Huan-te P. (2010). *Azospirillum* lipoferum strain AZm5 containing 1-aminocyclopropane-1-carboxylic acid deaminase improves early growth of tomato seedlings under nitrogen deficiency. *Plant Soil*, 337, 65–75. <https://doi.org/10.1007/s11104-010-0499-7>

Table 3. The effect of inoculation of spring wheat seeds with *A. brasilense* B-7318 on the yield of this culture (field experiments, 2017 to 2018)

Variants of experiment	Yield, t/ha			Yield increased, %
	2017	2018	mean for 2017–2018	
Control (without inoculation)	2.91	2.70	2.81	–
Inoculation with <i>A. brasilense</i> B-7318	3.46	3.03	3.25	15.7
LSD ₀₅	0.32	0.28	–	–

10. Manivannan, M., & Tholkappian, P. (2013). Prevalence of Azospirillum isolates in tomato rhizosphere soils of coastal areas of Cuddalore District, Tamil Nadu. *Int. J. Recent. Sci. Res.*, 4(10), 1610–1613.
11. Cohen, A. C., Bottini, R., Pontin, M., Berli, F. J., Moreno, D., Boccanlandro, H. ... Piccoli, P. N. (2015). Azospirillum brasilense ameliorates the response of Arabidopsis thaliana to drought mainly via enhancement of ABA levels. *Physiol Plant.*, 153(1), 79–90. <https://doi.org/10.1111/ppl.12221>
12. Cohen, A. C., Travaglia, C. N., Bottini, R., & Piccoli, P. N. (2009). Participation of abscisic acid and gibberelins produced by endophytic Azospirillum in the alleviation of drought effects in maize. *Botany*, 87(5), 455–462. <https://doi.org/10.1139/B09-023>
13. Ilyas N., & Bano A. (2010). Azospirillum strains isolated from roots and rhizosphere soil of wheat (*Triticum aestivum* L.) grown under different soil moisture conditions. *Biol. Fert. Soils.*, 46(4), 393–406.
14. Cassan, F., Maiale, S., Masciarelli, O., Vidal, A., Luna, V., & Ruiz, O. (2009). Cadaverine production by Azospirillum brasilense and its possible role in plant growth promotion and osmotic stress mitigation. *Eur. J. Soil. Biol.*, 45, 12–19. <https://doi.org/10.1016/j.ejsobi.2008.08.003>
15. Rodrigues-Salazar, J., Suarez, R., Caballero-Mellado, J., & Itturiaga, G. (2009). Trehalose accumulation in Azospirillum brasilense improves drought tolerance and biomass in maize plants. *FEMS Microbiol. Lett.*, 296(1), 52–59. <https://doi.org/10.1111/j.1574-6968.2009.01614.x>
16. Walker, V., Bertrand, C., Bellvert, F., Moëne-Loccoz, Y., Bally, R., & Comte, G. (2011). Host plant secondary metabolite profiling shows a complex, strain-dependent response of maize to plant growth-promoting rhizobacteria of the genus Azospirillum. *New Phytol.*, 189(2), 494–506. <https://doi.org/10.1111/j.1469-8137.2010.03484.x>
17. Chamam, A., Sanguin, H., Bellvert, F., Meiffren, G., Comte, G., Wisniewski-Dyé, F. ... Prigent-Combaret, C. (2013). Plant secondary metabolite profiling evidences strain-dependent effect in the Azospirillum – *Oriza sativa* association. *Phytochem.*, 87, 65–77. <https://doi.org/10.1016/j.phytochem.2012.11.009>
