Microbiology, Vol. 70, No. 3, 2001, pp. 316–318. Translated from Mikrobiologiya, Vol. 70, No. 3, 2001, pp. 370–373.
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EXPERIMENTAL ARTICLES

Bacterial Microflora on Disinfected Sugar Beet Seeds

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Received May 4, 2000

Abstract—Sugar beet seeds disinfected with the carbofuran-containing insecticide adifur and the fungicide tachygaren by seed-producing firms were found to be abundantly populated with bacterial microflora. The bacteria isolated from the seed surface were identified to a species level. The selection of bacteria with respect to pesticide resistance may lead to the obtaining of agronomically useful bacterial strains.

Key words: bacteria, sugar beet, disinfected sugar beet seeds, pesticides, carbofuran, tachygaren, TMTD, gaucho.

One of the topical agricultural problems is to increase crop production with the use of bacterial fertilizers. One of the ways to achieve it is the presowing bacterization of seeds. However, another presowing procedure (the pretreatment of seeds with pesticides in order to protect seedlings from infectious fungal diseases and damage by entomofauna) is believed to be harmful to bacteria. For this reason, it is recommended to use relatively nontoxic fungicides, such as fundazol and bavistin.

Many newly produced fungicides and insecticides are very toxic to fungi and insects and, therefore, should be thoroughly studied with respect to their toxicity to agronomically useful bacteria. The selection of new bacterial strains that could long remain viable on dry disinfected seeds would allow the bacterial pretreatment of seeds in earlier presowing terms.

Some promising results along this line of research were obtained in our laboratory [1, 2]. For instance, we succeeded in the isolation of two phosphate-mobilizing bacteria, *Bacillus polymyxa* KB (VNIISKhM B-324 D) and *Achromobacter album* 1122 (VNIISKhM B-322 D), which are tolerant to tetramethylthiuram disulfide (TMTD) and carbofuran (adifur) and remain long viable on the seeds disinfected with these pesticides. This made it possible to perform the concurrent disinfection and bacterization of sugar beet seeds in early presowing terms.

Furthermore, there is evidence that some bacterial strains can efficiently degrade some pesticides, such as the insecticide adifur [3] and the fungicide TMTD [4].

Sugar beet and maize are economically important agricultural crops. Therefore, searching for bacterial strains that can be efficiently used for the bacterization of the sugar beet and maize seeds in combination with their disinfection is a challenging problem.

All the foregoing calls for a closer investigation of the tolerance of bacterial strains to the pesticides presently used in agriculture. The creation of the collection of such bacterial strains should allow a more efficient selection of strains resistant to particular pesticides. It should be noted in this regard that disinfected sugar beet seeds are usually abundantly colonized by various bacteria.

The aim of the present work was to perform the quantitative and qualitative analysis of bacteria isolated from the surface of disinfected sugar beet seeds and to screen them for agronomically useful strains.

MATERIALS AND METHODS

We studied the bacterial microflora of the disinfected sugar beet cv. Belotserkovskaya 45 seeds obtained from the Vinnytsa seed-producing firm (Ukraine). The seeds were disinfected with a mixture containing the insecticide adifur (Ser-Italia, Italy) with the active substance carbofuran, the fungicide tachygaren (Sankyo, Japan) with the active substance hymexazol, and a gluing substance, SMAN [5]. Four months after seed disinfection, 100 seeds were placed in flasks containing 100 ml of water, the flasks were shaken for 1 h, the washings were serially diluted tenfold, and the dilutions were plated onto agar medium.

After five days of incubation, all the types of grown colonies were transferred to the respective nutrient media (a total of 78 isolates were obtained). The isolates were identified to a species level according to the general [6–8] and special [9, 10] manuals. The isolates were tested for purity and analyzed with respect to the morphology of colonies, the morphology and motility of cells grown in nutrient broth for 2 and 5 days, the formation of spores and flagella, gram staining, resistance to acids, requirement for oxygen, fermenting ability, the liberation of hydrogen sulfide and ammonia, the ability to assimilate citrate on Simpson agar, requirement for mineral nitrogen sources (NH_4^+, and NO_3^-),

0026-2617/01/7003-0316$25.00 © 2001 MAIK “Nauka / Interperiodica”

the liquefaction of gelatin, the hydrolysis of starch, the reduction of NO_3^-, oxidase and catalase activities, the ability to utilize various carbon sources, and so on.

In searching for agronomically useful strains, the isolates were tested for the ability to dissolve $Ca_3(PO_4)_2$, to degrade organic phosphates, and to fix the atmospheric nitrogen. In these studies, we used (1) liquid Muromtsev medium containing (g/l) glucose, 10.0; asparagine, 1.0; K_2SO_4, 0.2, $MgSO_4 \cdot 7H_2O$, 0.2; corn extract, 0.2; and $Ca_3(PO_4)_2$, 5.0 in tap water; (2) solid Menkina medium containing (g/l) glucose, 1.0; $(NH_4)_2SO_4$, 0.5; NaCl, 0.3; $MgSO_4 \cdot 7H_2O$, 0.3, $FeSO_4$, traces; $MnSO_4$, traces; $CaCO_3$, 5.0; Ca–Mg phytate, 1.0; and agar, 20 in distilled water; and (3) solid Fedorov medium containing (g/l) sucrose, 20; K_2HPO_4, 0.3, $CaHPO_4$, 0.2, K_2SO_4, 0.2, $MgSO_4 \cdot 7H_2O$, 0.3; NaCl, 0.3; $FeCl_3$, traces; $CaCO_3$, 5.0; and agar, 20 in distilled water.

