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EXPERIMENTAL ARTICLES

Biological Properties of the Wild Rhizosphere Strain *Pseudomonas fluorescens* 2137 and Its Derivatives Marked with the *gusA* Gene

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Abstract—he natural wild rhizosphere strain *P. fluorescens* 2137 was marked with the β-glucuronidase gene *gusA*. The introduction of this gene influenced the viability of the wild strain, as well as its certain physiological parameters, such as cultural characteristics, biochemical properties, and antagonistic activity against the phytopathogenic fungi *Fusarium culmorum, F. oxysporum, F. graminearum*, and *Verticillum nigrescens*. The *gusA*-marked derivative strains that deviate the least from the wild strain in biological properties can be used to monitor populations of *P. fluorescens* 2137 cells in the plant rhizosphere.

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Global changes in the ecological equilibrium in nature and the appearance of phytopathogenic strains tolerant to most agents currently used to control plant diseases call for the development of new preparations based on antagonistic rhizosphere microorganisms [1–5]. The lack of adequate knowledge of the interaction of plant growth-promoting rhizobacteria (PGPR) with other components of ecosystems may considerably reduce the agricultural efficiency of these preparations.

In recent years, microbial populations have been studied by using the specific and sensitive method of genetic marking with the genes of β-galactosidase, luciferase, catechol-2,3-dioxygenase, and β-glucuronidase [6]. A microorganism that receives the β-glucuronidase gene *gusA* acquires the ability to produce a deep blue color when grown in a nutrient medium with 5-bromo-4-chloro-3-indolyl β-D-glucuronide (X GlcA). Such a *gusA*-marked microorganism can easily be detected in soil extracts and on plant roots by treating them with X GlcA. The *gusA* gene of the *Tn*5 transposon carried by the *Escherichia coli* plasmid encoding spectinomycin resistance [7] is introduced into a recipient cell by means of conjugation [8]. Theoretically, the insertion of this gene into a bacterial chromosome may induce mutations leading to a reduction in the viability of rhizosphere bacteria, in their symbiotic and antagonistic activities, and in their ability to fix atmospheric nitrogen and to colonize the plant rhizosphere.

The aim of this work was to mark the rhizosphere strain *Pseudomonas fluorescens* 2137 with the β-glucuronidase gene *gusA* and to study how gene introduction can influence the physiological properties of the recipient organism.

MATERIALS AND METHODS

Experiments were carried out with the natural rhizosphere strain *Pseudomonas fluorescens* 2137 (courtesy of N.M. Makarova, Research Institute of Agricultural Machine Building), *Escherichia coli* strain S17-1 λ-pir carrying plasmid pmTn5SS*gusA*20 (courtesy of S. Akao, Japan State Institute of Agrobiological Sciences), and the phytopathogenic fungi *Fusarium culmorum*, *F. oxysporum*, *F. graminearum*, and *Verticillium nigrescens* (these fungi were obtained from the collection of microorganisms at the Research Institute of Plant Disease Control). The *P. fluorescens* strains were cultivated in LB broth at 28°C. The *E. coli* strains were cultivated at 37°C in LB broth supplemented with 1 μg/ml spectinomycin. The morphological, cultural, and biochemical properties of the *P. fluorescens* strains were studied by routine methods [9–11]. The *P. fluorescens* strain 2137 was marked with the *gusA*20 gene by means of conjugation using the *E. coli* strain S17-1 λ-pir as a recipient of the plasmid pmTn5SS*gusA*20 [12]. The one-day-old *E. coli* and *P. fluorescens* cul-

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tures were mixed in a proportion of 10 : 1 and 20-μl aliquots of the mixed suspension were placed on filter paper disks lying on the surface of LB agar plates. The plates were incubated at 28°C for 12 h. Simultaneously, serial dilutions of the mixed suspension were plated on minimal LO agar [13] with spectinomycin (in order to select transconjugants) and without it (in order to determine the total number of viable *P. fluorescens* cells and to calculate the frequency of transconjugation). The plates were incubated at 28°C for 5–7 days. The selected transconjugants were tested for the presence of the *gusA20* gene by cultivating their colonies on LO agar supplemented with X GlcA. If a colony produced blue color, it was grown on legume agar supplemented with 1 μg/ml spectinomycin. The growth rate and the size of *P. fluorescens* colonies grown on the legume agar were greater than in the case of cultivation on the LO agar. This circumstance facilitated the selection of 38 *P. fluorescens gusA* strains for further studies. The selected strains were maintained on LB broth containing 1 μg/ml spectinomycin. Every two months, the strains were transferred to the fresh medium. Twice a year, the strains were tested for the presence of the *gusA* gene by cultivating them on the legume agar with X GlcA.

