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Variation in Susceptibility to *Bacillus thuringiensis* Toxins among Unselected Strains of *Plutella xylostella*

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So far, the only insect that has evolved resistance in the field to *Bacillus thuringiensis* toxins is the diamondback moth (*Plutella xylostella*). Documentation and analysis of resistant strains rely on comparisons with laboratory strains that have not been exposed to *B. thuringiensis* toxins. Previously published reports show considerable variation among laboratories in responses of unselected laboratory strains to *B. thuringiensis* toxins. Because different laboratories have used different unselected strains, such variation could be caused by differences in bioassay methods among laboratories, genetic differences among unselected strains, or both. Here we tested three unselected strains against five *B. thuringiensis* toxins (Cry1Aa, Cry1Ab, Cry1Ac, Cry1Ca, and Cry1Da) using two bioassay methods. Tests of the LAB-V strain from The Netherlands in different laboratories using different bioassay methods yielded only minor differences in results. In contrast, side-by-side comparisons revealed major genetic differences in susceptibility between strains. Compared with the LAB-V strain, the ROTH strain from England was 17- to 170-fold more susceptible to Cry1Aa and Cry1Ac, respectively, whereas the LAB-PS strain from Hawaii was 8-fold more susceptible to Cry1Ab and 13-fold more susceptible to Cry1Da and did not differ significantly from the LAB-V strain in response to Cry1Aa, Cry1Ac, or Cry1Ca. The relative potencies of toxins were similar among LAB-V, ROTH, and LAB-PS, with Cry1Ab and Cry1Ac being most toxic and Cry1Da being least toxic. Therefore, before choosing a standard reference strain upon which to base comparisons, it is highly advisable to perform an analysis of variation in susceptibility among field and laboratory populations.

Insecticidal crystal (Cry) proteins of *Bacillus thuringiensis* are contained in the crystalline bodies produced during the sporulation phase. They are produced as full-length proteins (protoxins) that, upon solubilization in the insect midgut, are processed by midgut proteases to render a protease-resistant fragment that constitutes the active toxin. The active toxin binds to specific target sites in the insect midgut, creating pores in the midgut membranes that eventually kill the insect (19). Cry proteins are extremely useful because, compared with conventional insecticides, they are more specific and thus environmentally safer (2, 3). Transgenic crop plants that produce Cry proteins are being used widely (10). In addition, some insect populations resistant to chemical insecticides have been controlled with *B. thuringiensis* products (2).

So far, the only insect that has evolved resistance in the field to *B. thuringiensis* toxins is the diamondback moth (*Plutella xylostella*) (23). Documentation and analysis of resistant strains rely on comparisons with laboratory strains that have not been exposed to *B. thuringiensis* toxins. Previously published reports show considerable variation among laboratories in responses of unselected laboratory strains to *B. thuringiensis* toxins (Table 1). Such variation could affect not only the absolute assessment of toxicity but also the relative resistance levels detected for other strains. Because different laboratories have used different unselected strains, such variation could be caused by differences in bioassay methods among laboratories, genetic differences among unselected strains, or both.

Each of our three laboratories has been using a different unselected *P. xylostella* strain as a reference strain to determine the toxicity of *B. thuringiensis* products and individual toxins (1, 12, 25). Here we used side-by-side comparisons to test the hypothesis that differences in susceptibility to Cry proteins between strains are genetically based. We also evaluated the effects of differences in bioassay protocols, including differences in the duration of exposure to toxins and in the source and preparation of toxins used in bioassays. Finally, we examined variations in the relative potencies of Cry proteins caused by differences in strains and bioassay procedures.

MATERIALS AND METHODS

Insects. Each of the three susceptible strains had been reared for at least 10 years without exposure to Cry proteins. The LAB-V strain was collected in The Netherlands (5) and maintained in Spain; the ROTH strain was collected and maintained in the United Kingdom (18); and the LAB-PS strain was derived from the LAB-P strain, which was collected in Hawaii (13) and maintained in the United States. Larvae were reared on cabbage leaves.

Cry proteins. Cry1Aa, Cry1Ab, Cry1Ac, Cry1Ca, and Cry1Da were obtained from recombinant *B. thuringiensis* strains EG1273, EG7077, EG11070, EG1081, and EG7300, respectively (Ecogen Inc.). Protoxin purification, trypsin activation, and protein quantification were performed as described by Sayyed et al. (18) at the University of Valencia, Valencia, Spain. Activated toxins were sent frozen to the Imperial College of Science, Ascot, Berkshire, United Kingdom. The same batch of toxins was shared and used by the above two laboratories.

