AGRICULTURAL BIOLOGY,
2022, V. 57, Iss. 1, pp. 171-182
[SEL'SKOKHOZYAISTVENNAYA BIOLOGIYA]

ISSN 2412-0324 (English ed. Online)
ISSN 0131-6397 (Russian ed. Print)
ISSN 2313-4836 (Russian ed. Online)

UDC 579.64:57.083.13

doi: 10.15389/agrobiology.2022.1.171eng
doi: 10.15389/agrobiology.2022.1.171rus

CO-CULTURE OF *Pseudomonas chlororaphis* and *Saccharomyces cerevisiae* TO CREATE A COMPLEX BIOLOGICAL PRODUCT

A.S. PRONIN[1] ✉, T.S. KOLMYKOVA[2], A.S. LUKATKIN[1]

[1]*National Research Mordovia State University*, 68, ul. Bolshevistskaya, Saransk, 430005 Russia, e-mail proninbio@gmail.com (✉ corresponding author), aslukatkin@yandex.ru;
[2]*Lyceum No. 31*, 2, ul. Metallurgov, Saransk, 430034 Russia, e-mail: tskolmykova@yandex.ru
ORCID:
Pronin A.S. orcid.org/0000-0001-5196-9418
Lukatkin A.S. orcid.org/0000-0002-8174-5367
Kolmykova T.S. orcid.org/0000-0002-0581-8288
The authors declare no conflict of interests
Received November 24, 2021

Abstract

Currently, there has been a trend in agriculture towards an increase in the use of biological preparations, including plant protection means. Developments are actively underway to create and optimize technologies for the production of new biological preparations that contain plant growth stimulating lines of bacteria (Plant Growth Promoting Bacteria, PGPB) or fungi (Plant Growth Promoting Fungi, PGPF). Fungi and bacteria co-inhabit the rhizosphere of higher plants and fungal-bacterial interactions permanently occur. In recent years, biological preparations based on bacteria and fungi (insecticide Biostop, fungicide Sporobacterin) have entered the agrochemical and pesticide market. However, special requirements of bacteria and fungi for nutrient media, aeration, and culture regimes imped production of combined bacterial-fungal biologicals. In this work, we have established for the first time the positive effect of *Saccharomyces cerevisiae* Y-4317 on the growth of *Pseudomonas chlororaphis* subsp. *auerofaciens* B-5326 and revealed the optimal regimes and the composition of the nutrient medium for co-culture of microorganisms of different taxonomic groups (PGPB and PGPF). The high efficiency of using sugar beet molasses for co-culture of *P. chlororaphis* and *S. cerevisiae* has been shown. Our findings revealed the stimulating effect of the liquid culture (LC) on the seed germination energy and germination rate of cereals. The aim of this work was to develop a protocol for co-culture of bacteria *Pseudomonas chlororaphis* subsp. *auerofaciens* B-5326 and yeast *Saccharomyces cerevisiae* Y-4317 to create a biological preparation stimulating seed germination and initial growth of cereal plants. Lyophilized strains *P. chlororaphis* and *S. cerevisiae* were obtained from the All-Russian Collection of Industrial Microorganisms of the National Research Center Kurchatov Institute — GosNIIgenetica (Moscow). Seeds of maize (*Zea mays* L.) hybrid Delitop, wheat (*Triticum aestivum* L.) variety Mironovskaya 808, barley (*Hordeum vulgare* L.) variety Scepter served for testing bioactivity of the biological. After restoring viability, the strains were cultured for 48 hours on a shaker SPH-2102 (BIORUS, Belarus) using three nutrient media differing in the carbon source (glucose, fructose, and sugar beet molasses). Microbial growth (colony-forming units, CFU) and biomass were assessed during co-culture (from 0 to 72 h) on the shaker and in a lab fermenter BIORUS GJ (BIORUS, Belarus) at different temperatures (from 20 to 32 °C) and airflow rates (from 1 to 6 L/h). The seeds were treated by spraying with 1:200-1:25 serial dilutions of the liquid co-culture. After 12 h, the seeds were placed into Petri dishes with water. The germination energy and seed germination rate were determined in 3 and 7 days, respectively. The research data showed that media containing sugar beet molasses completely satisfies the need of co-cultured *P. chlororaphis* and *S. cerevisiae* for basic nutrients, and the titers did not fall below 6×10^8 and 3×10^6 CFU/ml, respectively. The total biomass was 20.4 g/l, or 17-22 % higher than on the media with glucose or fructose. For co-culture, the optimal conditions were 30°C, aeration mode 4 l/h and 24 h of growth. A positive effect of *S. cerevisiae* on the viability of *P. chlororaphis* during 72 h co-culture was demonstrated. The abundance of *P. chlororaphis* in the co-culture with *S. cerevisiae* was 1×10^6 CFU/ml vs. 5×10^4 CFU/ml in pure culture of *P. chlororaphis*. Probably, a higher viability of *P. chlororaphis* and stimulation of its growth is due to phytohormones produced by *S. cerevisiae* during co-culture. Preliminary testing revealed stimulating effect of the biological on the germination energy and seed germination rate for barley, wheat and corn. All serial dilutions of the liquid co-culture exhibited a clear trend towards an increase in seed germination in the cereals tested. The maize seed germination was most stimulated. A 1:200 dilution of the biological led to the maximum increase in seed germination of the crops. Our research data identify the

key parameters of the co-culture of PGPB *P. chlororaphis* and PGPF *S. cerevisiae* and thereby create the prerequisites for the development of a biological based on microorganisms of vadifferent taxonomic groups.