18. Walker, V., Couillerot, O., von Felten, A., Bellvert, F., Jansa, J., Maurhofer, M. ... Comte, G. (2012). Variation of secondary metabolite levels in maize seedling roots induced by inoculation with Azospirillum, Pseudomonas and Glomus consortium under field conditions. *Plant Soil.*, 356, 151–163. <https://doi.org/10.1007/s11104-011-0960-2>
19. Bulegon, L. G., Guimaraes, V. F., Klein, J., Battistus, A. G., Inagaki, A. M., Offemann, L. C., & Souza, A. K. P. (2017). Enzymatic activity, gas exchange and production of soybean co-inoculated with Bradyrhizobium japonicum and Azospirillum brasilense. *Aust J Crop Sci.*, 11, 888–896. <https://doi.org/10.21475/ajcs.17.11.07.pne575>
20. Galindo, F. S., Teixeira Filho, M. C. M., Buzetti, S., Santini, J. M. K., Alves, C. J., & Ludkiewicz, M. G. Z. (2017). Effects of nitrogen fertilization and inoculation with Azospirillum brasilense on wheat yield in the Cerrado region. *Pesq Agropec Bras.*, 52, 794–805. <https://doi.org/10.1590/s0100-204x2017000900012>
21. Galindo, F. S., Teixeira Filho, M. C. M., Buzetti, S., Santini, J. M. K., Alves, C. J., Nogueira, L. M. ... Bellotte, J. L. M. (2016). Corn yield and foliar diagnosis affected by nitrogen fertilization and inoculation with Azospirillum brasilense. *Rev Bras Cienc Solo.*, 40. e015036. <https://doi.org/10.1590/18069657rbc20150364>
22. de-Bashan, L. E., Hernandez, J. P., & Bashan, Y. (2012). The potential contribution of plant growth-promoting bacteria to reduce environmental degradation — a comprehensive evaluation. *Appl Soil Ecol.*, 61, 171–189. <https://doi.org/10.1016/j.apsoil.2011.09.003>
23. Nadkernychna, O. V. (2000). Vykorystannia azotfiksuichykh bakterii Azospirillum brasilense dlia polipshennia yakosti zerna ozymoho zhyta [The use of nitrogen-fixing bacteria Azospirillum brasilense to improve the quality of winter rye grain]. *Biulleten ISHM UAAN — Bulletin of the Institute of Agricultural Microbiology UAAS*, 8, 18–20 [in Ukrainian].
24. Mattos, M. L. T., Valgas, R. A., & Martins, J. F. da S. (2022). Evaluation of the agronomic efficiency of Azospirillum brasilense strains Ab-V5 and Ab-V6 in flood-irrigated rice. *Agronomy*, 12, 3047. <https://doi.org/10.3390/agronomy12123047>
25. Okon, Y. (1985). Azospirillum as a potential inoculant for agriculture. *Trends in Biotechnology*, 3(9), 223–228. [https://doi.org/10.1016/0167-7799\(85\)90012-5](https://doi.org/10.1016/0167-7799(85)90012-5)
26. Bashan, Y., Levanony, H. (1989). Factors, effecting adsorption of Azospirillum brasilense Cd to root hairs as compared with root surface of wheat. *Can. J. Microbiol.*, 35(10), 936–944. <https://doi.org/10.1139/m89-155>
27. Brenner, D. J., Krieg, N. R., Staley, J. T. (Eds.). Editor-in-chief G. M. Garrity (2005). *Bergey's Manual of Systematic Bacteriology. The Gammaproteobacteria*. Part A + B + C. 2nd ed. New York: Springer. <https://doi.org/10.1007/0-387-28021-9>
28. Kordyum, V. A., Moshinets, E. V., Tsapenko, M. V., Adamchuk-Chalaya, N. I., Irodov, D. M., & Andrienko, V. I. (2008). Mikroorganizmy rizosfery — polnyy monitoring [Rhizosphere Microorganisms — Full Monitoring]. *Gruntoznavstvo — Soil science*, 9(1–2), 53–63 [in Russian].

