Use of biosurfactants produced by *Bacillus subtilis* H1 and *Pseudomonas aeruginosa* PAO1 as a disinfectant and plant growth stimulation

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Abstract. Sustainable agriculture involves the maximum use of the resource potential of the earth, with the constant renewal of the fertility of the ecosystem. One method of transition to sustainable agriculture is the use of biological control agents, which include biosurfactants. We assessed the possibility of using crude biosurfactants obtained from Bacillus subtilis H1 and Pseudomonas aeruginosa PAO1 as dressing agents for untreated wheat seeds and the presence of seed germination stimulating properties. It was shown that crude rhamnolipids obtained from Pseudomonas aeruginosa PAO1 inhibited the area occupied by fungal mycelia at a concentration of 500 mg/l. Surfactin, derived from Bacillus subtilis H1, inhibited fungal growth at a concentration of 100 mg/L. Stimulation of germination of wheat seeds by 1.9 and 2 times was determined by rhamnolipid at a concentration of 500 mg/l and surfactin at a concentration of 100 mg/l for 168 hours of germination. The use of biocontrol agents is a promising method that can improve product quality while reducing the negative impact on the environment.

1 Introduction

The current phytosanitary condition of the fields forces agricultural enterprises to use a large amount of pesticides (fungicides, insecticides, antimicrobial) [1]. One of the main activities in preparation for sowing grain crops is the disinfection of seeds using various kinds of pesticides - dressing. At the same time, there is no universal remedy, and the choice of a treater depends on many factors: soil tillage system; predecessor; phytosanitary condition of the soil [2, 3]. For example, the amount of plant residues in the field, and, consequently, the level of infectious background for fungal diseases and the presence of pests, depends on the tillage system. With the active use of no- or mini-till technologies, which are so popular in the current conditions, the initial infectious background is much higher than during plowing with a layer turnover [4]. Depending on the totality of these factors, enterprises use mono-components or a variety of insect-fungicide mixtures. Most often, preparations based on four substances are used for insecticidal seed treatment: imidacloprid, thiamethoxam, clothianidin or acetamiprid, and substances of the triazole class are used as fungicidal agents. Research

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aimed at determining the impact of disinfectants on the environment and human health does not stop. For example, since 2018, thiamethoxam, imidacloprid and clonidine have been banned for use in field crops in EU countries due to the environmental aspects of their use [5].

Due to the wide variety of chemical pesticides, the presence of mutagenic and / or carcinogenic properties found for some substances with constant exposure to organisms even at low concentrations, of great interest are biopesticides with lower toxicity, a biodegradation period, while having the ability to stimulate growth and development plants [6].

One of the representatives of natural biopesticides are biosurfactants with antimicrobial and fungicidal properties [7]. Biosurfactants are secondary metabolites of various microorganisms - bacteria, fungi, etc., which have antimicrobial properties against various phytopathogens. For example, more than a hundred strains of *B. subtilis* are known to be capable of producing more than twenty different antibiotics [8]. In addition, B. subtilis are producers of biosurfactants of the lipopeptide group, which have high activity against insects, mites, phytopathogens, and are also capable of stimulating plant defense mechanisms [9]. It is suggested that lipopeptides produced by *B. subtilis* stimulate induced systemic plant resistance [10].

Bacteria of the genus *Pseudomonas*, in particular *P. aeruginosa*, are producers of another group of biosurfactants, rhamnolipids [11]. These amphiphilic compounds consist of one or two polar rhamnosoglycosyl heads linked by a beta-glycosidic bond to one or two hydrophobic 3-hydroxy fatty acid tails [12]. Rhamnolipids are used by bacteria for surface mobility, biofilm development, as well as for absorption or degradation of poorly soluble substrates [13]. Rhamnolipids are widely used in industry - in food production, cosmetics and pharmaceuticals, as well as for bioremediation [14]. Rhamnolipids are also considered as promising biocontrol agents and substances capable of stimulating plant growth. In the work of W. Patricio Luzuriaga-Loaiza et al, 2018, using structural and functional analysis, it was shown that rhamnolipids, depending on the length of the acyl chain, differently activate early and late defense reactions of plants and provide a local increase in resistance to phytopathogens [15].

