# Nitrogen use by plants and nitrogen flows after application of standard and biomodified nitrogen fertilizers on barley

**Alexey Zavalin[1], Vladimir Chebotar[2], Alexey Alferov[1], Lyudmila Chernova[1], Elena Shcherbakova[2], and Elena Chizhevskaya[2]**

[1]All-Russian Scientific Research Institute of Agrochemistry named after D. N. Pryanishnikov, ul. Pryanishnikova, 31a, Moscow, 127434, Russian Federation
[2]All-Russia Research Institute for Agricultural Microbiology, Shosse Podbel'skogo, 3, Saint Petersburg, 196608, Russian Federation

Address correspondence and requests for materials to Vladimir Chebotar, vladchebotar@rambler.ru

## Abstract

The aim of our study was to assess the efficiency of application of biomodified nitrogen fertilizers for barley, to reveal the sources of nitrogen used for biomass formation with the use of the $^{15}N$ stable isotope, and to study nitrogen flows in the system of fertilizers–soil–plants–atmosphere. We demonstrated in a model experiment the ability of the plant growth-promoting bacteria *Bacillus subtilis* Ch-13 to move from the granules of mineral fertilizers to plant roots and to colonize them effectively. The effectiveness of biomodified nitrogen fertilizers for barley, Nur variety, was assessed in a microfield trial. After the application of biomodified nitrogen fertilizers, the accumulation of $^{15}N$ in the plants increased by 2–5 %, its incorporation in the soil decreased and gaseous losses were decreased by 7 % as compared with the use of the usual forms of fertilizers. The application of biomodified nitrogen fertilizers can be used in agricultural practice as a novel technology to regulate nitrogen flows in the system of fertilizers–soil–plants–atmosphere.

**Keywords:** PGPB, nitrogen fertilizers, biomodified fertilizers, nitrogen isotope, barley biomass, nitrogen flows

**Citation:** Zavalin, A., Chebotar, V., Alferov, A., Chernova, L., Shcherbakova, E., and Chizhevskaya, E. 2021. Nitrogen use by plants and nitrogen flows after application of standard and biomodified nitrogen fertilizers on barley. Bio. Comm. 66(4): 283–289. https://doi.org/10.21638/spbu03.2021.401

**Authors' information:** Alexey Zavalin, Dr. of Sci. in Agricultural Sciences, Professor, Head of Laboratory, orcid.org/0000-0001-7717-877X; Vladimir Chebotar, PhD, Head of Laboratory, orcid.org/0000-0001-9762-989X; Alexey Alferov, Dr. of Sci. in Biology, Senior Researcher, orcid.org/0000-0001-7988-6809; Lyudmila Chernova, PhD, Senior Researcher, orcid.org/0000-0001-8424-1175; Elena Shcherbakova, PhD, Junior Researcher, orcid.org/0000-0002-7871-034X; Elena Chizhevskaya, PhD, Senior Researcher, orcid.org/0000-0002-7715-8696

**Manuscript Editor:** Alla Krasikova, Department of Cytology and Histology, Faculty of Biology, Saint Petersburg State University, Saint Petersburg, Russia

**Received:** October 13, 2020;

**Revised:** August 10, 2021;

**Accepted:** August 16, 2021.



**Funding:** The study was partly supported by the Russian Foundation for Basic Research, Grant No. 18-016-00048 A. The work of V. K. Chebotar and E. P. Chizhevskaya was supported by the project "Development of potato breeding and seed production in the Russian Federation" of the Federal Scientific and Technical Program for Agricultural Development for 2017–2025.

**Ethics statement:** This paper does not contain any studies involving human participants or animals performed by any of the authors.

**Competing interests:** The authors have declared that no competing interests exist.

## Introduction

About 3 million tons of the active ingredient (NPK) of mineral fertilizers are annually used in Russian agriculture, which corresponds to 40 kg of the active ingredient (NPK) per 1 ha of crops. Less than half of the nutrients introduced with mineral fertilizers is actually used by plants for yield formation (Zavalin and Sokolov, 2019). Increasing the efficiency of mineral fertilizers is important both economically and ecologically (Ohkama-Ohtsu and Wasaki, 2010). One of the ways to achieve this is to use microbial preparations based on plant growth-promoting bacteria for treatment of granules of mineral fertilizers (Hassan et al., 2019).

