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Designing of the Biological Product "AGROBIOLOG" for Mitigating Pesticide Stress in Agricultural Plants and Stimulating Their Growth

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Abstract. The results of the selection of new plant growth-promoting bacteria resistant to herbicides of different chemical structure are presented. Two methods were used for screening: sowing on microbiological media from freshly sampled soil and obtaining enrichment cultures from soil samples exposed to herbicides for a month at a temperature of 28 °C. Inoculation was carried out on selective nutrient media containing herbicides Octapon extra (2,5 g/l), Florax (2,5 g/l), Dicamba (1 g/l), Nanomet (1 g/l), Spetsnaz (1 g/l) or Chistalan (2,5 g/l). These herbicides are commonly used to control weeds in wheat crops. The isolated microorganisms belong to the genus *Pseudomonas,* fix nitrogen, mobilize phosphates, synthesize phytohormones and antimicrobial compounds. They also can mitigate pesticide stress of crops. An anti-stress biological product containing them has been developed and tentatively named "AGROBIOLOG". For its production, the optimal composition of the nutrient medium and the conditions for industrial submerged cultivation on reactors of various volumes were determined. In the laboratory fermenter FA-10 with a volume of 10 liters after 72 hours of cultivation at a temperature of 28 °C, a stirrer speed of 200 rpm, aeration of 0.5 volumes of air per 1 min per 1 volume of medium, the amount of viable cells was 28 billion CFU/ml of culture liquid. Cultivation on biological reactors with a volume of 1000 liters under the same conditions allowed to achieve a titer of 6.0 billion CFU/ml of culture liquid.

Keywords: plant stress neutralizer, biological product, PGPB, PSMB, plant productivity.

Introduction

The productivity of agricultural plants is directly related to the use of methods for their integrated protection from stress effects: diseases, pests, drought, and weeds. Pesticides are an important element in intensive plant cultivation systems. At least 60% of them are herbicides [1]. Their use, in turn, not only leads to environmental pollution, damages soil fertility and microbiota, but also significantly effects crops [2-4]. It is possible to mitigate these stressful effects with the use of bacterial antistress agents.

In crop production, there is a growing interest in the bacterial-based products that have a positive influence on the growth and development of agricultural plants. The plant growth stimulation is usually caused by a complex of beneficial properties of bacteria, such as the ability to nitrogen fixation, phosphate dissolution, synthesis of phytohormones and antimicrobial compounds, competition for nutrients and roots surface with pathogens [5-7]. According to the scientific review, the treatment of crops with growth-stimulating bacteria (plant growth promoting bacteria – PGPB) turns out to be the most effective in overcoming abiotic stresses [8, 9]. Their use can improve the plants growth exposed to herbicides and drought [3]. Still reports on the mitigation of pesticide stress of plants by bacteria are few [10-11]. In this case, the ability of bacteria to effectively combine with chemical herbicides comes to the fore. It assumes their resistance to herbicides [12], and the ability to have a complex positive effect on the plant, including protection from moisture deficiency [13] and pathogens [14] against the background of herbicides.

Therefore, the development of biological products based on pesticide stress mitigating bacteria (PSMB) for stimulating the growth of agricultural crops becomes relevant.

Materials and Methods

The purpose of the work is to screen new bacteria that are resistant to herbicides of different chemical structures and promote plant growth, to create on its basis an anti-stress biological product with the operational name "AGROBIOLOG" and optimizing the conditions of its industrial production.

Two methods were used for screening: sowing on microbiological media directly from freshly harvested soil and obtaining enrichment cultures from soil samples with addition of herbicides, of which, after a month of incubation at a temperature of 28 °C, inoculation on selective nutrient media

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containing herbicides was carried out. The Raymond mineral medium was used as the basis for the media with the addition of, as selective agents, herbicides based on 2,4-dichlorophenoxyacetic acid ususally used on wheat crops (Octapon extra (2,5 g/l), Florax (2,5 g/l), Dicamba (1 g/l), Chistalan (2,5 g/l)) and based on sulfonylureas (Nanomet (1 g/l), Spetsnaz (1 g/l)). The concentrations of herbicides in the medium were selected based on the concentration of their working solution according to the manufacturers recommendations.