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TABLE 1. Reported LC_{50} and FL_{95} values of different toxins for four unselected laboratory strains of *P. xylostella*

Protein	Result for the following strain:							
	LAB-V_{91}[a]		Geneva[b]		LAB-PS[c]		Reunion Island[d]	
	LC_{50}	FL_{95}	LC_{50}	FL_{95}	LC_{50}	FL_{95}	LC_{50}	FL_{95}
Cry1Aa	239	127–1,045	0.3	0.2–0.3	2.80	1.94–4.26	22.10	16.20–30.16
Cry1Ab	15	5–30	0.6	0.4–1.2	1.60	1.35–1.90	0.57	0.47–0.69
Cry1Ac	44	29–99	1.1	0.5–2.5	0.74	0.50–1.16	20.09	14.67–27.52
Cry1Ca	117	68–210	4.3	3.3–5.7	10.55	8.08–14.20	7.58	5.65–10.18
Cry1Da	>1,350	NA	0.2	0.1–0.2	19.10[e]	13.20–25.00	18.82	14.47–24.48

[a] Bioassays were performed with the diet overlay method. The larvae were intoxicated for 5 days, and mortality was scored at 5 days. Cry proteins were used in their activated form. Values are given in nanograms per square centimeter and are from references 1 (for Cry1Aa, Cry1Ab, and Cry1Ac), 5 (for Cry1Da), and 7 (for Cry1Ca), which contain the most recent data published for this strain. NA, not available.

[b] Bioassays were performed with the leaf dip method (22). The larvae were intoxicated for 3 days, and mortality was scored at 3 days. Cry1Aa, Cry1Ab, Cry1Ac, and Cry1Ca were used as solubilized protoxins, and Cry1Da was used as an activated toxin. Values are given in milligrams per liter.

[c] Bioassays were performed with the leaf dip method (16). The larvae were intoxicated for 2 days, and mortality was scored at 5 days. Cry proteins were used as a mixture of spores and crystals. Values are given in milligrams per liter.

[d] Bioassays were performed with the leaf dip method (17). The larvae were intoxicated for 2 days, and mortality was scored at 2 days. Cry proteins were used in their activated form. Values are given in milligrams per liter.

[e] Bioassay data are from this study (slope of the regression line obtained by probit analysis, and standard error, 2.10 ± 0.37).

Bioassays. Susceptibility to each Cry protein was tested with third-instar larvae by use of a leaf dip bioassay (21). At least five concentrations of each Cry protein were included. The replicates were performed on different days with larvae from different parents. We used three types of bioassays, each performed at a different laboratory: B1 (University of Valencia), B2 (Imperial College of Science), and B3 (University of Arizona, Tucson). The B1 bioassay used activated Cry proteins from Ecogen strains. Mortality was scored after larvae (10 per concentration) were exposed to Cry proteins for 2 days at 25°C. This bioassay was performed twice. The B2 bioassay also used activated Cry proteins from Ecogen strains, but mortality was scored after larvae (5 per concentration) were exposed to toxins for 5 days at 20°C. This bioassay was repeated eight times. The B3 bioassay used lyophilized powder containing spores and crystals from the strain that expresses Cry1Da (Ecogen strain EG7300). Two days after larvae (10 per concentration) were placed on treated leaf disks, fresh untreated leaf disks were added. Mortality was scored 5 days after the start of the bioassay. Rearing and tests for B3 were done at 28°C with 14 h of light and 10 h of dark. Four replicates of this bioassay were performed.

Before the side-by-side tests, strains were reared for at least two generations in the laboratory where the bioassays were performed at 25°C (B1, LAB-V versus LAB-PS) or 20°C (B2, LAB-V versus ROTH), at 70% relative humidity, and with a photoperiod of 16 h of light and 8 h of dark.

Mortality data were evaluated by probit analysis (6) using the POLO-PC program (LeOra Software, Berkeley, Calif.) to estimate the concentrations killing 50% of the larvae tested (LC_{50}s) and their 95% fiducial limits (FL_{95}). LC_{50}s were considered significantly different if their FL_{95}s did not overlap.

RESULTS

Differences between strains. Side-by-side comparisons revealed genetic differences in susceptibility to Cry proteins between unselected strains of the diamondback moth. Comparisons using the B1 bioassay showed that relative to LAB-V, LAB-PS was 8-fold more susceptible to Cry1Ab and 13-fold more susceptible to Cry1Da (Table 2). Significant differences in LC_{50} between LAB-V and LAB-PS were not observed for Cry1Aa, Cry1Ac, or Cry1Ca (Table 2). Comparisons using the B2 bioassay showed that relative to LAB-V, ROTH was significantly more susceptible to each of the five toxins tested (Table 3). The differences in LC_{50} ranged from 17-fold for Cry1Aa to 170-fold for Cry1Ac.