Keywords: *Pseudomonas chlororaphis*, *Saccharomyces cerevisiae*, biological preparation, biomass, culture procedure, maize, wheat, barley, seed germination

In recent years, there has been a trend in global agriculture towards an increase in the use of biological products, including plant protection products (1). Technologies for the production of new biological products are being actively optimized. To ensure the high efficiency of biological preparations, plant growth promoting bacteria (PGPB) and fungi (plant growth promoting fungi, PGPF) strains stimulating plant growth are used [2-4]. Microbiological preparations are living cells of microorganisms selected for useful properties and either in the culture liquid or adsorbed on a neutral carrier.

PGPBs of the genus *Pseudomonas* synthesize metabolites that have a growth-stimulating and antifungal effect on plants [5-9]. When using these biological products, an increase in the biological and economic yield of a number of crops has been shown [7, 8]. Of interest are preparations created on the basis of joint cultures of two or more PGPBs, in particular, a microbiological product based on *Pseudomonas aureofaciens* and *Azotobacter vinelandii* [10-13]. These types of bacteria are intensively used as growth regulators of cultivated plants, and also stimulate their resistance to pathogens [14]. However, under the conditions of an unstable climate and the systematic negative impact of external factors, the problem of reducing yields because of stressful impacts remains relevant. In this regard, it is fundamentally important to look for new approaches to create biological preparations that also have a stress-modulating effect.

Thus, attention is drawn to the possibility of using representatives of another taxonomic group of microorganisms, fungi, in the creation of biological preparations. Few studies have shown that certain yeast strains produce phytohormones [15]. It is known that biologically active substances with hormonal activity can not only regulate the growth and development of plants, but also participate in protection against abiotic factors [16-18]. The fact that the ability to synthesize phytohormones was found in yeast allows us to consider them as a potential component of biological preparations for plant growing practice.

Fungi and bacteria co-exist in the rhizosphere, there is constant interaction between them. Microorganisms allocated to the PGPF and PGPB groups can affect the growth and development of plants [19-23]. However, in modern literature, we have not come across information on their joint action on plants. There is evidence that representatives of the genus *Pseudomonas* improved the growth of the basidiomycete *Agaricus bisporus*, but the mechanism of this interaction is not known [24].

In recent years, biological preparations containing bacteria and fungi (Biostop insecticide, Sporobacterin fungicide) have entered the market of agrochemicals and pesticides [25]. The main difficulties in the production of such preparations are associated with the different demands of the components on the composition of the nutrient medium, aeration, and cultivation modes. In bacteria, the optimal growth temperature is lower and the duration of cultivation is shorter compared to fungi. In addition, fungi require free amino nitrogen for biosynthesis [26, 27]. The study of the conditions of co-cultivation of PGPB and PGPF is necessary for the development of fundamental principles for the subsequent creation of complex biological preparations that affect agricultural crops.

In this work, we have established for the first time the positive effect of

Saccharomyces cerevisiae Y-4317 on the growth of *Pseudomonas chlororaphis* subsp. *auerofaciens* and revealed the optimal cultivation regimes and the composition of the nutrient medium for the joint growth of microorganisms of various taxonomic groups (PGPB and PGPF). The high efficiency of using beet molasses for the joint cultivation of *P. chlororaphis* and *S. cerevisiae* has been shown. The stimulating effect of the obtained cultural liquid (CL) on the germination and germination energy of seeds of cultivated cereals was revealed.

The aim of this work is to study the possibility of co-cultivation of the bacterium Pseudomonas chlororaphis subsp. auerofaciens B-5326 and yeast *Saccharomyces cerevisiae* Y-4317 and optimize their cultivation parameters to obtain a culture fluid that stimulates seed germination and initial growth of cereal plants.

Materials and methods. Lyophilisated strains of the bacterium *Pseudomonas chlororaphis* subsp. *aureofaciens* B-5326 and yeast *Saccharomyces cerevisia*e Y-4317 were obtained in ampoules from the All-Russian Collection of Industrial Microorganisms of the Research Center Kurchatov Institute — GosNIIgenetika (Moscow). The strains were not genetically modified.

Seeds of corn (*Zea mays* L.) hybrid Delitop, wheat (*Triticum aestivum* L.) variety Mironovskaya 808, barley (*Hordeum vulgare* L.) variety Skipert were used.

To restore the viability of the culture, an ampoule with a dried strain of microorganisms was sterilely opened and suspended in a liquid nutrient medium (0.2-0.4 ml) was added, DMEM/F12 Merck (Sigma-Aldrich, USA) for *P. chlororaphis*, Malt extract (Pronadisa, Spain) for *S. cerevisiae*. The contents were sterilely transferred with a Pasteur pipette into Petri dishes on agar nutrient media of the same composition. Next, the strains were grown separately for 48 h in a thermostat TS-1/20 SPU (Smolenskoye SKTB SPU, Russia) at 28 °C.

When preparing inoculums, a single colony with a characteristic morphology of the strain was selected from each dish, transferred to 250 ml flasks, and cultivated on an SPH-2102 shaker-incubator (BIORUS, Belarus) at 28 °C and 130 rpm. The medium for growing *P. chlororaphis* contained 10 g/l of glucose and 0.5 g/l of $NaNO_3$, $(NH_4)_2SO_4$, NH_4NO_3, KH_2PO_4. The medium for growing *S. cerevisiae* is a synthetic modified Reader's medium: 10 g/l glucose, 0.5 g/l peptone, 1 g/l yeast extract, 0.5 g/l $NaNO_3$, $(NH_4)_2SO_4$, NH_4NO_3, KH_2NO_4 each. After 48 h, the titers of *P. chlororaphis* and *S. cerevisiae* were 10^7 and 10^5 CFU/ml, respectively.