Antagonism to phytopathogens was determined by the simultaneous cultivation of bacteria and fungi in Petri dishes. Testing was performed on the following mycelial fungi: *Bipolaris sorokiniana* (Sacc.) Shoemaker, *Fusarium culmorum* (W.G. Smith) Sacc. ВКМ F-844, *F. gibbosum* Appel et Wollenw ВКМ F-848, *F. graminearum* Schwabe ВКМ F-1668, *F. solani* (Mart) Sacc. ВКМ F-142, *F. oxysporum* Schltdl ВКМ F-137, *F. nivale* (Fr.) Ces. Ex Sacc. ВКМ F-3106, *F. semitectum* ВКМ F-1938, *F. avenaceum* ВКМ F-132, *Alternaria alternate* (Fr.) Keissl. ВКМ F-3047, *Rhizoctonia solani* J.G. Kuehn ВКМ F-895. To measure the nitrogenase activity, an acetylene assay was used, according to the methodology [15]. The ability of the strain to synthesize IAA was determined using the method of enzyme immunoassay [16]. The solubilization of phosphates was evaluated by the formation of a clear halo around the bacterial colony after five incubation days at 28 °C in the Pikovskaya agar as described [17].

When optimizing the cultivation conditions, we relied on the experience of obtaining biological products for agricultural purposes based on pseudomonads [4]. Biological reactors of various volumes were used for scaling (LLC firm "Prointech", Pushchino, Russia).

Results

A scheme for the selection of anti-stress bacterial agents is proposed (fig. 1), the main condition is the resistance of microorganisms to herbicides.

Stage 1. Herbicide resistance

Stage 2. Availability of PGPB properties: production of phytohormones, production of antibiotics, nitrogen fixation, phosphorus mineralization.

Stage 3. The presence of positive properties of PGPB in the presence of herbicides

Figure 1. Scheme of selection of potential anti-stress agents for the protection of agricultural plants

Рисунок 1. Схема подбора потенциальных антистрессовых средств для защиты сельскохозяйственных растений

Microbes resistant to herbicides were isolated from 178 soil samples taken on the territory of agricultural lands and industrial zones of the Republic of Bashkortostan, industrial area in the Komi Republic (Russia). After studying their properties, 11 isolates were selected from them (Table 1). The selection criteria were the presence of agronomically valuable characteristics, resistance to herbicides and the ability to grow using different herbicides as a source of nutrients. When testing new strains for resistance in tank mixtures, it was found that the most toxic of the tested for bacteria was the herbicide Chistalan (Table 1). Apparently, the toxicity of Chistalan is also due to the lower probability of detecting its destructors.

Table 1.

The ability of new isolates to grow in a medium with herbicides as the sole source of carbon and energy

Таблица 1.

Способность новых изолятов к росту в среде с гербицидами в качестве единственного источника углерода и энергии

Isolate	Herbicide					
	Dicamba	Octapon	Nanomet	Florax	Chistalan	Spetsnaz
CH.3.1	–	++	–	+	–	+
12N1	++	++	++	++	–	+
CH5% 1	+	++	+	++	+	+
CH5% 2	++	++	++	+	+	+
6CH1	++	++	–	++	+	+
6CH2	++	+	++	+	++	+
5N1	++	++	++	++	–	+
ДД4	+	+	+	–	+	+
DA1.2	++	++	++	++	++	+
NG	++	++	++	++	++	+
NEKR	++	++	+	++	–	++
Note: ++ active growth, + weak growth, – no growth						

The isolates DA1.2, 6CH2, CH5% 2, which were identified as *Pseudomonas protegens*, *P. avallanae* and *P. plecoglossicida*, respectively, were the most promising in the scope of the studied problem according to the set of abilities. Their agronomically valuable characteristics of PGPB are presented in Table 2, according to which and increased up to 200% level of antifungal activity (the diameters of the growth inhibition zone of the studied fungi are in the range of 30–40 mm) the strain DA1.2 is the best for creating the biological product "AGROBIOLOG".

Table 2.

Properties of potential bacterial antistressants

Таблица 2.