The relatedness of the selected *P. fluorescens gusA* strains was estimated in terms of the Dice similarity coefficient $S_D = 2n_{AB}/(n_A + n_B)$, where n_{AB} is the number of common traits for A and B, n_A is the number of traits in A, and n_B is the number of traits in B. A similarity dendrogram was constructed using the unweighted pair group mean arithmetic (UPGMA) algorithm realized in the Taxotron software package (P.A.D. Grimont, 1998).

The antagonistic activity of the *P. fluorescens* strains against the phytopathogenic fungi was determined in vitro on Czapek medium by the methods of wells (in the case of the *F. culmorum* and *F. oxysporum* fungi) and blocks (in the case of the *F. graminearum* and *V. nigrescens* fungi) [14, 15]. These measurements were carried out in triplicate. The phytopathogenic fungi were cultivated on Czapek medium at 24°C for seven days. Then, 10 ml of a suspension containing 10^6 fungal spores per ml was mixed with 1 l of the Czapek medium and the mixture was dispensed into petri dishes. An aliquot (50 μl) two-day-old *P. fluorescens* cultures containing 10^8 cells/ml was placed into a well with a diameter of 5 mm, which was made in the Czapek agar containing fungal spores. Alternatively, the same aliquot was used to prepare an LB agar block with a height of 3 mm and a diameter of 10 mm, which was laid on the surface of the Czapek agar with fungal spores. The diameter of the fungal growth inhibition zones formed around the wells and the blocks were measured in mm after seven days of incubation at 28°C.

RESULTS AND DISCUSSION

The conjugative transfer of the plasmid carrying the Tn5 transposon into the recipient *P. fluorescens* 2137 resulted in the strain being marked with the β-glucuronidase gene *gusA*. The transconjugants of *P. fluorescens* 2137 grown on media with X GlcA produced blue-colored colonies. The frequency of transconjugation was found to be 2×10^{-2}.

The 38 selected *gusA*-marked strains of *P. fluorescens* 2137 were studied with regard to the main biological properties ($n = 34$). All of these genetically marked strains were close to the wild strain *P. fluorescens* 2137 (which is a typical representative of *P. fluorescens* biovar II) in morphological, tinctorial, cultural, and biochemical properties, such as growth on nitrogen-deficient media, hydrolysis of starch and urea, the ability to use D-xylose, β-alanine, L-arginine, citrate, sucrose, and *meso*-inositol as sources of carbon, as well as nitrate as a source of nitrogen. For example, the wild and the *gusA*-marked *P. fluorescens* strains were able to grow on nitrogen-free media (Ashby, LO, and NFB), which are selective with respect to nitrogen-fixing bacteria. It should be noted that the ability of the *P. fluorescens* bacterium to fix atmospheric nitrogen has been reported only recently [16]. Furthermore, the wild and the *gusA*-marked *P. fluorescens* strains virtually did not differ in oxidase, urease, amilolytic, and denitrifying activities. At the same time, as is evident from Table 1, the *gusA*-marked *P. fluorescens* strains differed in a number of properties, including the intensity of the blue color of colonies grown on media with X GlcA. This fact implies that the *gusA*-marked strains are heterogeneous with respect to the degree of expression of the β-glucuronidase gene, the degree of expression depending on the stability of this gene and its location on the bacterial chromosome [6].

Thirteen of the 38 tested *P. fluorescens* strains (approximately 34%) showed a high intensity of fluorescence, probably due to a high content of pseudobactin (pyoverdin) (Table 1), a yellow–green pigment with siderophore activity, which may be responsible for the fungistatic action of *P. fluorescens* against phytopathogenic fungi (by inducing iron deficiency) [11].