29. Hardy, R. W. F., Burns, R. C., & Holsten, R. D. (1973). Application of the acetylene-ethylene assay for measurement of nitrogen fixation. *Soil Biol. Biochem.*, 5(1), 41–83.
30. Volkohon, V. V., Nadkernychna, O. V., Tokmakova, L. M., Melnychuk, T. M., & Chaikovska, L. O. (2010). *Eksperymentalna gruntova mikrobiolohiia* [Experimental soil microbiology]. Kyiv: Ahrarna nauka [in Ukrainian].
31. Sadasivam, S., Manickam, A. (1996). *Biochemical methods*. New Delhi: New Age International.
32. Lowry, O. H., Rosebrough, N. J., Farr, A. L., & Randall, R. J. (1951). Protein measurement with Folin phenol reagent. *J. Biol. Chem.*, 193(1), 265–275.
33. Hrytsaienko, Z. M., Hrytsaienko, A. O., & Karpenko, V. P. (2003). *Metody biolohichnykh ta ahrokhimichnykh doslidzhen roslyn ta gruntiv* [Methods of biological and agrochemical research of plants and soils]. Kyiv: Nichlava [in Ukrainian].
34. Volkohon, V. V., Vorobei, Yu. O., Nadkernychna, O. V. (Eds.). (2015). *Kataloh kultur mikroorhanizmiv* [Catalogue of cultures of microorganisms]. Chernihiv: publ. Brahynets O. V. [in Ukrainian].
35. Pat. 85924 UA, MPK C05F 11/08, C12N 1/20, C12R 1/00. Shtam bakterii Azospirillum brasilense dlia vyhotovlennia bakteriialnogo dobrovya pid pshenytsiu yaru [Bacterial strain Azospirillum brasilense for producing bacterial fertilizer for spring wheat], Kopylov, Ye. P., Publ. 10.03.2009 [in Ukrainian].
36. Pat. 104212 C2 UA, MPK C12N 1/20 (2006.01) C12R (2006.01) C05F11/08 (2006.01). Shtam bakterii Azospirillum brasilense dlia inokuliatsii nasinnia trytykale yarohe [Bacterial strain Azospirillum brasilense for inoculation of spring triticale seeds], Nadkernychna, O. V., Shakhovnina, O. O., Ushakova, M. A., Publ. 10.01.2014 [in Ukrainian].
37. Pat. 105118 UA, MPK C12N 1/20 (2006.01). Shtam aktyvnykh azotfiksuvalnykh bakterii Azospirillum brasilense dlia inokuliatsii nasinnia pshenytsi yaroï [Strain of active nitrogen-fixing bacteria Azospirillum brasilense for inoculation of spring wheat seeds], Nadkernychna, O. V., Vorobei Yu. O., Shakhovnina, O. O., Publ. 10.03.2016 [in Ukrainian].
38. Vorobei, Yu., Deriabin, O., & Usmanova, T. (2020). Phenotypic and molecular characterization of nitrogen-fixing bacteria Azospirillum brasilense 137 capable to root colonization of spring wheat. *Analele Universităţii din Oradea, Fascicula Biologie*, XXVII(2), 129–135.
39. Patriquin, D. G., Döbereiner, J., & Jain, D. K. (1983). Sites and processes of association between diazotrophs and grasses. *Can. J. Microbiol.*, 29(8), 900–915. <https://doi.org/10.1139/m83-146>
40. Umali-Garcia, M., Hubbel, D. H., Gaskins, M. H., & Dazzo, F. B. (1980). Association of Azospirillum with grass roots. *Applied and Environmental Microbiology*, 39(1), 219–226. <https://doi.org/10.1128/aem.39.1.219-226.1980>
41. Gafny, R., Okon, Y., Kapulnik, Y., & Fischer, M. (1986). Adsorption of Azospirillum brasilense to corn roots. *Soil Biol. Biochem.*, 18(1), 69–76. [https://doi.org/10.1016/0038-0717\(86\)90105-7](https://doi.org/10.1016/0038-0717(86)90105-7)
42. Merrick, M. J., & Edwards, R. A. (1995). Nitrogen control in bacteria. *Microbiol Rev.*, 59(4), 604–622. <https://doi.org/10.1128/mr.59.4.604-622.1995>
43. Miflin, B. J., & Habash, D. Z. (2002). The role of glutamine synthetase and glutamate dehydrogenase in nitrogen assimilation and possibilities for improvement in nitrogen utilization of crops. *J. Exp. Bot.*, 53(370), 979–987. <http://dx.doi.org/10.1093/jexbot/53.370.979>
44. Gallori, E., & Bazzicalupo, M. (1985). Effect of nitrogen compounds on nitrogenase activity in Azospirillum brasilense. *FEMS Microbiology Letters*, 28(1), 35–38. <https://doi.org/10.1111/j.1574-6968.1985.tb00759.x>
45. Okon, Y., Albrecht, S. L., & Burris, R. H. (1976). Carbon and ammonia metabolism of Spirillum lipoferum. *Journal of Bacteriology*, 128(2), 592–597. <https://doi.org/10.1128/jb.128.2.592-597.1976>
46. Gauthier, D., & Elmerich, C. (1977). Relationship between glutamine synthetase and nitrogenase in Spirillum lipoferum. *FEMS Microbiology Letters*, 2(2), 101–104. <https://doi.org/10.1111/j.1574-6968.1977.tb00917.x>
47. Zhang, Y., Burris, R. H., Ludden, P. W., & Roberts, G. P. (2006). Regulation of nitrogen fixation in Azospirillum brasilense. *FEMS Microbiology Letters*, 152(2), 195–204. <https://doi.org/10.1111/j.1574-6968.1997.tb10428.x>
48. Komok, M. S. (2012). Fiziolohichno aktyvni rechovyny yak faktor aktyvizatsii azotfiksuvalnykh symbioziv ta azotnoho obminu soi [Physiologically active substances as a factor in activation of nitrogen-fixing symbioses and nitrogen metabolism of soybeans]. *Silskohospodarska mikrobiolohiia — Agricultural Microbiology*, 14, 49–63 [in Ukrainian]. <https://doi.org/10.35868/1997-3004.14.49-63>
49. Kopylov, Ye. P., & Zhydenko, A. O. (2009). Vplyv hruntovoho saprotrofnogo hryba Chaetomium cochliodes na asotsiatyvnu systemu “Triticum aestivum – Azospirillum brasilense” [Influence of soil saprophyte fungus Chaetomium cochliodes on associative “Triticum aestivum – Azospirillum brasilense”]. *Visnyk Dnipropetrovskoho universytetu — Visnyk of Dnipropetrovsk University*, 17(3), 33–39 [in Ukrainian].