Свойства потенциального бактериального антистрессового средства

Strain	Nitrogenase activity, nmol $C_2H_4 \cdot h^{-1}ml^{-1}$	Synthesis of auxins, ng/ml	Phosphates solubilization
Pseudomonas plecoglossicida **CH5% 2**	20,7 ± 0,2	323 ± 16	+
P. avallanae **6CH2**	19,8 ± 0,2	169 ± 8	+
P. protegens **DA1.2**	20,8 ± 0,3	870 ± 44	+

The composition of the nutrient medium (g/l) was selected for the production of the biological product "AGROBIOLOG" : peptone-2, yeast extract-2, glycerin-5, NaCl-3, K_2HPO_4-2, $MgSO_4 \cdot 7H_2O$-1, as the most optimal in terms of the ratio of the cost of components and the final titer of the culture fluid. When cultured in a laboratory fermenter FA-10 with a volume of 10 liters at a temperature of 28 °C, stirrer speed-200 rpm, aeration-0,5 air volume per 1 min per 1 volume of medium for 72 hours, the concentration of viable cells was $2,8 \cdot 10^{10}$ CFU/ml. Cultivation in a reactor with a volume of 1000 liters under the same conditions allows achieving a titer of $6,0 \cdot 10^9$ CFU/ml.

Discussion

Almost all isolates were able to produce auxins. It is known that auxins accumulation in roots helps to increase the volume of the root system of plants and allows to accelerate the pathogen-sensitive stages of its development [18]. It also supports the plant having water deficiency [19].

The joint cultivation of the new bacterial strains with fungal mycelium showed their ability to antagonize phytopathogens. Their fungistatic activity was expressed as strongly as in previously isolated cultures of *Pseudomonas* [20]. However, unlike them, they are stable and able to grow in environments polluted by herbicides based on low-volatile esters of 2,4-D and sulfonylurea.

The optimization of the nutrient medium and cultivation conditions makes it possible to obtain the culture of *P. protegens* DA1.2 containing at least 5 billion CFU/ml of culture fluid without losing its characteristic properties. This meets the requirements of industrial cultivation of microorganisms and makes the biological product "AGROBIOLOG" promising for industrial production

Conclusion

An anti-stress biological product "AGROBIOLOG" based on PSMB has been developed. It can be used as a biofungicide, biofertilizer or safener in classic tank mixtures with herbicides. The useful properties (fixation of molecular nitrogen, immobilization of phosphorus, disease control), in addition to its effect on the mechanisms of stress in plants, increases its practical and commercial value.

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Conflict of interest

The authors declare no conflict of interest.

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Разработка биопрепарата "АГРОБИОЛОГ" для снижения пестицидного стресса сельскохозяйственных растений и стимулирования их роста

Аннотация. Представлены результаты селекции новых бактерий, стимулирующих рост сельскохозяйственных растений и устойчивых к гербицидам различной химической структуры. Для скрининга использовались два метода: посев на микробиологические среды из свежесобранной почвы и получение обогащенных культур микроорганизмов из образцов почвы, подвергнутых воздействию гербицидов в течение месяца при температуре 28°C. Бактерии изолировали на селективных питательных средах, содержащих гербициды Октапон экстра (2,5 г/л), Флоракс (2,5 г/л), Дикамба (1 г/л), Наномет (1 г/л), Спецназ (1 г/л) или Чисталан (2,5 г/л). Эти гербициды обычно используются для борьбы с сорняками в посевах пшеницы. Выделенные микроорганизмы принадлежат к роду Pseudomonas, фиксируют атмосферный азот, мобилизуют нерастворимые фосфаты, синтезируют фитогормоны и антимикробные соединения. Они также могут смягчать проявления пестицидного стресса у сельскохозяйственных растений. Был разработан содержащий новые штаммы бактерий антистрессовый биопрепарат, получивший предварительное название "АГРОБИОЛОГ". Для его производства был подобран оптимальный состав питательной среды и условия промышленного глубинного культивирования в биологических реакторах различного объема. В лабораторном ферментере FA 10 объемом 10 литров после 72 часов культивирования при температуре 28°C, скорости перемешивания 200 об/мин, аэрации 0,5 объема воздуха в 1 мин на 1 объем среды количество жизнеспособных клеток составило 28 млрд. КОЕ/мл жидкой культуры. Культивирование в биологических реакторах объемом 1000 литров при тех же условиях позволило достичь титра 6,0 млрд КОЕ/мл жидкой культуры.

Ключевые слова: нейтрализатор стресса растений, биопрепарат, PGPB, PSMB, продуктивность растений.

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