The *gusA*-marked *P. fluorescens* strains showed different abilities to grow at 37°C (a critical growth temperature for *P. fluorescens*) on potato agar. Fifty percent of the tested *P. fluorescens* strains (including 2137) showed good growth after one day of incubation at 37°C; 16 strains (approximately 42%) showed visible growth after two days; and one strain was found to grow after four days. Two strains (21*gusA* and 41*gusA*) were not able to grow at 37°C at all (Table 1). More than half of the 38 tested strains could utilize L-rhamnose, L-serine, and L-valine as carbon sources for growth. Seven *gusA*-marked strains (18%) showed poor growth (accompanied by an alkalization of the medium) on L-rhamnose; six strains (16%), on

Table 1. Some cultural and biochemical properties of the wild strain *P. fluorescens* 2137 and its *gusA*-marked derivatives

Strain	Characteristic										
	Intensity of blue color of colonies grown on X GlcA*	Intensity of fluorescence of yellow–green pigment**	Growth at 37°C on potato agar, days	Hydrolysis of gelatin, days	Growth on litmus milk		Utilization of carbon sources				
					Serum separation, days	Peptonization, days	α-oxoglutaric acid	L-serine	L-valine	D-tartrate	L-rhamnose
2137	–	+	1	11	12	12	–	+	+	+	+
1	+	+	2	25	15	12	–	+	+–	+	+
2	+	+	1	25	15	12	–	+	+–	+	+
3	+	+	1	25	12	12	+	+	+	+	+
4	+	+	1	25	43	12	+	+	+–	+	+
5	+–	+	1	25	15	15	+	+	+	+	+
6	+	++	2	25	15	15	+	+	+–	+	+
11	+	+	1	25	43	12	–	+	+–	+	–
12	++	++	1	25	15	12	+	+–	+	+–	+–
13	+	+	1	25	15	12	+	+	+	+	–
14	++	++	1	25	15	12	+	+	+	+–	–
15	++	++	1	25	19	12	+	+	+	+	+
16	++	+	2	25	15	12	+	+–	+–	+–	+
17	++	+	1	25	15	12	–	+	+	+–	+
18	++	++	1	18	19	15	+	+	+	+	+
19	++	++	1	25	15	12	+	+	+	+	+
21	++	+	–	25	15	15	+	+	+	+	+
22	+	+	4	25	15	15	+	+	+–	+-	+
23	+	++	1	25	15	12	+	+	+	+	+
24	+	+	1	25	15	12	–	+	+	+	+
25	+	++	2	25	15	12	–	+	+–	+	+–
26	+	++	2	25	15	12	+	+	+	+	+
27	+	+	1	25	43	15	–	+	+–	+	+
28	++	+	2	25	15	12	–	+	+	+	+
29	+	+	2	25	15	15	+	+	+	+	+
30	+	+	2	25	15	12	–	+	+	–	+–
31	+	++	2	25	15	12	+	+	+–	+	+–
32	+	+	1	25	15	12	+	+	+	+	+
33	+	++	1	25	15	12	+	+	+	–	+–
34	+	++	2	18	43	12	–	+	+–	+	+
41	+	+	–	25	15	12	–	+–	+–	+	+
42	+	+	2	25	15	12	+	+	+–	+	+–
43	+	++	2	18	15	15	+	+–	+–	+	+
44	+	+	1	25	43	19	–	+–	+	+	+
45	+	+	2	25	15	12	–	+	+–	+	+–
46	+	+	2	25	15	12	–	+	+	+	+
47	+	+	2	25	15	15	–	+	+	+	+
48	+	+	2	25	12	12	+	+	+–	+	+
49	+	+	1	25	12	12	–	+–	+–	+	+

* "+" and "++" stand for low and high intensity of blue color of colonies, respectively.

** "+" and "++" stand for low and high intensity of fluorescence of yellow–green pigment. In other cases, "–," "+–," and "+" stand for zero, low, and high levels of expression of a given characteristic, respectively.

