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Possible use of oil-degrading microorganisms for protection of plants growing under conditions of oil pollution

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Abstract. When entering soil, oil-degrading microorganisms come into contact with plants growing in polluted areas. We have studied the protective effect of oil-degrading microorganisms on plants growing under the conditions of model oil pollution. It has been shown that the treatment of the studied plants' seeds with a suspension of *Rhodococcus* at a concentration of 10^7 CFU eliminates the negative effect of oil and restores germination and morphological parameters of their seedlings. The content of chlorophyll a and b as well as carotenoids increased in plants treated with a suspension of *Rhodococcus* cells. That indicates the ability of *Rhodococcus* strain obtained to synthesize substances similar to cytokinins.

1. Introduction

A plant cannot choose conditions of existence; it is to transform the environment and improve it with the help of microorganisms. It forms its own microbiome, selectively isolating microorganisms with beneficial properties from the environment. Most of the rhizo- and endospheric microorganisms synthesize biologically active compounds that contribute to the improvement of plant nutrition and its protection against biotic and abiotic stress effects.

When entering soil, oil-degrading microorganisms come into contact with plants growing in polluted areas. Their interaction can be different. On the one hand, oil-degrading bacteria can help reduce the impact of oil and oil products on a plant and stimulate its growth due to the direct isolation of extracellular biologically active substances, such as phytohormones, enzymes, etc [1]. The indirect effect of microorganisms occurs when the oil film, which envelops the root surface, dissolves. That improves plant respiration and nutrient availability blocked by the oil film. On the other hand, the suppression of plant growth and development is not excluded due to the appearance of toxic low-molecular oil degradation products in immediate proximity to the root which are formed as a result of the activity of oil-degrading bacteria [2].

In this paper, the authors used the strains of oil-degrading microorganisms isolated from the endo- and rhizosphere of couch-grass (*Elytrigia repens*) which grew in the oil-polluted territory of the Irkutsk region [3, 4].

The purpose of the study was to evaluate the protective effect of oil-degrading strains on different types of plants and specify its mechanism.



2. Methods

Studying the effect of the strains on seed germination and plant growth under the conditions of oil pollution. To study the complex interaction of the plant, endo- and rhizosphere microorganisms, the authors used oil radish *Raphanus sativus* var. *oleiferus* (L.) Sazonova & Stank. (selection sample of SIPPB SB RAS “The line of IrSAA”), red daniel, Orpheus salad (Gavrish), Scherbet pea (Gavrish), and Sugar cob maize (Gavrish). Plastic containers with sterile sand were used for growing the plants in. The seeds were pre-sterilized, first with 96% ethanol for 1 min, and then with 3% hydrogen peroxide for 20 minutes. After that they were thoroughly washed and soaked for 24 hours in the bacteria suspension (10^7 CFU/ml). The plants whose seeds were soaked in sterile tap water served as control. Then the seeds were sown in containers with wet sand (60% of maximum water-holding capacity) with the addition of oil to a concentration of 2% (V/V). The plants were grown under the controlled conditions: t - 20°C, illumination - 2.1 kLq, photoperiod 14/10 h (day/night). On the 14th day of the experiment, the effect of the toxic effect of oil on the plant and its reduction in the presence of microorganisms was evaluated. Germination, mass and length of the aerial part of the plant and roots were analyzed for this purpose.

To stimulate the formation of auxins by cultures, D, L - tryptophan at a concentration of 200 mg/l was added to the liquid mineral medium (composition (g / l): NH_4NO_3 - 1.0, MgCl_2 - 0.1, KH_2PO_4 - 3.0, K_2HPO_4 - 7.0, CaCO_3 - 1.0, pH 7.0). The number of auxins was determined by the Salkowski method with the Gordon and Weber modification [5]. Their biological activity was determined using two biotests: with suppressing the growth of brown mustard seeds and with the intensity of rooting of beans cuttings [6, 7].