50. Avramenko, R. A., & Kirsanova, H. V. (2004). *Vyznachennia biolohichnoho vrozhaiu osnovnykh silskohospodarskykh kultur* [Determination of biological yield of major crops]. Dnipropetrovsk: Editorial and publishing department of Dnipropetrovsk State Agrarian University [in Ukrainian].

trovsk State Agrarian University [in Ukrainian].

51. Lykhochvor, V. V. (1999). *Struktura vrozhaiu ozymoї pshenytsi* [Structure of winter wheat yield]. Lviv: Ukrainski tekhnolohii [in Ukrainian].

Received 23.01.2023

Сільськогосподарська мікробіологія. 2023. Вип. 37. С. 48–60.
ISSN 1997-3004

<https://doi.org/10.35868/1997-3004.37.48-60>

УДК 631.461:633.11

ЕФЕКТИВНІСТЬ АСОЦІАЦІЇ «ЯРА ПШЕНИЦЯ – *AZOSPIRILLUM BRAZILENSE* B-7318»

Ю. О. Воробей, О. О. Шаховніна, О. В. Логоша, Т. О. Усманова

Інститут сільськогосподарської мікробіології та агропромислового виробництва НААН, м. Чернігів
e-mail: yu.a.vorobey@gmail.com

Мета. Дослідити здатність *Azospirillum brasilense* B-7318 — бактерії, що інтенсивно фіксує молекулярний азот — утворювати стійкі ефективні асоціації з рослинами пшениці ярої. **Методи.** Мікробіологічні, аплікаційний (для визначення інтенсивності розвитку азоспірил у корневих сферах рослин), ацетиленовий (для дослідження нітрогеназної активності), фосфатний (для визначення активності глутамінсинтетази в листках рослин), польового досліду, статистичні. **Результати.** Методом аналітичної селекції одержано новий штам *A. brasilense* B-7318, здатний інтенсивно фіксувати молекулярний азот. Показано, що за інокуляції насіння ярої пшениці діазотрофи ефективно колонізують поверхню коренів і ризосферний ґрунт (найбільшу щільність розташування бактерій спостерігали до 2 мм від поверхні кореня). Потенційна нітрогеназна активність у ризосферному ґрунті за використання для інокуляції *A. brasilense* B-7318 істотно не відрізнялася від значень контрольного варіанту (без інокуляції). Водночас потенційна нітрогеназна активність на відмитих коренях інокульованих рослин значно (в 4,9 раза) перевищувала цей показник в ризоплані рослин контрольного варіанту. *A. brasilense* B-7318 сприяє також істотному зростанню глутамінсинтетазної активності в листках рослин пшениці — на 57%. За інокуляції насіння пшениці бактеріальною суспензією *A. brasilense* B-7318 вміст білка у листках рослин підвищувався на 9,7%. Результати визначення структури урожаю у польовому досліді засвідчили, що азоспірили забезпечували достовірний приріст довжини колосу (на 8,1%), кількості зерен у колосі (на 4,1%), маси зерен з колосу (на 14,8%) та маси 1000 зерен (на 6,1%), а також підвищення урожайності на 15,7% проти контролю. **Висновки.** Бактерія *A. brasilense* B-7318, що характеризується високою нітрогеназною активністю в чистій культурі, здатна активно колонізувати кореневі сфери рослин пшениці ярої, підвищувати глутамінсинтетазну активність та вміст білка у листках, а також сприяти підвищенню потенційної нітрогеназної активності на коренях рослин і зростанню урожайності пшениці.