The quality and quantity of biosurfactants depend on many factors - the type of producer, the type of nutrient medium, the type of functional groups of biosurfactants. Therefore, it is important to conduct a comprehensive assessment of the various properties of the resulting biosurfactants. As part of this study, we evaluated the possibility of using two biosurfactants obtained from *P. aeruginosa* PAO1 and *B. subtilis* H1 as wheat seed protectants, as well as evaluated the biostimulation properties of these substances relative to wheat seeds.

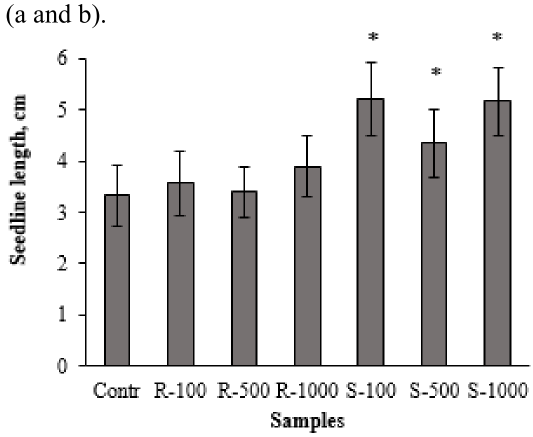
2 Materials and methods

Biosurfactant producing strains - B. subtilis H1 and P. aeruginosa PAO1 were obtained from oil-contaminated soil and soil contaminated with reservoir oil and «Devoroil», respectively. The strains are stored in the museum of the laboratory of the Institute of Ecological Sciences of the Kazan Federal University. To obtain biosurfactants, the strains were cultivated in a medium with glycerol nitrate, under the following conditions - 35°C and 180 rpm for 6 days in an INFORS HT fermenter (Sweetland). The composition of the medium corresponded to: (g/l): glycerol (40), $NaNO_3$ (4.0), $K_2HPO_4*3H_2O$ (4.0), KH_2PO_4 (3.0), $MgSO_4*7H_2O$ (0.5), KCl (0.5), NaCl (0.5), $CaCl_2*2H_2O$ (0.2). Supernatants were obtained by centrifugation at 8000 rpm for 10 min. The obtained biosurfactants were characterized by two methods: thin layer chromatography (TLC) and Fourier transform infrared spectroscopy (FTIR) [16].

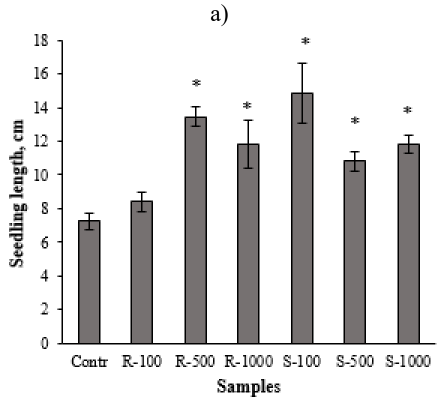
From an economic point of view, the use of crude mixtures of biosurfactants is more promising, therefore, crude mixtures of biosurfactants were used in this experiment. At the beginning, the effect of various concentrations of biosurfactants on seed disinfection was evaluated. For this, wheat seeds were treated with solutions of biosurfactans at three different concentrations - 100, 500, 1000 mg/l (samples R-100, R-500, R-1000 and S-100, S-500, S-1000 for rhamnolipid and surfactin respectively). The treatment was carried out under sterile conditions, the seeds were laid out in a Petri dish with a substrate of sterile filter paper, the solutions were applied to the seeds in 5 ml. In each variant, there were 6 replications of 10 seeds each. In the control sample, the seeds were treated with sterile water. At 72 hours, the morphometric data of the seedlings were evaluated, after which the seedlings were left in the Petri room for 21 days. The experiment was carried out in two stages, in the second stage the seeds were germinated for 168 hours.

3 Results

The effect of biosurfactants on seed germination was assessed at 72 h and 168 h after seed treatment with biosurfactants at three different concentrations. The data obtained are presented in Figure 1 (a and b).



a)



b)

Fig. 1. Wheat stem length after a) 72 h and b) 168 h when treated with sterile water, rhamnolipid and surfactin at concentrations of 100, 500, 1000 mg/l, respectively. Presented data correspond to means of at least thirty biological repetitions, error bars correspond to standard errors and asterisks correspond to significant differences ($P < 0.05$) according to Wilcoxon test.

Next, the germinated seeds were left in Petri dishes to determine the effect of biosurfactants on the degree of their overgrowing with fungi. Examples of photographs of variants of wheat seed sprouts treated with biosurfactants and a control variant are presented in Table 1.