For co-culture of two strains of microorganisms, 15 ml of the obtained starter culture was taken, transferred to 500 ml flasks, and grown for 48 h at 28 °C and 130 rpm on an SPH-2102 shaker-incubator. The composition of the medium for the co-culture of microorganisms was as follows: 0.5 g/l peptone, 1 g/l yeast extract, 0.5 g/l $NaNO_3$, $(NH_4)_2SO_4$, NH_4NO_3, KH_2PO_4, carbon source varied.

To determine the most acceptable composition, three variants of nutrient media were used, differing in the source of carbon: 1 — glucose (10 g/l), 2 — fructose (10 g/l), 3 — beet molasses (15 g/l). Variants with variation of some other components of nutrient media were considered (no significant differences were found with the main recipe). Subsequently, the influence of temperature (from 20 to 32 °C) on the co-culture of *P. chlororaphis* and *S. cerevisiae* on a shaker-incubator was determined, and the number of viable microorganisms (by the number of colony-forming units, CFU) in dynamics (from 0 to 48 h and from 48 to 72 h) was also estimated.

Co-culture of *P. chlororaphis* and *S. cerevisiae* was also carried out in a BIORUS GJ laboratory fermenter (BIORUS, Republic of Belarus), in which the

air supply rate through the filters was varied (from 1 to 6 l/h). Cultivation lasted 20 h at 30 °C. The medium was similar to that used in the previous experiment, with molasses as the main carbon source.

The amount of biomass obtained was estimated by drying the filters to constant weight in a UF75 oven (Memmert GmbH + Co. KG, Germany) for 1-2 h at 105 °C, separating microorganism cells from the culture liquid (CL volume 10 ml, with 3-fold washing with distilled water), and determining the dry weight on an Analytical Balance ME54T/A00 analytical balance (Mettler Toledo, USA) to a constant value (±0.1 mg) [28].

The viability of the cells of each microorganism under study in the culture liquid was determined by inoculation on an agar universal medium containing 10 g/l glucose, 0.5 g/l peptone, 1 g/l yeast extract, and 0.5 g/l $NaNO_3$, $(NH_4)_2SO_4$, NH_4NO_3, and KH_2PO_4. Samples of the culture fluid were taken with a 5 ml microbiological pipette. The number of microorganisms was determined by the method of serial 10-fold dilutions, counting the number of colonies of each species, the indication was carried out according to the morphological description of colonies of yeast and bacteria of the studied genera [29-31]. The microobjects were visualized using a microscope with a Fixed Microscope Adapter (Levenhuk, USA).

Seeds of agricultural plants were treated by spraying with cultural liquid in different dilutions (1:200, 1:150, 1:100, 1:50, 1:25), after 12 h, the seeds were germinated in water in Petri dishes (20 or 50 seeds per dish) at 25 °C in a thermostat TS-1/20 SPU. Seeds treated with water served as control. Germination energy and germination were determined by conventional methods after 3 and 7 days, respectively [32].

All experiments were carried out at least three times, each including 5 flasks or Petri dishes with the same material grown under the same type of conditions. When determining cell viability, each variant was analyzed in 10 replications. Seed germination was carried out in 5 repetitions (Petri dishes) for each variant of the experiment.

Statistical processing of the results was carried out according to standard methods [33] in the Microsoft Excel 2007 program. The data in tables and graphs are given as arithmetic mean values with standard errors (M±SEM). Differences in sample means were assessed by Student's t-test at $p \leq 0.05$ using Microsoft Excel 2007 and Statistica v.12 (StatSoft, Inc., USA).

Results. After 48 h of separate cultivation of *P. chlororaphis* subsp. *aureofaciens* B-5326 and *S. cerevisiae* Y-4317, their abundance reached 2×10^8 and 7×10^6 CFU/ml, respectively. Next, we studied the possibility of co-growth of two cultures in a liquid nutrient medium. Note that we did not find relevant data on such cultivation and its conditions in the available literature.

To optimize the co-culture of two strains of microorganisms, we studied the effect of the composition of the nutrient medium on changes in their biomass and abundance. Glucose was used as the main source of carbon, while fructose and beet molasses serbed as alternatives.

On media with glucose and fructose, a relatively weak accumulation of total biomass was observed (Fig. 1, A). The maximum value of the total biomass during the co-culture of the studied strains was recorded on the medium containing molasses, 20.4 g/l, or 17-22% higher than on the media with glucose and fructose.

During co-culture, the abundance of *P. chlororaphis* and *S. cerevisiae* did not fall below 6×10^8 and 3×10^6 CFU/ml, respectively (see Fig. 1, B). The CFU values for *S. cerevisiae* and *P. chlororaphis* did not change significantly on all the

studied media. The studied strains were able to grow together on molasses without a significant decrease in cell viability (see Fig. 1, B). Therefore, in all subsequent experiments, we used a nutrient medium containing molasses as a carbon source.