Ключові слова: *Azospirillum brasilense*, PGPR, яра пшениця, нітрогеназна активність.

ЦИТОВАНА ЛІТЕРАТУРА

1. Kloepper J., Leong J., Teintze M., Schroth M. N. Enhanced plant growth by siderophores produced by plant growth-promoting rhizobacteria. *Nature*. 1980. № 286. P. 885–886.

<https://doi.org/10.1038/286885a0>

2. Pereg L., de-Bashan L. E., Bashan Y. Assessment of affinity and specificity of *Azospirillum* for plants. *Plant Soil*. 2016. № 399. P. 389–414. <https://doi.org/10.1007/s11104-015-2778-9>

3. Bashan Y., Holguin G., de-Bashan L. E. Azospirillum – plant relationships: physiological, molecular, agricultural, and environmental advances (1997–2003). *Can. J. Microbiol.* 2004. Vol. 50. № 8. P. 521–577. <https://doi.org/10.1139/w04-035>
4. Воробей Ю. О., Надкернична О. В., Шаховніна О. О., Ушакова М. А., Леонов О. Ю. Створення ефективних асоціацій «Пшениця яра – діазотрофи роду *Azospirillum*». *Сільськогосподарська мікробіологія*. 2008. Вип. 8. С. 71–81. <https://doi.org/10.35868/1997-3004.8.71-81>
5. Шаховніна О. О. Ефективність використання асоціативних діазотрофів для підвищення урожайності тритикале ярого. *Вісник аграрної науки*. 2020. Т. 98, № 7. С. 25–30. <https://doi.org/10.31073/agrovisnyk202007-03>
6. Volkogon V. V., Dimova S. B., Volkogon K. I., Sydorenko V. P., Volkogon M. V. Biological nitrogen fixation and denitrification in rhizosphere of potato plants in response to the fertilization and inoculation. *Front. Sustain. Food Syst.* 2021. Vol. 5. <https://doi.org/10.3389/fsufs.2021.606379>
7. Cassán F., Vanderleyden J., Spaepen S. Physiological and agronomical aspects of phytohormone production by model Plant-Growth-Promoting Rhizobacteria (PGPR) belonging to the genus *Azospirillum*. *J Plant Growth Regul.* 2014. № 33. P. 440–459. <https://doi.org/10.1007/s00344-013-9362-4>
8. Malhotra M., Srivastava S. Stress-responsive indole-3-acetic acid biosynthesis by *Azospirillum brasilense* SM and its ability to modulate plant growth. *Eur. J. Soil Biol.* 2009. Vol. 45, № 1. P. 73–80. <https://doi.org/10.1016/j.ejsobi.2008.05.006>
9. Esquivel-Cote R., Ramírez-Gama R. M., Tsuzuki-Reyes G., Orozco-Segovia A., Huante P. *Azospirillum lipoferum* strain AZm5 containing 1-aminocyclopropane-1-carboxylic acid deaminase improves early growth of tomato seedlings under nitrogen deficiency. *Plant Soil.* 2010. № 337. P. 65–75. <https://doi.org/10.1007/s11104-010-0499-7>
10. Manivannan M., Tholkappian P. Prevalence of *Azospirillum* isolates in tomato rhizosphere soils of coastal areas of Cuddalore District, Tamil Nadu. *Int. J. Recent. Sci. Res.* 2013. Vol. 4, Is. 10. P. 1610–1613.
11. Cohen A. C., Bottini R., Pontin M., Berli F. J., Moreno D., Boccanlandro H. ... Piccoli P. N. *Azospirillum brasilense* ameliorates the response of *Arabidopsis thaliana* to drought mainly via enhancement of ABA levels. *Physiol Plant.* 2015. Vol. 153, Is. 1. P. 79–90. <https://doi.org/10.1111/ppl.12221>