The mechanism of their action is based on the fact that the microorganisms used for pelleting of granules of mineral fertilizers increase the availability of nutrients contained in mineral fertilizers and mobilize their reserves in soil; they produce amino acids, vitamins, hormones and organic acids promoting plant growth and enhancing its immune defences; and they synthesize substances blocking the development of phytopathogenic microorganisms (Adesemoye and Kloepper, 2009; Chebotar et al., 2009; Bhat 2019). After treatment with bacteria, a "biocapsule" is formed on the surface of pelleted mineral fertilizers. It has several functions at the same time: it fertilizes, protects and stimulates.

Such a combined beneficial effect makes it possible to achieve a considerable increase in the yield of agricultural crops and thus, in the pay-off of mineral

fertilizers (Kozhemyakov et al., 2015; Chebotar et al., 2016b). Such microorganisms enhance plant resistance to phytopathogens (Barraquio et al., 1997; Rothballer et al., 2007; Chebotar et al., 2015) and their ability to synthesize phytohormones (Bloemberg and Lugtenberg, 2001), stimulate root system growth thus improving mineral nutrition (Okon and Vanderleyden, 1997; Bertrand et al., 2000; Ruby and Raghunath, 2011; Chebotar et al., 2016a), and regulate the rate of water uptake (Dobbelaere et al., 2001; Shaposhnikov et al., 2011). Plant growth-promoting bacteria (PGPB) *Bacillus subtilis* Ch-13, used in Russia for the production of the microbiological fertilizer Extrasol, stimulated plant growth, producing phytohormones — auxin derivatives (Chebotar et al., 2009). Inoculation with *Bacillus subtilis* Ch-13 reduced plant stress induced by heavy metals exposure and salinity (Pishchik et al., 2009).

The aim of our study was: 1) to assess the efficiency of application of nitrogen fertilizers biomodified by the *Bacillus subtilis* Ch-13 for barley (*Hordeum vulgare* L.), and 2) to reveal the sources of nitrogen used for biomass formation with the use of the $^{15}N$ stable isotope and to study nitrogen flows in the system of fertilizers–soil–plants–atmosphere.

## Materials and methods

### Gnotobiotic system for plants

A 15-cm-long tube with holes on the surface was placed in a glass beaker with calcined moistened sand. Granules of mineral fertilizer Nitroammophoska (3 g per beaker) treated with a sterile mineral carrier (negative control) and granules treated with the microbiological powder fertilizer on the basis of *Bacillus subtilis* Ch-13 ($50\times10^3$ CFU/g) were placed into the tube. Barley seeds (*Hordeum vulgare* L.) of the universal Nur variety were used for the study. Seeds (40–50) were sterilized for 20 minutes in 70 % ethanol, followed by 35 min in a sterilizing mixture containing 20 ml of sodium hypochlorite, 50 ml of sterile water, and 1 ml of 10 % SDS. The seeds were then washed thrice with sterile water for 3 min, placed into Petri dishes with tryptic soy nutrient agar (TSA, Difco Laboratories, MI, USA) and placed in an incubator at 28 °C for 24 hours to check their sterility. Sterile seeds were transferred into Petri dishes with sterile moistened filter paper and germinated in an incubator at 28 °C for 24 hours. Sterile plant seedlings were transferred to the system and grown for 3 days. Then the gnotobiotic system was disassembled under sterile conditions, and the seedlings were washed from the sand in sterile water. The experiment was performed in three replications with five plants in each beaker.

Plants roots from each variant were used to isolate bacteria that had colonized the root surface. The plant roots were additionally washed with sterile 0.85 % NaCl solution, ground in a mortar with 2 ml of 0.85 % NaCl solution, and used for preparation of serial dilutions. Aliquots of 100 µl of the cell suspension were plated on tryptic soy agar plates. Plates were incubated at 28 °C for 3 days. Colonies derived from cell suspension were identified and subjected to Amplified Fragment Length Polymorphism (AFLP) analysis.

### Identification of bacterial strains

Total genomic DNA of all *Bacillus* strains was isolated using the lysozyme-sodium dodecyl sulfate method (Laguerre et al., 1992). The following primers were used for PCR amplification of 16S rRNA gene: (5'-3') fD1 (AGAGTTTGATCCTGGCTCAG) and rD1 (CTTAAGGAGGTGATCCAGCC). PCR amplification was performed according to standard protocol (Weisburg et al., 1991). The obtained PCR fragments were isolated from agarose gel (Onishchuk et al., 2015) and sequenced using the ABI PRISM 3500xl (Applied Biosystems, Waltham, MA, USA). The sequences were compared with the sequence of the 16S rRNA gene of the strain Ch-13 *B. subtilis*, available in the GenBank database (Accession Number: MW050985).