L-serine; and 17 strains (45%), on L-valine. Three strains could not grow on L-rhamnose at all. Unlike the wild strain, sixteen *gusA*-marked strains were able to grow on α-oxoglutaric acid as the carbon source. The wild strain and most of the *gusA*-marked strains could grow on the medium with ammonium tartrate (Table 1).

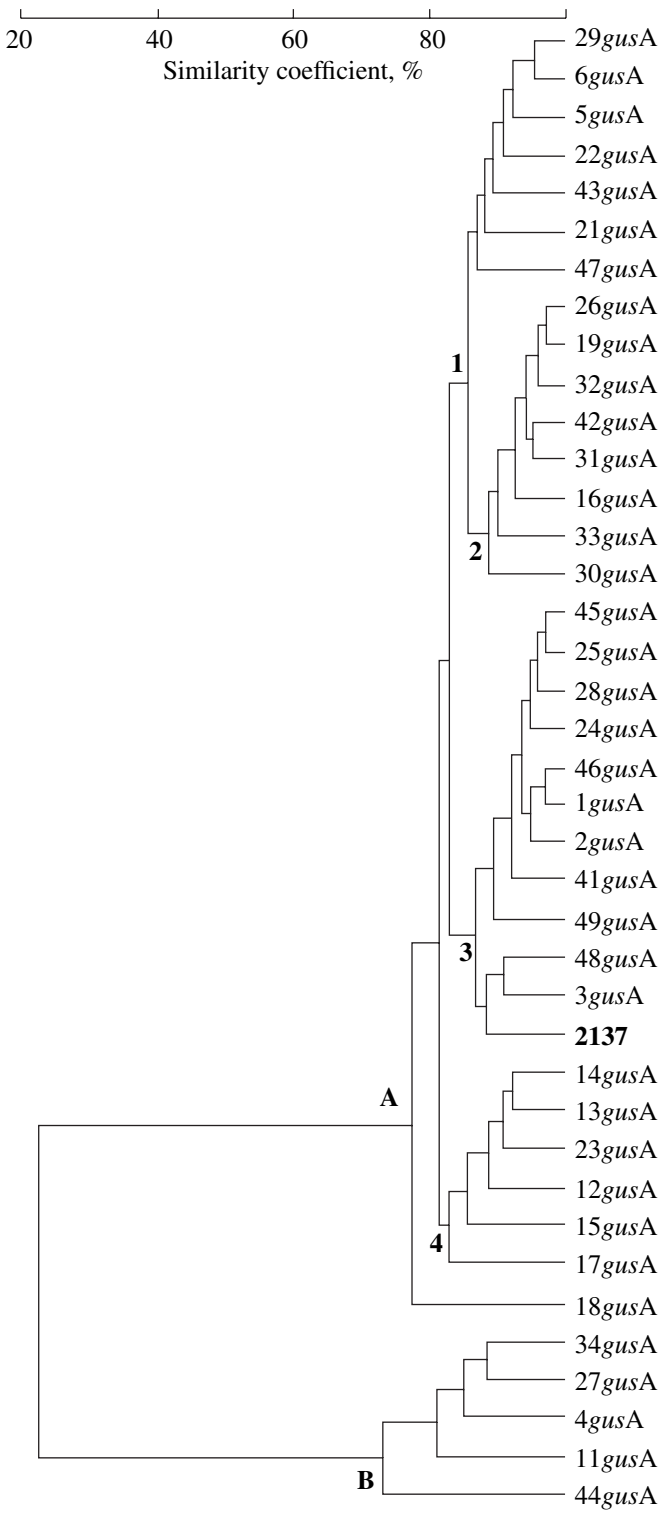
The proteolytic activity of the *gusA*-marked strains was lower than in the case of the wild strain. Indeed, *P. fluorescens* 2137 liquefied gelatin after 11 days of incubation, whereas 3 *gusA*-marked strains liquefied it after 18 days of growth, and 35 strains (92%), after 25 days of growth (Table 1).