Differences between bioassays. The LC_{50} of Cry1Ab was significantly higher for the LAB-V strain in the B1 bioassay (exposure for and scoring at 2 days) than in the B2 bioassay (exposure for and scoring at 5 days) (Tables 2 and 3). Significant differences in LC_{50} did not occur for the other four toxins considered individually. However, for all five toxins, the LC_{50} was higher for the B1 bioassay than for the B2 bioassay (one-tailed sign test; $P = 0.03$). The differences in LC_{50} between bioassays ranged from 1.3-fold for Cry1Ac to 4.3-fold for Cry1Ab.

Relative potencies of Cry proteins. The analysis of bioassay data from this work showed that the relative potencies of Cry1 proteins for the LAB-V strain followed a regular pattern. With either the B1 bioassay or the B2 bioassay, Cry1Ab and Cry1Ac were the most potent, followed in order by Cry1Ca, Cry1Aa, and Cry1Da (Table 4). In addition, data reported for the same strain in 1991 (5), 1994 (1), and 1996 (7) but with a diet overlay bioassay and toxins from a different source showed the same pattern. Cry1Ab and Cry1Ac were also the most toxic for LAB-PS and ROTH. However, Cry1Ab and Cry1Ac showed

TABLE 2. Susceptibility of LAB-V and LAB-PS strains of *P. xylostella* to several Cry1 proteins in the B1 bioassay

Protein	Result[a] for the following strain:						Toxicity ratio (LC_{50} for LAB-V/LC_{50} for LAB-PS)[b]
	LAB-V			LAB-PS			
	LC_{50}	FL_{95}	Slope ± SE[c]	LC_{50}	FL_{95}	Slope ± SE	
Cry1Aa	3.82	1.53–6.98	1.75 ± 0.35	1.61	0.89–2.70	1.19 ± 0.20	NS
Cry1Ab	0.60	0.40–0.86	1.46 ± 0.26	0.079	0.031–0.38	1.90 ± 0.30	8
Cry1Ac	0.22	0.14–0.31	2.17 ± 0.37	0.23	0.16–0.32	3.34 ± 0.53	NS
Cry1Ca	1.50	0.52–3.83	1.37 ± 0.22	0.74	0.29–1.17	1.62 ± 0.39	NS
Cry1Da	45.31	14.30–95.50	0.64 ± 0.17	3.38	1.55–6.24	2.47 ± 0.38	13

[a] Values are given in milligrams per liter.

[b] NS, LC_{50}s were not significantly different.

[c] Slope of the regression line obtained by probit analysis, and standard error.

TABLE 3. Susceptibility of LAB-V and ROTH strains of *P. xylostella* to several Cry1 proteins in the B2 bioassay

Protein	Result[a] for the following strain:						Toxicity ratio (LC_{50} for LAB-V/LC_{50} for ROTH)
	LAB-V			ROTH			
	LC_{50}	FL_{95}	Slope ± SE[b]	LC_{50}	FL_{95}	Slope ± SE	
Cry1Aa	2.72	1.97–4.62	1.21 ± 0.15	0.160	0.100–0.290	0.95 ± 0.12	17
Cry1Ab	0.14	0.08–0.24	0.95 ± 0.12	0.002	0.000–0.005	0.74 ± 0.23	70
Cry1Ac	0.17	0.09–0.47	1.06 ± 0.21	0.001	0.001–0.002	1.61 ± 0.30	170
Cry1Ca	1.00	0.74–1.49	1.11 ± 0.14	0.030	0.006–0.059	1.79 ± 0.60	33
Cry1Da	15.21	12.15–19.19	1.05 ± 0.35	0.270	0.180–0.290	1.01 ± 0.19	56

[a] Values are given in milligrams per liter.
[b] Slope of the regression line obtained by probit analysis, and standard error.

an inverse pattern of potencies for these two strains compared with the LAB-V strain in the same type of bioassay (Table 4). Relative potencies for the Geneva (22) and Reunion Island strains (17) differed greatly from those for the three strains that we tested. For the Geneva strain, Cry1Da had the highest potency of the five toxins tested. For the Reunion Island strain, Cry1Ab was much more potent than the other four toxins.

DISCUSSION

The results presented here show major genetic differences in susceptibility to Cry toxins between unselected laboratory strains of *P. xylostella* from The Netherlands (LAB-V), England (ROTH), and Hawaii (LAB-PS). In this study, genetic differences between strains in side-by-side comparisons were much larger than effects caused by differences in bioassays between laboratories.