Table 1. Seed sprouts in Petri dishes for 21 days of keeping in Petri dishes, treated with biosurfactants and control variant without treatment.

Consentration	**100 mg/l**	**500 mg/l**	**1000 mg/l**
Ramnolipid			
Surfactin			
Control			

The area occupied by fungal mycelium in Petri dishes with wheat seedlings, calculated using the ImagJ program, is shown in Figure 2.

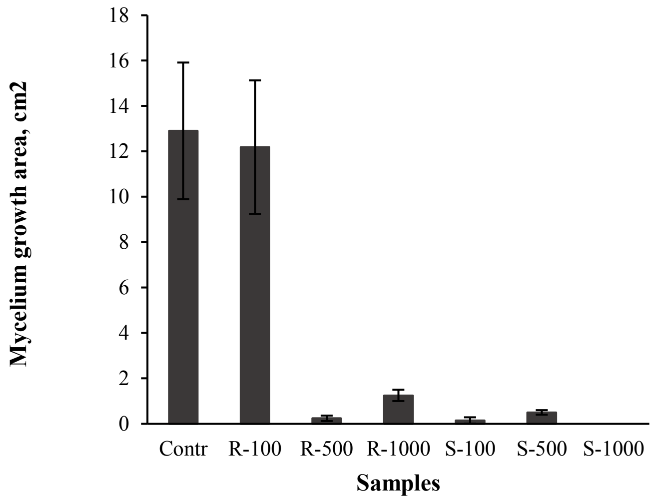


Fig. 2. The area occupied by fungal mycelium in Petri dishes with wheat seedlings treated with sterile water, rhamnolipids and surfactin at concentrations of 100, 500, 1000 mg/l, respectively.

4 Discussion

The use of biosurfactants is a promising strategy for replacing synthetic chemical growth promoters and seed treatments in agriculture with more environmentally friendly alternatives. Stimulation of germination of wheat seeds for 72 hours showed that the effectiveness of the control treated with surfactin has already reached 100 mg/L. 1.9-fold increase in germination observed for wheat germination for 168 hours for seeds treated with 500 mg/l rhamnolipids compared to control. Biosurfactants are known to be direct sources of environmental pollution, so in a study by Adnan et al. 2018, treatment of pepper seeds with a biosurfactant produced by the endophytic fungus *Xylaria regalis* led to an increase in the mass and use of seedlings, which led to faster plant growth, also increased the content of the content of chlorophyll, nitrogen, phosphorus in the treated plants [17]. Khare and Arora, et al, 2021 showed to stimulate the growth of sunflower plants, an 80.8% yield increase due to increased nutrient availability with biosurfactants, and antagonistic activity against *Macrophomina phaseolina* [18].

Plant seeds can contain a wide range of microorganisms, which can also be passive carriers of plant diseases. Fungal phytopathogens carried by seeds can significantly reduce seed germination and affect plant yields [19]. A detailed study of the functional potentials of biosurfactants showed that various biosurfactants have antimicrobial and fungicidal properties [20]. In Díaz De Rienzo et al., 2016; Valotteau et al., 2017; Kumar et al., 2021 ; Handore et al., 2022 described that rhamnolipid, surfactin, mannosylerythritollipids, sophorolipids, trihalose lipids and cellobiase lipids have antimicrobial activity against various phytopathogens - bacteria, fungi, viruses, nematodes [21–24]. This work also shows the ability of biosurfactants derived from bacteria to inhibit the growth of fungal mycelia. Surfactins derived from *B. subtilis* H1 inhibited fungal growth on wheat seedlings at a concentration of 100 mg/L. Rhamnolipids derived from *P. aeruginosa* PAO1 inhibited fungal growth at a concentration of 500 mg/L. Thus, the studied biosurfactants have fungicidal properties, and help to stimulate the germination of wheat seeds.

5 Conclusion

This work showed the presence of fungicidal properties in the tested biosurfactants, a significant decrease in the growth of fungal mycelium on wheat seedlings was determined, from concentrations of 100 mg/l for surfactin, and 500 mg/l for rhamnolipid. In addition, the ability to stimulate the germination of wheat seeds was determined for these concentrations. Thus, the use of biocontrol agents is a promising method that can improve product quality while reducing the negative impact on the environment.

The work is carried out in accordance with the Strategic Academic Leadership Program «Priority 2030» of the Kazan Federal University of the Government of the Russian Federation.

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