### AFLP analyses

Genomic DNA of bacterial strains was digested with *Eco*RI and *Mse*I and ligated to EcoRI and MseI adapters, as described by Vos et al. (1995). Three sets of primers were used in separate PCRs: (5'-3') EcoRI-0 (GACTGCGTACCAATTC) and MseI-A (FAM-GATGAGTCCTGAGTAAA), EcoRI-0 and MseI-CA (FAM-GATGAGTCCTGAGTAACA), EcoRI-0 and MseI-GC (FAM-GATGAGTCCTGAGTAAGC). All PCRs were performed with the following temperature profile: denaturation for 2 min at 72 °C, 20 cycles of denaturation (20 s at 94 °C), annealing (30 s at 55 °C) and extension (2 min at 72 °C). The results of PCRs were analyzed using the sequencer ABI PRISM 3500xl according to the manufacturer's instructions (AFLP™ Microbial Fingerprinting [Applied Biosistems, USA]).

### Microfield trial

The effectiveness of biomodified nitrogen fertilizers, ammonium nitrate (Nan) and urea (Nu) for barley (universal Nur variety) was assessed in a microfield trial in bottomless pots in 2017–2019. Soil moisture was sustained at a level of 60–70 % of the total field water holding capacity. We used Soddy Retisol (Loamic) soil (IUSS Working Group WRB. 2015) with the following characteristics: humus content = 1.98–2.04 %; $pH_{kcl}$ = 5.1–5.2; total N content (according to Kjeldahl) 0.11–0.12 %; content of mobile forms of $P_2O_5$ and $K_2O$ = 58–67 and 153–161 mg/kg, respectively. The pots were filled with


AFLP analyses by three sets of PCRs primers: **a** — primers EcoRI-0 and MseI-A, **b** — primers EcoRI-0 and MseI-CA, **c** — primers EcoRI-0 and MseI-GC; lines: 1–3 strains isolated from the barley roots grown in "gnotobiotic system" (variants 1–3, respectively), 4 — strain Ch13 *B. subtilis* (positive control), 5 — strain 1.5A *B. pumilus* (negative control).

soil and nitrogen fertilizers were applied there in the form of salts: ammonium nitrate — Nan ($^{15}NH_4{}^{15}NO_3$) 47.29 atomic %, and urea — Nu ($CO(^{15}NH_4)_2$) with an enrichment of 47.85 atomic %, both at rates of 96 and 193 mg/pot, which corresponds to N45 kg and N90 kg per hectare. Two forms of nitrogen fertilizers were added separately to each pot in four replications (4 pots).

Double superphosphate and potassium chloride (P) were also applied to all pots at rates equivalent to P60K60 per hectare. The experiment was conducted in six replications. In the tillering, tubing, and earing phases, the weight of plants was measured and soil samples were taken in one replication. In the dead-ripe phase, the biomass of plants was measured in three replications.

### Biomodification of nitrogen fertilizers

For biomodification, granulated mineral fertilizers were treated with microbiological powder fertilizer on the basis of *Bacillus subtilis* Ch-13 (MF) at a rate of 10 g/kg of the fertilizer to achieve a cell number not less than $50\times10^3$ CFU/g (Chebotar et al., 2017).

### Agrochemical analysis

Total nitrogen and its isotopic composition in the plants and in the soil were measured using Delta V mass spectrometer (Thermo Fisher Scientific, USA). Phosphorus and potassium in plants were measured using infrared spectroscopy.

### Statistic analysis

The data in the trial were assessed by Fisher's least significant difference (LSD) method (ANOVA).

## Results

### Colonization of barley roots by the PGPB *B. subtilis* Ch-13 in a model experiment

A gnotobiotic system was used to study the colonization of the barley roots by the strain *B. subtilis* Ch-13. Bacteria were isolated from the roots of barley seedlings and identified as belonging to the type *B. subtilis* by the 16S rRNA method. AFLP analyses demonstrated that all isolates belonged to the strain *B. subtilis* Ch-13 (Figure). The number of cells of *B. subtilis* Ch-13 on the barley roots in the variant with biomodified Nitroammophoska was $5.19\times10^6$ CFU per plant. In the control with Nitroammophoska, *B. subtilis* Ch-13 was not detected. Our experiments with the use of the "gnotobiotic system" showed that the strain *B. subtilis* Ch-13 could effectively colonize the barley roots, moving from the tube with biomodified Nitroammophoska to the plant seedlings.