12. Cohen A. C., Travaglia C. N., Bottini R., Piccoli P. N. Participation of abscisic acid and gibberelins produced by endophytic *Azospirillum* in the alleviation of drought effects in maize. *Botany.* 2009. Vol. 87, № 5. P. 455–462. <https://doi.org/10.1139/B09-023>
13. Ilyas N., Bano A. *Azospirillum* strains isolated from roots and rhizosphere soil of wheat (*Triticum aestivum* L.) grown under different soil moisture conditions. *Biol. Fert. Soils.* 2010. Vol. 46, № 4. P. 393–406.
14. Cassan F., Maiale S., Masciarelli O., Vidal A., Luna V., Ruiz O. Cadaverine production by *Azospirillum brasilense* and its possible role in plant growth promotion and osmotic stress mitigation. *Eur. J. Soil. Biol.* 2009. № 45. P. 12–19. <https://doi.org/10.1016/j.ejsobi.2008.08.003>
15. Rodrigues-Salazar J., Suarez R., Caballero-Mellado J., Itturiaga G. Trehalose accumulation in *Azospirillum brasilense* improves drought tolerance and biomass in maize plants. *FEMS Microbiol. Lett.* 2009. Vol. 296, № 1. P. 52–59. <https://doi.org/10.1111/j.1574-6968.2009.01614.x>
16. Walker V., Bertrand C., Bellvert F., Moëne-Loccoz Y., Bally R., Comte G. Host plant secondary metabolite profiling shows a complex, strain-dependent response of maize to plant growth-promoting rhizobacteria of the genus *Azospirillum*. *New Phytol.* 2011. Vol. 189, № 2. P. 494–506. <https://doi.org/10.1111/j.1469-8137.2010.03484.x>
17. Chamam A., Sanguin H., Bellvert F., Meiffren G., Comte G., Wisniewski-Dyé F. ... Prigent-Combaret C. Plant secondary metabolite profiling evidences strain-dependent effect in the *Azospirillum* – *Oriza sativa* association. *Phytochem.* 2013. № 87. P. 65–77. <https://doi.org/10.1016/j.phytochem.2012.11.009>
18. Walker V., Couillerot O., von Felten A., Bellvert F., Jansa J., Maurhofer M. ... Comte G. Variation of secondary metabolite levels in maize seedling roots induced by inoculation with *Azospirillum*, *Pseudomonas* and *Glomus* consortium under field conditions. *Plant Soil.* 2012. Vol. 356. P. 151–163. <https://doi.org/10.1007/s11104-011-0960-2>
19. Bulegon L. G., Guimaraes V. F., Klein J., Battistus A. G., Inagaki A. M., Offemann L. C., Souza A. K. P. Enzymatic activity, gas exchange and production of soybean co-inoculated with *Bradyrhizobium japonicum* and *Azospirillum brasilense*. *Aust J Crop Sci.* 2017. № 11. P. 888–896. <https://doi.org/10.21475/ajcs.17.11.07.pne575>
20. Galindo F. S., Teixeira Filho M. C. M., Buzetti S., Santini J. M. K., Alves C. J., Ludkiewicz M. G. Z. Effects of nitrogen fertilization and inoculation with *Azospirillum brasilense* on wheat yield in the Cerrado region. *Pesq Agropec Bras.* 2017. № 52. P. 794–805. <https://doi.org/10.1590/s0100-204x2017000900012>
21. Galindo F. S., Teixeira Filho M. C. M., Buzetti S., Santini J. M. K., Alves C. J., Nogueira L. M. ... Bellotte J. L. M. Corn yield and foliar diagnosis affected by nitrogen fertilization and inoculation with *Azospirillum brasilense*. *Rev Bras*