Like the wild strain, the *gusA*-marked strains could hydrolyze casein during growth on litmus milk (Table 1). *P. fluorescens* 2137 and three *gusA*-marked strains (3*gusA*, 48*gusA*, and 49*gusA*) separated milk serum after 12 days of growth. Most of the *gusA*-marked strains (79%) separated serum after 15–19 days of growth, and five strains (13%), after 43 days of growth. *P. fluorescens* 2137 and most of the *gusA*-marked strains (74%) completely peptonized the milk after 12 days of growth (Table 1).

Thus, the introduction of the *gusA* gene influenced, to some degree, 11 of the 34 studied cultural and biochemical properties of *P. fluorescens* 2137. The number of affected properties in particular strains varied from three (in the case of strains 3*gusA* and 24*gusA*) to nine (in the case of strain 43*gusA*) (Table 1).

The results obtained from the study of the biological properties of the wild *P. fluorescens* 2137 and the *gusA*-marked strains are presented as a dendrogram (see figure), which clearly demonstrates that particular *gusA*-marked strains differ from the wild strain and from each other. Strains with similar cultural and biochemical properties form four subclusters (1 to 4) with similarity coefficients higher than 80%. These subclusters, together with the strain 18*gusA*, comprise a big cluster A, which includes 33 of the 38 tested *gusA*-marked strains (87%) and the wild strain 2137 (figure). Strains 48*gusA*, 3*gusA*, and other strains of subcluster 3 are the most similar to the wild strain. Cluster B with its five *gusA*-marked strains (figure) considerably differs from cluster A in some properties, especially proteolytic activity during growth on litmus milk (Table 1). It should be noted that, after one year of maintenance, the 4*gusA* and 11*gusA* strains of cluster B became nonviable, whereas the *gusA*-marked strains of cluster A remained viable even after two years of maintenance on the selective medium with spectinomycin.

The study of the antagonistic activity of the wild *P. fluorescens* 2137 and the *gusA*-marked strains showed that the diameter of the lysis zone varied from 7 to 30 mm for the phytopathogenic fungus *F. culmorum*; from 7 to 16 mm for *F. oxysporum*; and from 12 to 21 mm for *F. graminearum* and *V. nigrescens* (Table 2).



Dendrogram showing similarity between the wild strain *P. fluorescens* 2137 and its *gusA*-marked derivatives

Eighteen of the 38 *gusA*-marked strains showed lower antagonistic activity against *F. culmorum* than the wild strain (Table 2), five of these strains being

Table 2. Antagonistic activity of the wild strain *P. fluorescens* 2137 and its *gusA*-marked derivatives against phytopathogenic fungi

P. fluorescens			Phytopathogenic fungi			
Cluster		Strain	*F. culmorum**	*F. oxysporum**	*F. graminearum***	*V. nigrescens***
A	1	5	+++	++	++	++
		6	+	ND	+	0
		21	++	0	++	++
		22	+	0	ND	+
		29	++	0	++	++
		43	0	0	+	++
		47	+++	+	++	+
	2	16	+	ND	ND	0
		19	++	ND	+	+
		26	+	ND	0	ND
		30	ND	ND	0	0
		31	+++	ND	+	+
		32	+++	ND	+	0
		33	+++	+	ND	0
		42	+++	+	+	0
	3	Wild 2137	+++	+++	+++	+++
		1	++++	+++	++	ND
		2	+++	+	+	0
		3	0	0	0	ND
		24	0	0	0	0
		25	+++	++	++	++
		28	+	ND	0	ND
		41	++++	+++	++	+++
		45	++	ND	ND	0
		46	++++	++	0	ND
		48	++	ND	0	ND
		49	0	++	0	ND
	4	12	+	ND	+	+
		13	+++	++	0	ND
		14	++	ND	+	++
		15	++	ND	ND	0
		17	0	ND	0	ND
		23	+	ND	0	ND
		18	+++	+	+	+
B		4	ND	ND	ND	ND
		11	+	0	ND	ND
		27	+	ND	0	0
		34	++	0	0	0
		44	+	0	0	0

Note: ND stands for "no data available." 0 indicates the absence of fungal growth inhibition zone.

* In the method of agar wells, "+", "++" ,"+++", and "++++" indicate the fungal growth inhibition zone less than 10, 11–15, 16–20, and more than 20 mm in diameter, respectively.