## Microfield trial

***The effect of biomodified fertilizers on the barley yield.*** The agronomical efficiency of nitrogen fertilizers was assessed based on the grain and the straw weight and other production parameters such as height of plants, productive tilling capacity, number of plants, head length, thousand-kernel weight and protein content in grain. When Nan was applied at a rate of N45, the grain weight of barley statistically significantly increased as compared with the PK control, while the application of the biomodified Nan at this rate had no positive effect (Table 1). The use of standard Nu at a rate of N45 increased the grain weight as compared with the PK background. The application of its biomodified form at the same rate statistically significantly increased the grain weight as compared with the standard Nu.

Increasing the rates of the usual forms of nitrogen fertilizers had a positive effect on the grain weight, which was associated with improved nitrogen nutrition of the plants. The application of biomodified Nan at a rate of N90 had a positive effect on the increase of the barley grain weight (Table 1).

After the application of standard and biomodified nitrogen fertilizers on barley, the straw weight increased up to two times as compared with the PK control. A positive effect was observed after the use of biomodified Nu at both rates and the use of biomodified Nan at a rate of N90.

We revealed differences in the barley yield structure under standard and biomodified nitrogen fertilizers (Table 1). Owing to improved nitrogen nutrition associated with nitrogen fertilizers, the head length of barley increased from 4.5 to 5.2–6.1 cm and showed a tendency to grow in the case of application of biomodified forms of nitrogen fertilizer. The content of raw protein in the barley grain increased after the application of standard nitrogen fertilizers. This same effect from their biomodified forms was registered only in the case of Nu (at both rates), the added protein in the yield being 1 % (Table 1).

***Accumulation of nutrition elements in barley***. Accumulation of nitrogen, phosphorus and potassium in the plants and the proportion of different sources of nitrogen in the formation of barley biomass have been analysed. Owing to the improved nitrogen nutrition, the biomass of grain and straw increased, while the accumulation of nitrogen, phosphorus and potassium in the plants increased considerably (Table 2). Using the stable isotope $^{15}N$, we identified the proportion of nitrogen from the soil, nitrogen from the fertilizer, biological (fixed from atmosphere) nitrogen and "extra" (mineralization of organic matter in the soil) nitrogen in the formation of barley biomass (grain + straw) (Table 2). In the experiment without the application of nitrogen fertilizers, the biomass of barley was formed only at the expense of soil nitrogen (100 %). In the experiment with the application of Nan and Nu, the biomass accumulated not only the soil nitrogen but also nitrogen from fertilizers, identified based on the $^{15}N$ isotope (Table 2). A positive effect was observed after the use of biomodified Nu at both rates and the use of biomodified Nan at a rate of N90. It is interesting to note that biomodification of mineral fertilizers considerably increased the proportion of "biological" nitrogen in the proportion of