- Cienc Solo*. 2016. № 40. e015036. <https://doi.org/10.1590/18069657rbcs20150364>
22. de-Bashan L. E., Hernandez J. P., Bashan Y. The potential contribution of plant growth-promoting bacteria to reduce environmental degradation — a comprehensive evaluation. *Appl Soil Ecol*. 2012. № 61. P. 171–189. <https://doi.org/10.1016/j.apsoil.2011.09.003>
23. Надкернична О. В. Використання азотфіксуєючих бактерій *Azospirillum brasilense* для поліпшення якості зерна озимого жита. *Бюл. ІСГМ УААН*. 2000. № 8. С. 18–20.
24. Mattos M. L. T., Valgas R. A., Martins J. F. da S. Evaluation of the agronomic efficiency of *Azospirillum brasilense* strains Ab-V5 and Ab-V6 in flood-irrigated rice. *Agronomy*. 2022. № 12. 3047. <https://doi.org/10.3390/agronomy12123047>
25. Okon Y. *Azospirillum* as a potential inoculant for agriculture. *Trends in Biotechnology*. 1985. Vol. 3, № 9. P. 223–228. [https://doi.org/10.1016/0167-7799\(85\)90012-5](https://doi.org/10.1016/0167-7799(85)90012-5)
26. Bashan Y., Levanony H. Factors, effecting adsorption of *Azospirillum brasilense* Cd to root hairs as compared with root surface of wheat. *Can. J. Microbiol*. 1989. Vol. 35, № 10. P. 936–944. <https://doi.org/10.1139/m89-155>
27. Bergey's Manual of Systematic Bacteriology. The Gammaproteobacteria. Part A + B + C. 2nd ed. / Eds. D. J. Brenner, N. R. Krieg, J. T. Staley. Editor-in-chief G. M. Garrity. New York: Springer, 2005. 1388 p. <https://doi.org/10.1007/0-387-28021-9>
28. Кордюм В. А., Мошинец Е. В., Цапенко М. В., Адамчук-Чалая Н. И., Иродов Д. М., Андриенко В. И. Микроорганизмы ризосферы — полный мониторинг. *Грунтознавство*. 2008. Т. 9, № 1–2. С. 53–63.
29. Hardy R. W. F., Burns R. C., Holsten R. D. Application of the acetylene-ethylene assay for measurement of nitrogen fixation. *Soil Biol. Biochem*. 1973. Vol. 5, № 1. P. 41–83.
30. Волкогон В. В., Надкернична О. В., Токмакова Л. М., Мельничук Т. М., Чайковська Л. О. Експериментальна ґрунтова мікробіологія : монографія / за наук. ред. В. В. Волкогона. К. : Аграрна наука, 2010. 464 с.
31. Sadasivam S., Manickam A. Biochemical methods. New Delhi: New Age International, 1996. 272 p.
32. Lowry O. H., Rosebrough N. J., Farr A. L., Randall R. J. Protein measurement with Folin phenol reagent. *J. Biol. Chem*. 1951. Vol. 193, № 1. P. 265–275.
33. Грицаєнко З. М., Грицаєнко А. О., Карпенко В. П. Методи біологічних та агрохімічних досліджень рослин та ґрунтів. К. : Нічлава, 2003. 320 с.
34. Каталог культур мікроорганізмів / за ред. В. В. Волкогона, Ю. О. Воробей, О. В. Надкерничної. Чернігів : видавець Брагинець О. В., 2015. 48 с.
35. Штам бактерій *Azospirillum brasilense* для виготовлення бактеріального добрива під пшеницю яру: пат. 85924 Україна. МПК C05F 11/08, C12N 1/20, C12R 1/00, Є. П. Копилов; заявник і патентовласник: Інститут сільськогосподарської мікробіології УААН. № а 2007 06203; заявл. 04.06.2007; опубл. 10.03.2009, Бюл. № 5.
36. Штам бактерій *Azospirillum brasilense* для інокуляції насіння тритикале ярого: пат. 104212 C2 Україна. МПК C12N 1/20 (2006.01) C12R (2006.01) C05F11/08 (2006.01), О. В. Надкернична, О. О. Шаховніна, М. А. Ушакова; заявник і патентовласник: Інститут сільськогосподарської мікробіології та агропромислового виробництва НААН. № а 2012 03817; заявл. 29.03.2012; опубл. 10.01.2014, Бюл. № 1.
37. Штам активних азотфіксувальних бактерій *Azospirillum brasilense* для інокуляції насіння пшениці ярої: пат. 105118 Україна МПК C12N 1/20 (2006.01), О. В. Надкернична, Ю. О. Воробей, О. О. Шаховніна, М. А. Ушакова; заявник і патентовласник Інститут сільськогосподарської мікробіології та агропромислового виробництва НААН. № u 2015 07326; заявл. 21.07.2015; опубл. 10.03.2016, Бюл. № 5.
38. Vorobei Y., Deriabin O., Usmanova T. Phenotypic and molecular characterization of nitrogen-fixing bacteria *Azospirillum brasilense* 137 capable to root colonization of spring wheat. *Analele Universității din Oradea, Fascicula Biologie*. 2020. Vol. XXVII, Is. 2. P. 129–135.
39. Patriquin D. G., Döbereiner J., Jain D. K. Sites and processes of association between diazotrophs and grasses. *Can. J. Microbiol*. 1983. Vol. 29, № 8. P. 900–915. <https://doi.org/10.1139/m83-146>
40. Umali-Garcia M., Hubbel D. H., Gaskins M. H., Dazzo F. B. Association of *Azospirillum* with grass roots. *Applied and Environmental Microbiology*. 1980. Vol. 39, № 1. P. 219–226. <https://doi.org/10.1128/aem.39.1.219-226.1980>
41. Gafny R., Okon Y., Kapulnik Y., Fischer M. Adsorption of *Azospirillum brasilense* to corn roots. *Soil Biol. Biochem*. 1986. Vol. 18, № 1. P. 69–76. [https://doi.org/10.1016/0038-0717\(86\)90105-7](https://doi.org/10.1016/0038-0717(86)90105-7)
42. Merrick M. J., Edwards R. A. Nitrogen Control in Bacteria. *Microbiol Rev*. 1995. Vol. 59, № 4. P. 604–622. <https://doi.org/10.1128/mr.59.4.604-622.1995>
43. Mifflin B. J., Habash D. Z. The role of glutamine synthetase and glutamate dehydrogenase in nitrogen assimilation and possibilities for improvement in nitrogen utilization of crops. *J. Exp. Bot*. 2002. Vol. 53, № 370. P. 979–987. <http://dx.doi.org/10.1093/jexbot/53.370.979>
44. Gallori E., Bazzicalupo M. Effect of nitro-