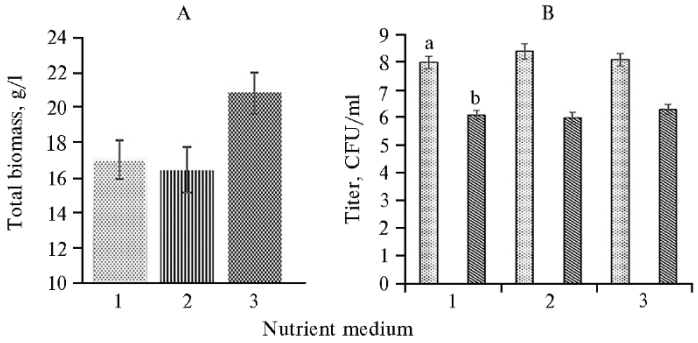

FIg. 1. Total biomass (A) **and the cell number** (B) **in co-culture of *Pseudomonas chlororaphis* subsp. *aureofaciens* B-5326** (a) **and yeasts *Saccharomyces cerevisiae* Y-4317** (b) **depending on liquid medium composition:** 1 — glucose (10 g/l), 2 — fructose (10 g/l), 3 — beet molasses (15 g/l) (N = 10, M±SEM).

The successful use of beet molasses for the co-culture of strains of microorganisms can be explained by the fact that, along with other components, it contains about 1% raffinose. On the one hand, raffinose can inhibit the growth and biofilm formation of *Pseudomonas* bacteria [34]; on the other hand, it also affects bacterial phenotypes, colony morphology, matrix formation, and colony motility [35]. The use of beet molasses for the co-culture of *P. chlororaphis* subsp. *aureofaciens* B-5326 and *S. cerevisiae* Y-4317 can reduce the economic load in the commercial production of the created biologicals.

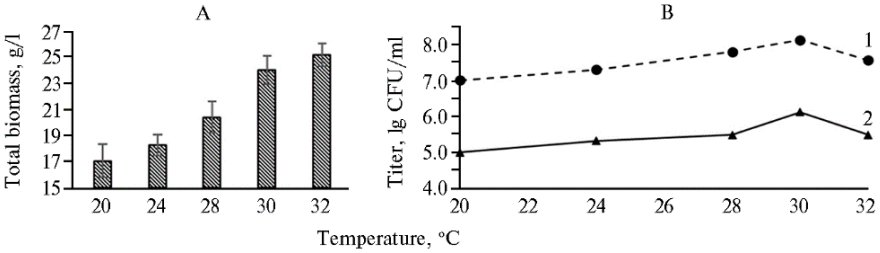

Fig. 2. Total biomass (A) **and the cell number** (Б) **in co-culture of *Pseudomonas chlororaphis* subsp. *aureofaciens* B-5326** (1) **and yeasts *Saccharomyces cerevisiae* Y-4317** (2) **on beet molasses** (15 g/l) **medium depending on temperature** (N = 5, M±SEM). Markers on Fig. 2, indicate the ±SEM values.

The temperature regime during the co-culture of bacteria and yeast affected the change in the total biomass of these microorganisms (Fig. 2, A). At temperatures of 20 and 24 °C, the total biomass turned out to be minimal (we did not record any statistically significant differences between these options). At 28 °C, the total biomass was 20.5 g/l, at 30 °C it was 24.3 g/l. A further increase in temperature (up to 32 °C) did not significantly increase the total biomass of bacteria and fungi compared to that at 30 °C.

The temperature regime also influenced the viability of bacteria and fungi (see Fig. 2, B). From 20 °C, the CFU values of *P. chlororaphis* and *S. cerevisiae* increased, and the maximum was recorded at 30 °C (10^8 and 10^6 CFU/ml, respectively). With a further increase in temperature (up to 32 °C), the number of colonies decreased significantly, to 7.5×10^7 and 5.4×10^5 CFU/ml for *P. chlororaphis* and *S. cerevisiae*. Therefore, in the joint cultivation of microorganisms of

different taxonomic groups, the optimum temperature is 30 °C.

Yeast by type of nutrition are facultative anaerobes; the presence of oxygen inhibits alcoholic fermentation and they actively accumulate biomass [36]. Therefore, the task of the next stage of the study was to select the optimal aeration regime for the joint cultivation of yeast and bacteria in a bioreactor (Table 1). We have found that the optimal aeration regimen is 4 l/h. It should be noted that the presence of oxygen in the medium had a greater effect on the viability of yeast, since the number of viable *P. chlororaphis* cells under all aeration modes did not fall below 2×10^8 CFU/ml. The data obtained at this stage are comparable with the results of previous experiments, when cultivation was carried out on a shaker-incubator in flasks with cotton-gauze stoppers. From this, we can conclude that the studied microorganisms are not very demanding on aeration regimes and there is no obvious competition between them.

1. The number of viable cells (CFU/ml) **of *Pseudomonas chlororaphis* subsp. *aureofaciens* B-5326 and *Saccharomyces cerevisiae* Y-4317 in co-culture** (a BIORUS GJ bioreactor, BIORUS, Republic of Belarus) **in a medium with beet molasses** (15 g/l)**, depending on the aeration regime** ($N = 5$, $M\pm$SEM)

Aeration, l/h	*P. chlororaphis*, $\times10^8$	*S. cerevisiae*, $\times10^6$
1	4.2±0.31	0.8±0.05
2	2.1±0.18	2.4±0.09
3	3.6±0.24	3.6±0.18
4	5.9±0.36	5.9±0.12
5	3.2±0.17	2.1±0.26
6	2.4±0.08	4.2±0.16