Soluble phosphates in liquid media were assayed with the phosphomolybdate reagent.

RESULTS AND DISCUSSION

The plating of washings from both untreated and disinfected seeds onto nutrient agar led to the formation of bacterial colonies, which were different in shape, consistency, and color: flat and convex, smooth and wrinkled, transparent and opaque, brilliant and dull, colorless, white, pale to bright yellow, indicating the abundance and diversity of the microflora present on the disinfected seeds. Pigmented bacteria, typical of epiphytic microflora, were dominant. It is known that pigmented nonspore-forming bacteria are tolerant to high concentrations of salts (NaCl) and to ultraviolet light [11]. The enumeration of microcolonies showed that, surprisingly, the washings from disinfected seeds produced many more colonies than the washings from undisinfected seeds. Generally, one disinfected seed contained 100–160 thousand bacterial cells, while one undisinfected seed contained only 30–40 thousand bacterial cells. This unexpected result can be explained by the better survival of indigenous microflora on disinfected seeds due to the presence of the gluing substance SMAN in the disinfecting mixture. The ability of some gluing substances to enhance bacterial survival was reported by Mal'tseva *et al.* [12]: adhesive substances, such as gelatin, carboxymethylcellulose (Na-CMC), polyvinyl acetate, and molasses, which were added to bacterial suspensions, favored bacterial survival on seeds. The colloidal film formed around bacterial cells protects them from drying and the action of bactericidic substances. This finding allowed Mal'tseva *et al.* to suggest that bacterized seeds can be stored for a relatively long time until be sown [13, 14].

This suggestion was confirmed in our experiments with two bacteria, *Flavobacterium* sp. L-30 (this bacterium is the primary component of the biocontrol agent flavobacterin) and *Achromobacter album* 1122 (this strain is the primary component of the biocontrol agent albobacterin). These experiments showed that Na-CMC prolonged the survival period of bacteria on sugar beet seeds by 2 months.

Bacterial species isolated from the surface of disinfected sugar beet seeds

Species	Number of strains
Aureobacterium saperdae	1
Bacillus alcalophilus	1
B. cereus	2
B. firmus	2
B. laterosporus	1
B. megaterium	1
Bacillus sp.	2
Cellulomonas sp.	2
Comamonas sp.	1
Enterobacter sakazakii	3
Erwinia amylovora	1
E. salicis	1
Microbacterium abrorescenes	2
M. imperiale	1
M. lacticum	1
Micrococcus nishinomiyaensis	1
Micrococcus sedentarius	1
Micrococcus roseus	1
Pseudomonas alkaligenes	2
P. facilis	1
P. mendosina	1
P. vesicularis	1

The table presents the results of the determination of the taxonomic composition of bacteria isolated from disinfected seeds. It can be seen that most isolated bacteria belong to epiphytic microflora, while only some of them might be brought to the seeds with dust. Analysis of the disinfecting mixture and its components (adifur and tachygaren) showed that they contained only spore-forming bacteria in an amount of no more than 1000 cells per g (or ml); therefore, they could have brought few bacteria on the disinfected seeds.

Thus, adifur and tachygaren do not considerably affect the survival of the bacterial species on the disinfected seed. Similar results were obtained for TMTD and the novel insecticide gaucho.

The tolerance of bacteria to the very toxic insecticides carbofuran and gaucho and to the relatively nontoxic fungicide tachygaren can be explained by the considerable difference of prokaryotic bacteria from eukaryotic fungi, animals, and protozoans. The specific organization of the bacterial cell envelope, which contain many hydrolytic enzymes, the presence of the

slime capsule, and the specific structure of the cytoplasmic membrane and various subcellular organelles, may be responsible for the high tolerance of bacterial cells to the toxic effect of pesticides. Nevertheless, some of the fungicides (e.g., sulfocarbation) were found to exert a bactericidal effect.

The isolated bacterial strains were studied with respect to their ability to dissolve mineral phosphates, to degrade organic phosphates (the so-called phosphorus mobilization ability), and to fix the atmospheric nitrogen. Three of the isolates were found to be rather active: when grown in the glucose–asparagine Muromtsev medium containing tricalcium phosphate, they accumulated 40–50 mg of mobile P_2O_5 in 100 ml. They also grew well on the calcium phytate-containing agar with the formation of clearing zones and on the nitrogen-free Fedorov medium. Further studies are needed to evaluate the potential of the bacterial isolates as biocontrol agents which would be used in combination with chemical disinfecting agents.

To conclude, many epiphytic bacteria turned out to be resistant to the action of carbofuran, which is highly toxic to insects, nematodes, mites, warm-blooded animals, and humans, and many can tolerate the effect of the fungicides TMTD and tachygaren and the insecticide gaucho: seeds treated with the pesticides mentioned retain sufficient amounts of viable indigenous bacterial cells for at least several months. The selection of agronomically useful bacterial strains should be performed with respect to their tolerance to particular pesticides.

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