The study of gibberellin-like activity of strains. The study of gibberellin-like activity (GLA) was carried out according to the endospermic test on the germ-free halves of barley seeds [8]. The sterile nutrient medium without bacteria was the control. GLA was calculated according to a calibration graph obtained for a series of concentrations (0.001-0.1%) of sodium salts of gibberellic acids.

Pigment determination. Photosynthetic pigments were extracted from fresh plant material with 100% acetone. The content of chlorophylls and carotenoids was determined spectrophotometrically using SF-56 (LOMO, Russia) in an acetone extract at wavelengths of 662 and 644 nm (chlorophylls) and 440.5 nm (carotenoids). The experiment was carried out in three biological and three analytical replicates [9].

3. Results

To obtain data on the nature of the effect of the strains under study on plants is an important point before the stage of industrial use of oil-degrading strains in the processes of soils cleanup from oil pollution. In this regard, the purpose of this work stage was to study the effect of oil-degrading bacteria isolated from the endo- and rhizosphere of the plants on seed germination and plant development under the conditions of oil pollution.

To study the effect of endospermic and rhizospheric bacteria on plant development, experiments with oil radish (*Raphanus sativus* var. *oleiferus*) seeds were carried out.

First of all, the oil use rate for the experiments was determined. For this purpose, 0.5, 1.0, 2.0, 3.0% of oil was used. The growth and development of the plants turned out to be significantly inhibited at 3% oil concentration. The concentrations of 0.5 and 1% did not have a negative impact on the development of the plant. The plant growth was suppressed by about 50% at 2% oil concentration (figure 1). This concentration was used for the further experiments.

Five (*Pseudomonas* (90), *Pseudomonas* (102), *Pseudomonas* (109), *Acinetobacter* (112, 114) of the six strains studied did not show any effect on the growth and development of oil radish. *Rhodococcus* strain (108) had a positive effect on the plants. Therefore, this strain was used for the further experiments.

At the next stage of the research, the protective effect of *Rhodococcus* sp. on monocotyledonous and dicotyledonous plants was evaluated taking into account the size of their seeds (maize, red daniel, pea, salad, and radish). Pretreatment of the seeds with this strain showed a significant decrease in the toxic effect of oil on all the plants under study. In the presence of oil, the plants grown from seeds treated with *Rhodococcus* sp. are visually marked to gain better leaf mass than the untreated ones.

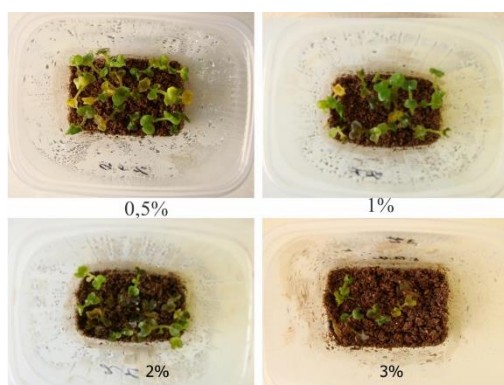
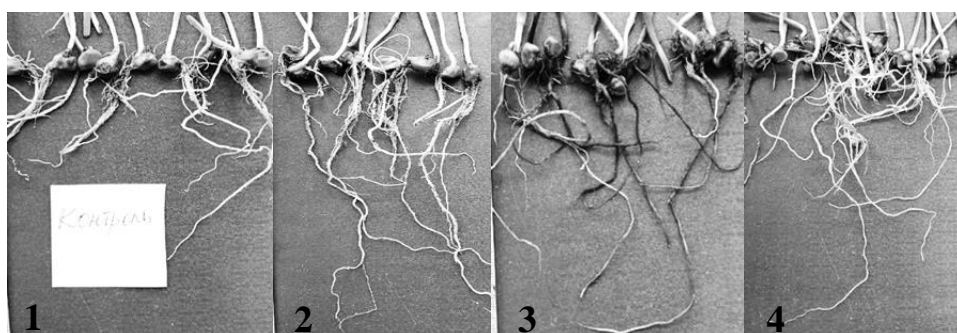


Figure 1. The effect of different concentrations of oil on the development of oil radish seedlings after 14 days of growing.