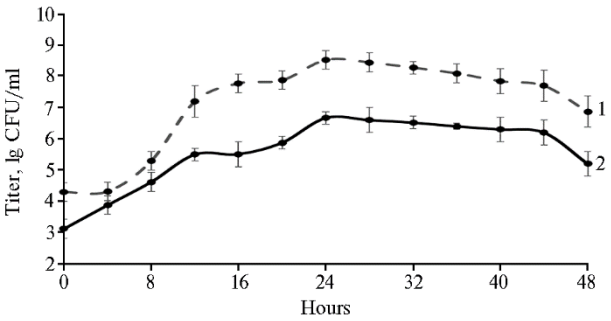

Fig. 3. The number of *Pseudomonas chlororaphis* subsp. *aureofaciens* B-5326 (1) **and *Saccharomyces cerevisiae* Y-4317** (2) **in co-culter in a medium with beet molasses** (15 g/l) **at 30 °C** ($N = 5$, $M\pm$SEM)**.**

Further, we studied the dynamics of changes in culture titers (CFU value) in the co-culture of *P. chlororaphis* and *S. cerevisiae* in a medium with molasses at 30 °C (Fig. 3). In the first 8 h, the CFU value in yeast increased much faster and more significantly than in bacteria, and the titers of *P. chlororaphis* and *S. cerevisiae* were 6×10^4 and 3.8×10^4 CFU/ml, respectively. Obviously, growth during the logarithmic phase in the first hours of cultivation in yeast was more intense than in *Pseudomonas*. After 12 h, the growth curve of *S. cerevisiae* entered the stationary phase, after which the number of yeasts slightly increased. After 24 h, it began to decrease. *Pseudomonas* showed the most active growth in the period from 8 to 24 h of culture, which correlates with literature data [37]. The maximum number of *P. chlororaphis* cells was recorded after 24 h of co-culture with yeast (8.4×10^8 CFU/ml). In the period from 36 to 48 h, the number of bacterial cells gradually decreased and the titer of *P. chlororaphis* decreased by almost 100 times (to 8.8×10^6 CFU/ml). The elongation of the logarithmic growth phase of *P. chlororaphis* observed in the co-culture of yeast and bacteria can be explained by the inclusion of molasses containing complex carbohydrates in the substrate composition, the use of which increases the duration of exponential cell growth. The subsequent slowdown in growth may be due to competition for nutrients [38].

In order to test this assumption, we extended the co-cultivation of microorganisms up to 72 h, while taking into account the growth of P. chlororaphis in pure culture (Fig. 4). At the same time, paradoxically, it turned out that in the mixed culture with S. cerevisiae yeast, there was no further decrease in the bacterial titer, while in the pure culture of Pseudomonas, after 72 h, the titer of viable cells decreased to the limiting minimum (5×104 CFU/ml).

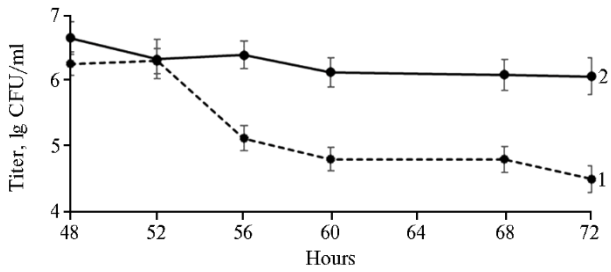

Fig. 4. ***Pseudomonas chlororaphis*** **subsp.** ***aureofaciens*** **B-5326 growth in pure culture** (1) **and co-culture with** ***Saccharomyces cerevisiae*** **Y-4317** (2) **for 72 h in a medium with beet molasses** (15 g/l) **at 30 °C** ($N = 5$, M±SEM).

Thus, a positive effect of S. cerevisiae on the viability of P. chlororaphis h was shown, since after 72 of co-cultivation the bacterial titer was 1×10^6 CFU/ml, which is approximately two orders of magnitude higher than in pure bacterial culture. J.D. Romano et al. [39] described similar effects of fungal metabolites on the viability of bacteria during the interaction of *Pseudomonas* and *Saccharomyces.*

It is known that the mechanism of interaction between bacteria and fungi is based on the ability of yeast to synthesize gluconate from glucose. Gluconate, in turn, increases the viability of bacteria of the genus *Pseudomonus* [40]. This can partly explain the revealed positive effect of *S. cerevisiae* on the growth of *P. chlororaphis.* In addition, in recent years, data have appeared that yeast are active producers of the auxin indolyl-3-acetic acid (possibly, other phytohormones as well). Approximately 90% of all known yeast species are capable of synthesizing auxins: yeasts of the genus *Cyberlindnera*, *Rhodotorula graminis*, *Rhodosporidium fluviale*, *Rhodosporidium paludigenum*, *Aureobasidium pullulans*, *Saccharomyces cerevisiae* [41-43]. In terms of the activity of synthesis of hormonal compounds, yeast is practically not inferior to bacteria and algae. It has been shown that yeast culture liquid can influence the growth and development of plants [44]. It can be assumed that it was the production of phytohormones by the yeast *S. cerevisiae* in co-culture that determined the active growth and high viability of *P. chlororaphi*s.

We preliminary evaluated the effectiveness of the complex bio-preparation by determining the germination of seeds of cereal plants (barley, wheat, corn) after treatment with the culture liquid, obtained by co-culturing yeast and *Pseudomonas*, in four dilutions (from 1:200 to 1:25) (Table 2). In the control, the germination energy and seed germination were quite high and ranged from 79 to 89%.