**Table 1. Barley productivity after application of usual and biomodified nitrogen fertilizers**

| Variant | Grain weight, g/pot | Straw weight, g/pot | Height of plants, cm | Productive tilling capacity, number of plants | Head length, cm | Thousand-kernel weight, g | Protein content in grain, % |
|---|---|---|---|---|---|---|---|
| 1. PK (background -P) | 15.0$^{a}$ | 12.9$^{a}$ | 44.7$^{a}$ | 1.66$^{a}$ | 4.5$^{a}$ | 42.6$^{a}$ | 8.3$^{a}$ |
| 2. P + MF | 15.7$^{a}$ | 14.0$^{a}$ | 48.5$^{b}$ | 1.55$^{a}$ | 5.4$^{a}$ | 45.0$^{a}$ | 8.9$^{b}$ |
| 3. P + Nan45 | 19.2$^{b}$ | 17.5$^{b}$ | 50.0$^{b}$ | 1.79$^{a}$ | 5.2$^{a}$ | 46.7$^{b}$ | 8.7$^{b}$ |
| 4. P + Nan45 + MF | 19.1$^{b}$ | 16.2$^{b}$ | 51.3$^{b}$ | 1.88$^{a}$ | 5.4$^{a}$ | 46.8$^{b}$ | 8.2$^{a}$ |
| 5. P + Nu45 | 18.3$^{b}$ | 16.6$^{b}$ | 49.5$^{b}$ | 1.72$^{a}$ | 5.5$^{a}$ | 48.1$^{b}$ | 8.3$^{a}$ |
| 6. P + Nu45 + MF | 21.3$^{b}$ | 19.8$^{b}$ | 50.3$^{b}$ | 1.76$^{a}$ | 5.8$^{b}$ | 50.0$^{b}$ | 9.1$^{b}$ |
| 7. P + Nan90 | 23.3$^{b}$ | 23.2$^{b}$ | 55.2$^{b}$ | 1.93$^{a}$ | 5.7$^{b}$ | 46.4$^{b}$ | 9.2$^{b}$ |
| 8. P + Nan90 + MF | 26.0$^{b}$ | 27.0$^{b}$ | 54.6$^{b}$ | 2.04$^{b}$ | 6.4$^{b}$ | 45.9$^{b}$ | 9.0$^{b}$ |
| 9. P + Nu90 | 22.1$^{b}$ | 21.1$^{b}$ | 50.9$^{b}$ | 2.04$^{b}$ | 5.3 | 46.6$^{b}$ | 8.7$^{b}$ |
| 10. Nu90 + MF | 24.3$^{b}$ | 25.3$^{b}$ | 52.2$^{b}$ | 2.04$^{b}$ | 6.1$^{b}$ | 47.7$^{b}$ | 9.2$^{b}$ |
| LSD $_{05}$ | *2,7* | *3,1* | *5,7* | *2,8* | *0,28* | *1,1* | *2,6* |

LSD (Least Significant Difference)

Letters by values denote significant differences among the treatments in a trial as assessed by Fisher LSD (ANOVA). Different letters indicate significant differences ($P \leq 0.05$).

**Table 2. Accumulation of nutrition elements in barley and the proportion of different sources of nitrogen in the formation of barley biomass**

| Variant | Accumulation in plants, mg/pot (average values for 2017–2019 years) | | | Proportion of nitrogen sources in plants, % (average values for 2017–2018 years) | | | |
|---|---|---|---|---|---|---|---|
| | N | $P_2O_5$ | $K_2O$ | N soil | $^{15}$N fertilizer | N biological | N "extra" |
| 1. PK (background -P) | 313[a] | 157[a] | 355[a] | 100 | - | - | - |
| 2. P + MF | 407[b] | 206[b] | 353[a] | 77 | - | 23 | - |
| 3. P + Nan45 | 447[b] | 264[b] | 417[a] | 70 | 9 | - | 21 |
| 4. P + Nan45 + MF | 473[b] | 234[b] | 414[a] | 66 | 9 | 20 | 5 |
| 5. P + Nu45 | 489[b] | 233[b] | 412[a] | 64 | 11 | - | 25 |
| 6. P + Nu45 + MF | 534[b] | 267[b] | 498[b] | 59 | 11 | 18 | 12 |
| 7. P + Nan90 | 564[b] | 307[b] | 542[b] | 56 | 15 | - | 29 |
| 8. P + Nan90 + MF | 609[b] | 326[b] | 628[b] | 51 | 16 | 15 | 18 |
| 9. P + Nu90 | 604[b] | 328[b] | 541[b] | 52 | 18 | - | 30 |
| 10. P + Nu90 + MF | 686[b] | 352[b] | 641[b] | 45 | 17 | 14 | 24 |
| $LSD_{0505}$ | *49* | *32* | *64* | | | | |

Letters by values denote significant differences among the treatments in a trial as assessed by Fisher LSD (ANOVA). Different letters indicate significant differences ($P \le 0.05$).

**Table 3. Flows of $^{15}$N nitrogen from fertilizers during barley cultivation, % of the applied amount (average values for 2017–2018 years)**