The roots of plants grown in sand with the addition of oil differed in the dark color which is characteristic of oil films and in a decrease in the number of lateral roots. Pretreatment of seeds with the suspension of microorganisms significantly improved the root system development (figure 2).

(a)



(b)

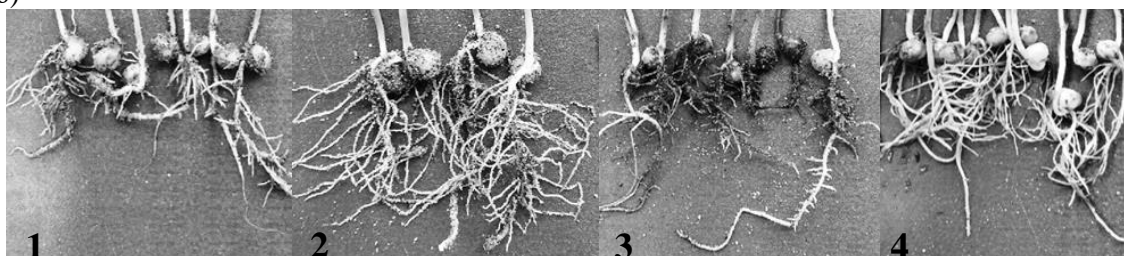


Figure 2. Development of the plant root system: A) maize; B) pea. 1. Control plants grown in the sand without the addition of 2% oil; 2. Plants grown from seeds treated with the suspension of *Rhodococcus* sp. in the sand without oil; 3. Plants grown in the sand from untreated seeds with 2% oil; 4. Plants grown in the sand from seeds treated with *Rhodococcus* sp. with 2% oil.

Oil pollution reduces the germination of seeds of all the plants studied by 20–50% depending on the plant species. The seedlings also suffer from the effects of oil: their total length, weight, and the number of pigments are significantly reduced. This is due to the insufficient ingress of water and oxygen into the germinating seed associated with the formation of an oil film on the seed surface (table 1).

The presence of microorganisms eliminated the negative effect of oil and restored germination almost to the control level (table 1). The morphological features of such plants were also close to the control. The number of pigments restored to the initial level and exceeded it in some cases. This is most likely due to the synthesis of biologically active substances similar to plant cytokinins by microorganisms.

Table 1. Morphological and physiological parameters of the plants depending on seed treatment with *Rhodococcus* sp. suspension and addition of 2% oil into the sand.

Culture	Germination, %	Aerial part of the plant length, cm	Roots length, cm	Mass, g	Chlorophyll a	Chlorophyll b	Carotenoids
Maize							
control	77	14,13	11,76	7,9	1,11±0,1 ¹ / 14,42±1,67 ²	0,28±0,03/ 3,70±0,4	0,74±0,1/ 9,64±1,3
108	83,3	13,3	11,9	4,8	1,25±0,1/ 12,98±1,7	0,35±0,04/ 3,59±0,4	0,80±0,1/ 8,23±1,1
Oil	60,4	5,4	7,8	5,5	0,55±0,01/5, 87±0,1	0,07±0,007/ 0,71±0,07	0,40±0,1/ 4,30±0,1
Oil+108	62,4	9,63	12,6	9,01	0,85±0,08/ 8,79±0,9	0,29±0,01/ 3,01±0,07	0,60±0,06/6,1 0±0,1
Pea							
control	29,7	10,35	6,75	4,45	1,2±0,1/ 10,36±1,6	0,54±0,07/ 3,95±0,6	0,77±0,1/ 7,53±1,1
108	33,3	11,75	6,5	6	1,2±0,1/ 12,43±0,9	0,46±0,02/ 3,88±0,2	0,84±0,05/ 8,96±0,5
Oil	11,7	6,27	4,17	3,35	0,84±0,1/ 7,00±0,8	0,32±0,04/ 2,89±0,2	0,62±0,07/ 5,20±0,5
Oil +108	35,3	11,2	5,05	4,8	0,96±0,1/ 9,26±0,8	0,35±0,05/ 3,38±0,57	0,71±0,1/ 6,88±0,5