2. Germination energy and germination (%) **of treated cereal seeds at different dilutions of the culture fluid from co-culture of** ***Pseudomonas chlororaphis*** **subsp.** ***aureofaciens*** **B-5326 and** ***Saccharomyces cerevisiae*** **Y-4317** (M±SEM)

Parameter	Dilutions					Control (water)
	1:200	1:150	1:100	1:50	1:25	
Barley (*Hordeum vulgare* L.) cv. Skipetr						
Germination energy	90±2.1	88±2.5	91±3.1	89±2.8	87±2.5	85±2.4
Germination	96±2.9*	92±3.4	95±3.8	92±2.2	89±3.2	87±3.0
Wheat (*Triticum aestivum* L.) cv. Mironovskaya 808						
Germination energy	93±3.2	91±2.9	95±3.7	89±2.6	85±2.8	86±3.2
Germination	94±3.6	95±3.1	96±4.1	92±2.8	90±3.1	89±2.7
Corn (*Zea mays* L.) hybrid Delitop						
Germination energy	89±2.9*	87±3.6	86±4.1	80±3.8	81±2.9	79±3.1
Germination	96±4.6*	89±3.6	90±2.8	85±3.0	88±3.3	83±3.4

* Differences from control are statistically significant at $p \le 0.05$.

Pre-sowing treatment with a biological product stimulated the germination of seeds of all studied cereal crops. The efficiency of treatment depended on the concentration (dilution) of the culture liquid. Germination of barley seeds statistically significantly ($p = 0.05$) increased by 9% compared to the control when using CL at a dilution of 1:200. A clear trend towards an increase in the germination energy and germination of barley seeds was noted at all dilutions (differences from the control are not significant). Similar relationships were found for wheat, where the highest germination rates were observed for dilutions of 1:200-1:100, but the differences with the control were not significant.

In the experiment with corn seeds, the lowest concentrations of the biological product also turned out to be the most effective. When using a dilution of 1:200, the germination energy significantly ($p = 0.05$) increased by 10%, and germination by 13% compared to the control. Dilutions of 1:150 and 1:100 showed a tendency to stimulate seed germination (differences from control are not significant). Higher doses of the biological product (dilutions 1:50 and 1:25) slightly increased the germination energy and the germination rate.

An increase in germination energy and high seed germination are controlled by phytohormones which activate cell division and elongation and increase stress resistance [16, 17].

Thus, we have shown the possibility of co-cultivation of the bacterium *Pseudomonas chlororaphis* subsp. *aureofaciens* B-5326 and yeast *Saccharomyces cerevisiae* Y-431, which will make it possible to create a biological product based on microorganisms of different taxonomic groups. Beet molasses (waste of sugar production) fully satisfies the needs of the studied microorganisms in basic nutrients. The optimal conditions for co-culture were selected, i.e., a temperature of 30 °C, aeration mode 4 l/h, culture duration 24 h. A positive effect of *S. cerevisiae* on the viability of *P. chlororaphis* was revealed during long-term (72 h) co-culture. Therefore, in the presence of *S. cerevisiae*, the titer of *P. chlororaphis* was 10^6 CFU/ml, in pure culture it was 10^4 CFU/ml. Pre-sowing treatment of seeds with a preparation obtained in co-culture of these microorganisms stimulated the vigor of germination and germination rate in barley, wheat and corn. The complex preparation showed the highest efficiency in relation to corn seeds. The maximum increase in the energy of seed germination and the germination rate for all the studied crops was revealed at a dilution of the preparaion 1:200.

REFERENCES

1. Kocira S., Hara P., Szparaga A., Czerwińska E., Beloev H., Findura P., Bajus P. Evaluation of the effectiveness of the use of biopreparations as seed dressings. *Agriculture*, 2020, 10(4): 90 (doi: 10.3390/agriculture10040090).
2. Yadav A.N., Verma P., Singh B., Chauhan V.S., Suman A., Saxena A.K. Plant growth promoting bacteria: Biodiversity and multifunctional attributes for sustainable agriculture. *Advances in Biotechnology & Microbiology*, 2017, 5(5): 555671 (doi: 10.19080/AIBM.2017.05.5556671).
3. El-Maraghy S.S., Tohamy A.T., Hussein K.A. Plant protection properties of the plant growth-promoting fungi (PGPF): Mechanisms and potentiality. *Current Research in Environmental & Applied Mycology (Journal of Fungal Biology)*, 2021, 11: 391-415 (doi: 10.5943/cream/11/1/29).
4. Kumar A., Singh S., Gaurav A.K., Srivastava S., Verma J.P. Plant growth-promoting bacteria: Biological tools for the mitigation of salinity stress in plants. *Frontiers in Microbiology*, 2020, 11: 1216 (doi: 10.3389/fmicb.2020.01216).
5. Burova Yu.A., Ibragimova S.A., Revin V.V. *Izvestiya Tul'skogo gosudarstvennogo universiteta. Estestvennye nauki*, 2012, 3: 198-206 (in Russ.).
6. Babenko L.M., Romanenko E.A., Yungin, O.S., Kosakovskaya I.V. Acyl-homoserine lactones for crop production and stress tolerance of agricultural plants (review). *Sel'skokhozyaistvennaya biologiya* [*Agricultural Biology*], 2021, 56(1): 3-19 (doi: 10.15389/agrobiology.2021.1.3eng).
7. Bertani I., Zampieri E., Bez C., Volante A., Venturi V., Monaco S. Isolation and characterization