| Variant | Utilized by plants | | | | Fixed in soil | | | | Losses | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 1* | 2 | 3 | 4 | 1 | 2 | 3 | 4 | 1 | 2 | 3 | 4 |
| Nan45 | 11 | 22 | 35 | 42 | 81 | 63 | 47 | 36 | 8 | 15 | 18 | 22 |
| Nan45 + MF | 10 | 25 | 40 | 45 | 85 | 63 | 46 | 35 | 5 | 12 | 14 | 20 |
| Nu45 | 20 | 28 | 42 | 57 | 78 | 69 | 47 | 28 | 2 | 3 | 11 | 15 |
| Nu45 + MF | 26 | 34 | 45 | 59 | 73 | 64 | 44 | 27 | 1 | 2 | 11 | 14 |
| Nan90 | 13 | 19 | 39 | 45 | 75 | 62 | 40 | 30 | 12 | 19 | 22 | 25 |
| Nan90 + MF | 18 | 24 | 41 | 46 | 70 | 58 | 38 | 31 | 12 | 18 | 21 | 23 |
| Nu90 | 25 | 30 | 41 | 57 | 64 | 56 | 43 | 25 | 11 | 14 | 16 | 18 |
| Nu90 + MF | 27 | 32 | 45 | 59 | 66 | 59 | 45 | 30 | 7 | 8 | 10 | 11 |

*Note: 1 — tillering, 2 — booting, 3 — ear emergence, 4 — dead-ripe grain.

nitrogen sources in plants, due to the enhanced activity of native nitrogen-fixing bacteria in the barley rhizosphere (Table 2).

***Flows of $^{15}$N nitrogen from fertilizers during barley cultivation.*** We studied the flows of nitrogen from fertilizers in the agroecosystem during barley vegetation by determining the amount of $^{15}$N in the plants and in the soil (Table 3). After application of fertilizers at a rate of N90, the use of $^{15}$N by plants increased by 10–12 % as compared to N45, and a greater biomass of barley was formed. When biomodified nitrogen fertilizers were applied, the plants accumulated $^{15}$N more intensively than in the case of standard nitrogen fertilizers. The accumulation was greater by 2–5 % in the tillering phase, by 3–5 % in the booting phase and by 2–5 % in the ear emergence phase (Table 3). A more intensive accumulation of $^{15}$N in different phases after the application of biomodified Nan and Nu as compared with the usual forms can be explained by the activity of PGPB *B. subtilis* Ch-13 on the plant roots. The application of biomodified nitrogen fertilizers on barley decreased the incorporation of $^{15}$N in the soil by 2–5 % and decreased the gaseous losses of nitrogen by 2–7 % as compared with the usual forms.

## Discussion

Microbial inoculants are promising components for integrated solutions to agro-environmental problems because inoculants possess the capacity to promote plant growth, enhance nutrient availability and uptake, and support the health of plants (Vessey, 2003; Han and Lee, 2005; Adesemoye et al., 2008). We demonstrated in a model experiment the ability of the strain *B. subtilis* Ch-13 to move from the granules of mineral fertilizers to plant roots and to colonize them effectively. Thus PGPB *B. subtilis* Ch-13 can improve the growth and productivity of plants (Chebotar et al., 2009). The specific mechanism involved in PGPB-elicited enhanced nutrient uptake was proposed by Adesemoye and Kloepper (2009). It was proposed that PGPB promoted the growth of the plant and increased the root surface area or the general root architecture, better roots then released higher amounts of C in root exudates, the increase prompted more microbial activity, and the cycle of events made more N available for plants' uptake. The effect of nitrogen fertilizers biomodified with PGPB *B. subtilis* Ch-13 was demonstrated in a microfield trial with barley. We revealed differences in the barley yield and production parameters under standard and biomodified nitrogen fertilizers. An increase in the protein content is considered as a positive effect for fodder grain. At the same time, increased protein content associated with the use of nitrogen fertilizers corresponds to a higher brewing quality of the barley grain (Zavalin, 2000). When nitrogen fertilizers are applied, the mineralization of organic matter in the soil resulted in the formation of "extra" nitrogen (Zavalin et al., 2015; Chebotar et al., 2017), which is used by plants. Biomodification of mineral fertilizers with PGPB *B. subtilis* Ch-13 can considerably decrease the proportion of gaseous losses of nitrogen, thus improving the efficiency of fertilizers.

## Conclusion

The application of biomodified nitrogen fertilizers in all the vegetative phases increased the accumulation of $^{15}N$ in the barley plants, decreased its incorporation in the soil and reduced gaseous losses as compared with the usual forms. The application of biomodified nitrogen fertilizers can be used in agricultural practice as a novel technology to regulate nitrogen flows in the system of fertilizers–soil–plants–atmosphere and can improve the efficiency of fertilizers.