** In the method of agar blocks, "+", "++", "+++", and "++++" indicate the fungal growth inhibition zone less than 15, 16–20, 21–30, and more than 30 mm in diameter, respectively. The natural rhizosphere strain *P. fluorescens* 2137 gave rise to a growth inhibition zone with a diameter of 16–20 mm in the method of agar wells and 21–30 mm in the method of agar blocks.

completely inactive. Of interest is the fact that three inactive strains of subcluster 3 (3*gusA,* 24*gusA*, and 49*gusA*) are very close to the wild strain in cultural and biochemical properties (figure). On the other hand, the antagonistic activity of three strains (1*gusA*, 41*gusA*, and 46*gusA*) of subcluster 3 was higher than that of *P. fluorescens* 2137 (Table 2). The other ten (28%) of the *gusA*-marked strains of cluster A showed the same antagonistic activity as the wild strain.

The phytopathogenic fungus *F. oxysporum* turned out to be less susceptible to the *gusA*-marked strains than the fungus *F. culmorum.* Namely, nine (43%) strains, including 3*gusA* and 24*gusA*, exhibited no antifungal activity, and 10 strains (48%) were slightly inhibitory with respect to *F. oxysporum.* Only two strains of subcluster 3 (1*gusA* and 41*gusA*) showed the same antifungal activity against *F. oxysporum* as the wild strain did.

The antagonistic activity of 17 *gusA*-marked strains (55%) against *F. graminearum* was lower than in the case of the wild strain. Fourteen strains (45%) showed no activity against *F. graminearum* (Table 2).

Half of the *gusA*-marked strains lost antagonistic activity against *V. nigrescens* and 12 *gusA*-marked strains (46%) showed lower activity than the wild strain did. Only one strain from subcluster 3 (41*gusA*) retained a high antifungal activity (Table 2).

On the whole, the highest antagonistic activity against the studied phytopathogenic fungi was exhibited by three strains of subcluster 3 (1*gusA*, 25*gusA*, and 41*gusA*) and one strain of subcluster 1 (5*gusA*) from cluster A (Table 2). The *gusA*-marked strains of cluster B showed a low viability rate (so that two of the five strains of this cluster became nonviable after one year of subculturing), as well as weak biochemical (Table 1) and antiphytopathogenic (Table 2) activities.

The development of efficient and ecologically safe biological preparations on the basis of PGPR, *P. fluorescens* in particular, requires knowledge of their behavior in the plant rhizosphere in situ. Such knowledge can be obtained by studying *P. fluorescens* strains marked with the β-glucuronidase *gusA* gene. In the present study, the natural rhizosphere strain *P. fluorescens* 2137 was marked with this gene and the selected genetically marked strains were investigated with reference to cell viability and other physiological parameters. In general, the selected *gusA*-marked strains were found to differ from the wild strain and from each other in cultural characteristics, biochemical properties, and antagonistic activity against phytopathogenic fungi from the genera *Fusarium* and *Verticilium* [15]. Until now, the effect of the introduction of the *gusA* gene on the biological properties of recipient bacteria has been considered only theoretically possible [6].

To conclude, we have demonstrated the importance of the preliminary study of the major biological properties of genetically modified bacteria intended for use as components of model rhizosphere communities. The strains 5*gusA* and 41*gusA*, which are the most close to the wild strain *P. fluorescens* 2137, can be used for monitoring the population of *P. fluorescens* cells in soil–bacterium–plant ecosystems.