¹ The content of pigments in the plants, mg/g wet weight; *Rhodococcus*² The content of pigments in the plants, mg chl/1g dry weight not determined

When growing plants treated with microorganisms in unpolluted soil, a small growth-promoting effect was observed with an increase in germinating ability of seeds and the number of pigments, especially carotenoids. This fact provides an opportunity to hope for the prospects of using *Rhodococcus* as a bio-fertilizer.

The outflow of extracellular biologically active substances, such as phytohormones, may be a possible mechanism for positive effects of *Rhodococcus* sp. In order to verify these assumptions, microbial synthesis of phytohormones in all the strains of the determined oil-degrading bacteria was studied.

When assessing extracellular phytohormones, the biotesting method is most commonly used since the quantitative content of phytohormones is not as important as their biological activity. The strain 102 showed the highest gibberellin-like activity. Its rate of glucose mobilization in the endospermic test corresponded to 0.038% concentration of industrial gibberellins (table 2).

Auxin-like activity was determined by two biological tests and quantitatively.

The strains 102 and 109 turned out to contain about 0.03% of auxins. These strains showed rather high activity during the biotests. These cultures belong to the genus *Pseudomonas*, which has a high adaptive capacity, according to literary data [10], and should have a large variety of biologically active substances. The culture 108 (the genus *Rhodococcus*) synthesized a large number of auxins, but their biological activity was not shown in all the biotests.

Table 2. Activity of phytohormones in the oil-degrading bacteria supernatant.

Strain	GLA, % of control	Number of auxins, % of control	Biological activity (auxin-like)		
			Number of beans roots, % of control	Height of beans petiole rooting, % of control	Mustard germination, % of control
102	0.038±0.002	0.003±0.0003	173.1±1.2	268.0±2.3	38
112	0.0074±0.0006	0.0015±0.0002	100.0	63.0±3.1	89
108	0	0.051±0.0009	110.0±1.4	245.6±3.2	14
114	0.0052±0.0003	0.0011±0.0001	89.8±1.3	103.5±2.1	93
109	0.0065±0.0003	0.0035±0.0003	220.5±1.8	301.8±1.8	34
90	0	-	230.7±2.1	224.6±1.9	24

Note: control is a culture medium without bacteria.

The same phytohormone is known to be active in one biotest and completely inactive in the other [8]. It should be noted that the authors were unable to detect auxin-like substances determined with Salkowski reagent in the culture 90. At the same time, the supernatant of this strain was characterized by very high activity in the auxin biotests.

4. Conclusion

To sum up, the study of the influence of oil-degrading bacteria isolated from plant endo- and rhizosphere on the plant development under the conditions of oil pollution showed that the only one strain (108, genus *Rhodococcus*) of the six ones studied performed the best phytoprotective properties. Moreover, it stimulated the growth and development of the plants grown in the absence of oil. Reducing the toxic effect of oil led to an increase in the length of the roots and seedlings as well as the weight of the plant. All the plants showed an increase in the pigment content that contributes to the improvement of photosynthesis. High stimulating activity of *Rhodococcus* is associated with its ability to produce substances similar to plant phytohormones. Using biotest methods, auxins, gibberellins and cytokinins were detected. It can be concluded from the foregoing that the studied *Rhodococcus* strain is a promising microorganism based on which it is possible to produce a new bio-fertilizer.

Acknowledgement

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