### References


Adesemoye, A. O., Torbert, H. A., and Kloepper, J. W. 2008. Enhanced plant nutrient use efficiency with PGPR and AMF in an integrated nutrient management system. *Canadian Journal of Microbiology* 54:876–886. https://doi.org/10.1139/W08-081

Adesemoye, A. O. and Kloepper, J. W. 2009. Plant-microbes interactions in enhanced fertilizer-use efficiency. *Applied Microbiology and Biotechnology* 85:1–12. https://doi.org/10.1007/s00253-009-2196-0

Barraquio, W. L., Revilla, L., and Ladha, J. K. 1997. Isolation of endophytic diazotrophic bacteria from wetland rice. *Plant and Soil* 194:15–24. https://doi.org/10.1023/A:1004246904803

Bertrand, H., Plassard, C., Pinochet, X., Touraine, B., Normand, P., and Cleyet-Marel, J. C. 2000. Stimulation of the ionic transport system in *Brassica napus* by a plant growth-promoting rhizobacterium (*Achromobacter* sp.). *Canadian Journal of Microbiology* 46:229–236. https://doi.org/10.1139/w99-137

Bhat, M. A. 2019. Plant growth promoting rhizobacteria (PGPR) for sustainable and eco-friendly agriculture. *Acta Scientific Agriculture* 3:23–25.

Bloemberg, G. and Lugtenberg, B. J. 2001. Molecular basis of plant growth promotion and biocontrol by rhizobacteria. *Current Opinion in Plant Biology* 4:343–350. https://doi.org/10.1016/S1369-5266(00)00183-7

Chebotar, V. K., Makarova, N. M., Shaposhnikov, A. I., and Kravchenko, L. V. 2009. Antifungal and phytostimulating characteristics of *Bacillus subtilis* Ch-13 rhizospheric strain, producer of bioprepations. *Applied Biochemistry and Microbiology* 45:419–423. https://doi.org/10.1134/S0003683809040127

Chebotar, V. K., Shcherbakov, A. V., Shcherbakova, E. N., Maslennikova, S. N., Zaplatkin, A. N., and Malfanova, N. V. 2015. Biodiversity of endophytic bacteria as a promising biotechnological resource. *Sel'skokhozyaistvennaya Biologiya* 50:648–654. https://doi.org/10.15389/agrobiology.2015.5.648eng

Chebotar, V. K., Shcherbakov, A. V., Maslennikova, S. N., Zaplatkin, A. N., Kanarsky, A. V., and Zavalin, A. A. 2016a. Endophytic bacteria of woody plants as the basis of complex microbial preparations for agriculture and forestry. *Russian Agricultural Sciences* 42:339–342. https://doi.org/10.3103/S1068367416050037

Chebotar, V. K., Zaplatkin, A. N., Shcherbakov, A. V., Malfanova, N. V., Startseva, A. A., and Kostin, Ya. V. 2016b. Microbial preparations on the basis of endophytic and rhizobacteria to increase the productivity in vegetable crops and spring barley (*Hordeum vulgare* L.), and the mineral fertilizer use efficiency. *Sel'skokhozyaistvennaya Biologiya* 51:335–342. https://doi.org/10.15389/agrobiology.2016.3.335eng

Chebotar, V. K., Zavalin, A. A., and Aritkin, A. G. 2017. Biomodified mineral fertilizers: efficiency of use and mode of actions. LAMBERT Academic Publishing, Saarbrucken, Germany.

Dobbelaere, S., Croonenborghs, A., and Thys, A. 2001. Responses of agronomically important crops to inoculation with *Azospirillum*. *Australian Journal of Plant Physiology* 28:871–879. https://doi.org/10.1071/PP01074

Han, H. S. and Lee, K. D. 2005. Phosphate and potassium solubilizing bacteria effect on mineral uptake, soil availability, and growth of egg plant. *Research Journal of Agriculture and Biological Sciences* 1:176–180.

Hassan, M. K., McInroy, J. A., and Kloepper, J. W. 2019. Review the interactions of rhizodeposits with plant growth-promoting rhizobacteria in the rhizosphere: A Review. *Agriculture* 9:142. https://doi.org/10.3390/agriculture9070142

IUSS Working Group WRB. 2015 World Reference Base for Soil Resources 2014, update 2015 International soil classification system for naming soils and creating legends for soil maps. World Soil Resources Reports No. 106 FAO, Rome.