of *Pseudomonas chlororaphis* strain ST9 and its potential as a bioinoculant for agriculture. *BioRxiv*, 2020, 10(7): 1466 (doi: 10.1101/2020.12.23.424151).
8. Jiménez J.A, Novinscak A., Filion M. Inoculation with the plant-growth-promoting rhizo-bacterium *Pseudomonas fluorescens* LBUM677 impacts the rhizosphere microbiome of three oilseed crops. *Frontiers in Microbiology*, 2020, 11: 569366 (doi: 10.3389/fmicb.2020.569366).
9. Zhang Y., Li T., Xu M., Guo J., Zhang C., Feng Z., Peng X., Li Z., Xing K., Qin S. Antifungal effect of volatile organic compounds produced by *Pseudomonas chlororaphis* subsp. *aureofaciens* SPS-41 on oxidative stress and mitochondrial dysfunction of *Ceratocystis fimbriata*. *Pesticide Biochemistry and Physiology*, 2021, 173: 104777 (doi: 10.1016/j.pestbp.2021.104777).
10. Salim H.A., Aziz A.K., Mahdi M.H., Ali M.A.K., Salman M.H., Hussein M.M., Mohammed L.K., Ahmed M.S., Khalil A.Y., Hadi T.A. Effect of bio-fertilizers *Azotobacter chroococcum* and *Pseudomonas fluorescens* on growth of broccoli (*Brassica oleracea* L. var. *italica*). *Journal of Advances in Biology*, 2018, 11(01): 2236-2240 (doi: 10.24297/jab.v11i1.7590).
11. Martínez-Carranza E., Ponce-Soto G.-Y., Servín-González L., Alcaraz L.D., Soberón-Chávez G. Evolution of bacteria seen through their essential genes: the case of *Pseudomonas aeruginosa* and *Azotobacter vinelandii*. *Microbiology,* 2019, 165(9): 976-984 (doi: 10.1099/mic.0.000833).
12. Cidorenko M.L., Sleptsova N.A., Bykovskaya A.N., Berezhnaya V.V., Klykov A.G. Effects of nitrogen-fixing and phosphate-solubilizing microorganisms from the Far East agricultural soils on the cereal seed germination. *Sel'skokhozyaistvennaya biologiya* [*Agricultural Biology*], 2021, 56(1): 146-157 (doi: 10.15389/agrobiology.2021.1.146eng).
13. Naserzadeh Y., Nafchi A.M., Mahmoudi N., Nejad D.K., Gadzhikurbanov A.S. Effect of combined use of fertilizer and plant growth stimulating bacteria *Rhizobium, Azospirillum, Azotobacter and Pseudomonas* on the quality and components of corn forage in Iran. *RUDN Journal of Agronomy and Animal Industries*, 2019, 14(3): 209-224 (doi: 10.22363/2312-797x-2019-14-3-209-224).
14. Ali S., Hameed S., Shahid M., Iqbal M., Lazarovits G., Imran A. Functional characterization of potential PGPR exhibiting broad-spectrum antifungal activity. *Microbiological Research*, 2020, 232: 126389 (doi: 10.1016/j.micres.2019.126389).
15. Streletskii R.A., Kachalkin A.V., Glushakova A.M., Demin V.V., Chernov I.Y. Quantitative determination of indole-3-acetic acid in yeasts using high performance liquid chromatography—tandem mass spectrometry. *Microbiology*, 2016, 85: 727-736 (doi: 10.1134/S0026261716060187).
16. Lukatkin A.S., Zauralov O.A. *Doklady Rossel'khozakademii*, 2009, 6: 20-22 (in Russ.).
17. Kolmykova T.S., Lukatkin A.S. *Agrokhimiya*, 2012, 1: 83-94 (in Russ.).
18. Gruznova K.A., Bashmakov D.I., Miliauskienė J., Vaštakaitė V., Duchovskis P., Lukatkin A.S. The effect of a growth regulator Ribav-Extra on winter wheat seedlings exposed to heavy metals *Zemdirbyste-Agriculture*, 2018, 105(3): 227-234 (doi: 10.13080/z-a.2018.105.029).
19. Yamagiwa Y., Inagaki Y., Ichinose Y., Toyoda K., Hyakumachi M., Shiraishi T. *Talaromyces wortmannii* FS2 emits β-caryphyllene, which promotes plant growth and induces resistance. *Journal of General Plant Pathology*, 2011, 77(6): 336-341 (doi: 10.1007/s10327-011-0340-z).
20. Bhattacharyya P.N., Jha D.K. Plant growth-promoting rhizobacteria (PGPR): emergence in agriculture. *World Journal of Microbiology and Biotechnology*, 2012, 28(4): 1327-1350 (doi: 10.1007/s11274-011-0979-9).
21. Khan A.L., Waqas M., Lee I.-J. Resilience of *Penicillium resedanum* LK6 and exogenous gibberellin in improving *Capsicum annuum* growth under abiotic stresses. *Journal of Plant Research*, 2015, 128(2): 259-268 (doi: 10.1007/s10265-014-0688-1).
22. Tian P., Razavi B.S., Zhang X., Wang Q., Blagodatskaya E. Microbial growth and enzyme kinetics in rhizosphere hotspots are modulated by soil organics and nutrient availability. *Soil Biology and Biochemistry*, 2020, 141: 107662 (doi: 10.1016/j.soilbio.2019.107662).
23. Ozimek E., Hanaka A. *Mortierella* species as the plant growth-promoting fungi present in the agricultural soils. *Agriculture*, 2021, 11(1): 7 (doi: 10.3390/agriculture11010007).
24. Taparia T., Krijger M., Haynes E., Elphinstone J.G., Noble R., van der Wolf J. Molecular characterization of *Pseudomonas* from *Agaricus bisporu*s caps reveal novel blotch pathogens in Western Europe. *BMC Genomics*, 2020, 21(1): 505 (doi: 10.1186/s12864-020-06905-3).
25. *Spravochnik pestitsidov i agrokhimikatov, razreshennykh k primeneniyu na territorii Rossiiskoi Federatsii* [Directory of pesticides and agrochemicals permitted for use on the territory of the Russian Federation]. Moscow, 2011 (in Russ.).
26. Jacob F.F., Striegel L., Rychlik M., Hutzler M., Methner F.-J. Spent yeast from brewing processes: a biodiverse starting material for yeast extract production. *Fermentation*, 2019, 5(2): 51 (doi: 10.3390/fermentation5020051).
27. Anderson A.J., Kim Y.C. Insights into plant-beneficial traits of probiotic *Pseudomonas chlororaphis* isolates. *Journal of Medical Microbiology*, 2020, 69(3): 361-371 (doi: 10.1099/jmm.0.001157).
28. Netrusov A.I., Egorova M.A., Zakharchuk L.M. *Praktikum po mikrobiologii* /Pod redaktsiei A.I. Netrusova [Workshop on microbiology. A.I. Netrusov (ed.)]. Moscow, 2005 (in Russ.).
29. Casalone E., Barberio C., Cappellini L., Polsinelli M. Characterization of *Saccharomyces cerevisiae* natural populations for pseudohyphal growth and colony morphology. *Research in Microbiology*, 2005, 156(2): 191-200 (doi: 10.1016/j.resmic.2004.09.008).