REFERENCES

1. Leshchinskaya, I.B., Modern Industrial Microbiology, *Biologiya,* 2000, vol. 6, no. 4, pp. 14–18.
2. Grishechkina, L.D., Microbiological Control of Cucumber Root Rot, *Materialy dokl. Mezhd. nauch.-prakt. konf. posvyashch. 75-letiyu RASKhN "Biologicheskaya zashchita rastenii - osnova stabilizatsii agroekosistem,"* (Proc. Int. Conf. on the Biological Control of Plant Diseases), Nadykty, V.D. et al., Issue 3, Krasnodar, 2004, pp. 225–228.
3. Pal, K., Tilak, K., Saxena, A., Dey, R., and Singh, C., Suppression of Maize Root Diseases Caused by *Macrophomina phaseolina, Fusarium moniliforme*, and *Fusarium graminearum* by Plant Growth Promoting Rhizobacteria, *Microbiol. Res.,* 2001, vol. 156, pp. 209–223.
4. Nagarajkumar, M., Bhaskaran, R., and Velazhahan, R., Involvement of Secondary Metabolites and Extracellular Lytic Enzymes Produced by *Pseudomonas fluorescens* in Inhibition of *Rhizoctonia solani,* the Rice Sheath Blight Pathogen, *Microbiol. Res.,* 2004, vol. 159, pp. 73–81.
5. Dey, R., Pal, K., Bhatt, D., and Chauhan, S., *Growth Promotion and Yield Enhancement of Peanut* (*Arachis hypogaea* L.) by Application of Plant Growth-Promoting Rhizobacteria, *Microbiol. Res.,* 2004, vol. 159, pp. 371–394.
6. Wilson, K., *GusA* as a Reporter Gene to Track Microbes, *Molecular Microbial Ecology Manual*, Akkermans, A.D. et al., Eds., Dordrecht: Kluwer Academic, 1995, pp. 85–100.
7. Herrero, M., de Lorenzo, V., and Timmis, K., Transposon Vectors Containing Non-Antibiotic Resistance Selection Markers for Cloning and Stable Chromosome Insertion of Foreign Genes in Gram-Negative Bacteria, *J. Bacteriol.,* 1990, vol. 172, pp. 6557–6567.
8. Wilson, K., Giller, K., and Jefferson, R., β-Glucuronidase (GUS) Operon Fusions as a Tool for Studying Plant–Microbe Interactions, *Kluwer Academic,* 1991, pp. 226–229.
9. *Bergey's Manual of Determinative Bacteriology*, 9th ed., Holt, J.G. et al., Eds., Baltimore: Williams & Wilkins, 1994. Translated under the title *Opredelitel' bakterii Berdzhi*, Moscow: Mir, 1997.
10. Voznyakovskaya, Yu.M. and Popova, Zh.P., *Metodicheskie ukazaniya po identifikatsii nesporovykh bakterii, dominiruyushchikh v rizosfere rastenii* (Methodological Guide on the Identification of Dominant Non-Spore-Forming Bacteria in the Plant Rhizosphere), Leningrad: VIR, 1985.
11. Smirnov, V.V. and Kiprianova, E.A., *Bakterii roda Pseudomonas* (Bacteria of the Genus *Pseudomonas*), Kiev: Naukova Dumka, 1990.
12. Limeshchenko, E.V. and Chebotar', V.K., The Use of the Genetic *gus* Marking Approach in Soil Microbiology

with Reference to *Pseudomonas fluorescens, Tez. dokl. Vseross. konf. "Mikrobiologiya pochv i zemledelie"* (Proc. Conf. "Soil Microbiology and Agriculture"), St. Petersburg, 1998.

13. Dreyfus, B.L., Elmerich, C., and Dommergues, Y.R., Free-Living *Rhizobium* Strain Able to Grow on N_2 as the Sole Nitrogen Source, *Appl. Environ. Microbiol.*, 1983, vol. 45, pp. 711–713.
14. Egorov, N.S., *Praktikum po mikrobiologii* (Practical Course on Microbiology), Moscow: Mosk. Gos. Univ., 1976.
15. Vyazovaya, A.A., Limeshchenko, E.V., and Buren', V.M., The Fungicidal Activity of Genetically Modified Derivatives of *Pseudomonas fluorescens* 2137, *Gertsenovskie chteniya: Materialy mezhvuz. konf. molodykh uchenykh* (Proc. Int. Conf. of Young Scientists), St. Petersburg: TESSA, 2003, Issue 3, pp. 10–12.
16. Chan, Y.-K., Barraquio, W.L., and Knowles, R., N_2-Fixing Pseudomonads and Related Soil Bacteria, *FEMS Microbiol. Rev.*, 1994, vol. 13, pp. 95–118.