- gen compounds on nitrogenase activity in *Azospirillum brasilense*. *FEMS Microbiology Letters*. 1985. Vol. 28, № 1. P. 35–38. <https://doi.org/10.1111/j.1574-6968.1985.tb00759.x>
45. Okon Y., Albrecht S. L., Burris R. H. Carbon and ammonia metabolism of *Spirillum lipoferum*. *Journal of Bacteriology*. 1976. Vol. 128, № 2. P. 592–597. <https://doi.org/10.1128/jb.128.2.592-597.19761976>
46. Gauthier D., Elmerich C. Relationship between glutamine synthetase and nitrogenase in *Spirillum lipoferum*. *FEMS Microbiology Letters*. 1977. Vol. 2, № 2. P. 101–104. <https://doi.org/10.1111/j.1574-6968.1977.tb00917.x>
47. Zhang Y., Burris R. H., Ludden P. W., Roberts G. P. Regulation of nitrogen fixation in *Azospirillum brasilense*. *FEMS Microbiology Letters*. 2006. Vol. 152, № 2. P. 195–204. <https://doi.org/10.1111/j.1574-6968.1997.tb10428.x>
48. Комок М. С. Фізіологічно активні речовини як фактор активізації азотфіксувальних симбіозів та азотного обміну сої. *Сільськогосподарська мікробіологія*. 2012. Вип. 14. С. 49–63. <https://doi.org/10.35868/1997-3004.14.49-63>
49. Копилов Є. П., Жиденко А. О. Вплив ґрунтового сапротрофного гриба *Chaetomium cochliodes* на асоціативну систему «*Triticum aestivum* – *Azospirillum brasilense*». *Вісник Дніпропетровського університету. Біологія. Екологія*. 2009. Вип. 17, № 3. С. 33–39.
50. Авраменко Р. А., Кірсанова Г. В. Визначення біологічного врожаю основних сільськогосподарських культур. Дніпропетровськ : Редакційно-видавничий відділ Дніпропетровського держагроуніверситету, 2004. 47 с.
51. Лихочвор В. В. Структура врожаю озимої пшениці: монографія. Львів : Українські технології, 1999. 200 с.

Отримано 23.01.2023