Kozhemyakov, A. P., Laktionov, Yu. V., Popova, T. A., Orlova, A. G., Kokorina, A. L., Vayshlya, O. B., Agafonov, E. V., Guzhvin, S. A., Churakov, A. A., and Yakovleva, M. T. 2015. The scientific basis for the creation of new forms of microbial biochemical. *Sel'skokhozyaistvennaya Biologiya* 50:369–376. https://doi.org/10.15389/agrobiology.2015.3.369eng

Laguerre, G., Masurier, S. I., and Amarger, N. 1992. Plasmid profiles and restriction fragment length polymorphism of *Rhizobium leguminosarum* bv. *viceae* in field populations. *FEMS Microbiology Ecology* 10:17–26. https://doi.org/10.1111/j.1574-6941.1992.tb01644.x

Ohkama-Ohtsu, N. and Wasaki, J. 2010. Recent progress in plant nutrition research: cross-talk between nutrients, plant physiology and soil microorganisms. *Plant and Cell Physiology* 51:1255–1264. https://doi.org/10.1093/pcp/pcq095

Okon, Y. and Vanderleyden, J. 1997. Root-associated *Azospirillum* species can stimulate plants. *ASM News* 63:364–370.

Onishchuk, O. P., Chizhevskaya, E. P., Kurchak, O. N., Andronov, E. E., and Simarov, B. V. 2015. Identification of new genes of nodule bacteria *Sinorhizobium meliloti* involved in the control of efficiency of symbiosis with alfalfa *Medicago sativa*. *Russian Journal of Genetics: Applied Research* 5:126–131. https://doi.org/10.1134/S2079059715020070

Pishchik, V. N., Provorov, N. A., Vorobyov, N. I., Chizevskaya, E. P., Safronova, V. I., Kozhemyakov, A. P., and Tuev, A. N. 2009. Interactions between plants and associated bacteria in soils contaminated with heavy metals. *Microbiology* 78:785–793. https://doi.org/10.1134/S0026261709060162

Rothballer, M., Schmid, M., and Hartmann, A. 2007. Diazotrophic bacterial endophytes in Gramineae and other plants; pp. 273–302 in Pawlowski, K. (ed.), Prokaryotic symbionts in plants. Microbiology monographs, vol. 8. Springer, Berlin, Heidelberg. https://doi.org/10.1007/7171_2007_103

Ruby, E. J. and Raghunath, T. M. 2011. A Review: Bacterial endophytes and their bioprospecting. *Journal of Pharmacy Research* 4:795–799.

Shaposhnikov, A. I., Belimov, A. A., Kravchenko, L. V., and Vivanko, D. M. 2011. Interaction of rhizosphere bacteria with plants: mechanisms of formation and factors of efficiency in associative symbiosis (review). *Sel'skokhozyaistvennaya Biologiya* 46:16–22. (In Russian)

Vessey, J. K. 2003. Plant growth promoting rhizobacteria as biofertilizers. *Plant and Soil* 255:571–586. https://doi.org/10.1023/A:1026037216893

Vos, P., Hogers, R., Bleeker, M., Reijans, M., van de Lee, T., Hornes, M., Frijters, A., Pot, J., Peleman, J., Kuiper, M., and Zabeau, M. 1995. AFLP: a new technique for DNA fingerprinting. *Nucleic Acids Research* 23:4407–4414. https://doi.org/10.1093/nar/23.21.4407

Weisburg, W. G., Barns, S. M., Pelletier, D. A., and Lane, D. J. 1991. 16S ribosomal DNA amplification for phylogenetic study. *Journal of Bacteriology* 173(2):697–703. https://doi.org/10.1128/jb.173.2.697-703.1991

Zavalin, A. A., Chernova, L. S., Gavrilova, A. Yu., and Chebotar, V. K. 2015. The influence of mineral fertilizers biomodification by microbial preparation bisolbifit on spring barley yield. *Agrokhimiya* 4:21–32.

Zavalin, A. A. and Sokolov, O. A. 2019. Utilization by plants of nitrogen fertilizer and its regulation. *International Agricultural Journal* 4:71–75.

Zavalin, A. A., Dukhanina, T. M., Chistotin, M. V., Ladonin, V. F., Vinogradova, L. V., Afanasyev, R. A. Sologub, D. B., Kozhemyakov, A. P., Vasyuk, L. V., Khotyanovich, A. V., Tsygutkin, A. S., and Pasynkov, A. V. 2000. Assessment of effectiveness of microbial preparations in agriculture. 82 pp. Tipografiya Rossel'hozakademii, Moscow. (In Russian)