30. Kasana R.C., Sharma U.K., Sharma N., Sinha A.K. Isolation and identification of a novel strain of *Pseudomonas chlororaphis* capable of transforming isoeugenol to vanillin. *Current Microbiology*, 2007, 54(6): 457-461 (doi: 10.1007/s00284-006-0627-z).
31. Jain R., Pandey A. A phenazine-1-carboxylic acid producing polyextremophilic *Pseudomonas chlororaphis* (MCC2693) strain, isolated from mountain ecosystem, possesses biocontrol and plant growth promotion abilities. *Microbiological Research*, 2016, 190: 63-71 (doi: 10.1016/j.micres.2016.04.017).
32. *GOST 12038-84. Semena sel'skokhozyaistvennykh kul'tur. Metody opredeleniya vskhozhesti* [GOST 12038-84. Seeds of agricultural crops. Germination methods]. Moscow, 2004 (in Russ.).
33. Lakin G.F. *Biometriya* [Biometrics]. Moscow, 1990 (in Russ.).
34. Šarić L.Ć., Filipčev B.V., Šimurina O.D., Plavšić D.V., Šarić B.M., Lazarević J.M., Milovanović I.L. Sugar beet molasses: properties and applications in osmotic dehydration of fruits and vegetables. *Food and Feed Research*, 2016, 43(2): 135-144 (doi: 10.5937/FFR1602135S).
35. Kim H.-S., Cha E., Kim Y., Jeon Y.H., Olson B.H., Byun Y., Park H.-D. Raffinose, a plant galactoside, inhibits *Pseudomonas aeruginosa* biofilm formation via binding to LecA and decreasing cellular cyclic diguanylate levels. *Scientific Reports*, 2016, 6(1): 25318 (doi: 10.1038/srep25318).
36. Kuriyama H., Kobayashi H. Effects of oxygen supply on yeast growth and metabolism in continuous fermentation. *Journal of Fermentation and Bioengineering*, 1993, 75(5): 364-367 (doi: 10.1016/0922-338X(93)90135-U).
37. Shen X., Wang Z., Huang X., Hu H., Wang W., Zhang X. Developing genome-reduced *Pseudomonas chlororaphis* strains for the production of secondary metabolites. *BMC Genomics*, 2017, 18: 715 (doi: 10.1186/s12864-017-4127-2).
38. Moreno-Avitia F., Utrilla J., Bolívar F., Nogales J., Escalante A. Metabolic reconstruction of *Pseudomonas chlororaphis* ATCC 9446 to understand its metabolic potential as a phenazine-1-carboxamide-producing strain. *Applied Microbiology and Biotechnology*, 2020, 104(23): 10119-10132 (doi: 10.1007/s00253-020-10913-4).
39. Romano J.D., Kolter R. *Pseudomonas-Saccharomyces* interactions: Influence of fungal metabolism on bacterial physiology and survival. *Journal of Bacteriology*, 2005, 187(3): 940-948 (doi: 10.1128/JB.187.3.940-948.2005).
40. Schleissner C., Reglero A., Luengo J.M. Catabolism of D-glucose by *Pseudomonas putida* occurs via extracellular transformation into D-gluconic acid and induction of a specific gluconate transport system. *Microbiology*, 1997, 143(5): 1595-1603 (doi: 10.1099/00221287-143-5-1595).
41. Limtong S., Koowadjanakul N. Yeasts from phylloplane and their capability to produce indole-3-acetic acid. *World Journal of Microbiology and Biotechnology*, 2012, 28(12): 3323-3335 (doi: 10.1007/s11274-012-1144-9).
42. Nutaratat P., Srisuk N., Arunrattiyakorn P., Limtong S. Plant growth-promoting traits of epiphytic and endophytic yeasts isolated from rice and sugar cane leaves in Thailand. *Fungal Biology*, 2014, 118(8): 683-694 (doi: 10.1016/j.funbio.2014.04.010).
43. Pirog T.P., Iutynska G.O., Leonova N.O., Beregova K.A., Shevchuk T.A. Microbial synthesis of phytohormones. *Biotechnologia Acta*, 2018, 11(1): 5-24 (doi: 10.15407/biotech11.01.005).
44. Streletskii R.A., Kachalkin A.V., Fedotov G.N. *Sovremennye problemy nauki i obrazovaniya*, 2017, 1: 130 (in Russ.).