The B1 and B2 bioassays compared here used activated toxins from the same source but differed in that the B1 bioassay was done at 25°C for 2 days whereas the B2 bioassay was done at 20°C for 5 days. As expected and in confirmation of previous results obtained with the diamondback moth (14, 20), LC_{50}s were generally higher in shorter tests. Relative to previous studies, in the present study the extent of the difference between two time intervals might have been reduced somewhat because the temperature was higher for the shorter bioassay (B1) than for the longer bioassay (B2).

Despite differences in source of toxin and bioassay procedure and genetic differences in absolute susceptibility between strains, the patterns of relative potencies among the five toxins tested were similar for the three unselected strains tested here. For example, the pattern of relative potency for LAB-V remained similar for at least 10 years and was not affected much by the type of bioassay (leaf dip or diet overlay) or the source of toxin. Also, for LAB-PS, relative potencies were similar in bioassays with activated toxin and bioassays with crystals and spores. The use of protoxin involves additional steps over the use of activated toxins, and these have an influence on the final toxicity (8, 15). The presence of spores may also enhance the effects of toxins (12, 22). In contrast to the similar patterns seen for the three unselected strains tested here, the Geneva and Reunion Island strains showed unique relative potencies. However, in these instances, we cannot make strong inferences about the differences among strains because toxin sources and bioassay procedures varied. Side-by-side tests would be needed to determine if the differences in relative potencies were genetically based.

Side-by-side experiments performed with LAB-V and LAB-PS and with LAB-V and ROTH in different bioassay protocols revealed important variations due to genetic differences among strains. The greatest differences were obtained between LAB-V and ROTH. LAB-V and LAB-PS were rather similar with respect to their spectrum of susceptibility and also in terms of absolute LC_{50}s.

Significant differences among conspecific populations have also been reported for other insect species. An analysis of Cry1Aa toxicity against two unselected strains of *Heliothis virescens*, carried out in different laboratories following similar protocols, showed about a 30-fold variation in absolute LC_{50}s, while the toxicities of Cry1Ab and Cry1Ac showed just minor

TABLE 4. Relative potencies of several Cry1 proteins against unselected strains of *P. xylostella*[a]

Protein	Result in the indicated test for the following strain:							
	LAB-V			LAB-PS		ROTH (bioassay B2)	Geneva (leaf dip)[d]	Reunion Island (leaf dip)[e]
	Bioassay B1	Bioassay B2	Diet overlay[b]	Bioassay B1	Bioassay B3[c]			
Cry1Aa	6	5	6	5	26	0.6	67	3
Cry1Ab	37	100	100	100	46	50	33	100
Cry1Ac	100	82	34	34	100	100	18	3
Cry1Ca	15	14	13	11	7	3	5	8
Cry1Da	0.5	0.9	<1	2	4	0.4	100	3

[a] Relative potencies were estimated by assigning a value of 100 to the most potent Cry protein (the one that showed the lowest LC_{50}). The potency of all others was calculated by dividing the lowest LC_{50} by their LC_{50} and multiplying the result by 100.
[b] Data are from Ballester et al. (1), Ferré et al. (5), and Granero et al. (7). See also Table 1.
[c] Data are from Liu et al. (16). See also Table 1.
[d] Data are from Tang et al. (22). See also Table 1.
[e] Data are from Monnerat et al. (17). See also Table 1.

differences. These were not side-by-side studies, but they used similar protocols to test toxicity (11, 24). Moreover, two studies performed with two unselected strains of *Trichoplusia ni* showed about 100-fold differences in absolute LC_{50}s for activated Cry1Ab and Cry1Ac. Although these were not side-by-side studies, they were performed in the same laboratory following essentially the same protocol and using Cry proteins from the same source (4, 9).

In conclusion, susceptibility to Cry proteins may vary among unselected populations of a given insect species. This variation affects the criteria for resistance, because a treated field population might be considered resistant or not resistant depending on the unselected reference strain used. Further, such variation could affect the standardization of potency for products based on *B. thuringiensis*. We strongly recommend an analysis of variation in susceptibility among unselected field and laboratory populations before a standard reference strain upon which to base comparisons is chosen.

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REFERENCES

1. **Ballester, V., B. Escriche, J. L. Ménsua, G. W. Riethmacher, and J. Ferré.** 1994. Lack of cross-resistance to other *Bacillus thuringiensis* crystal proteins in a population of *Plutella xylostella* highly resistant to Cry1A(b). Biocontrol Sci. Technol. **4:**437–443.
2. **Charles, J. F., A. Delécluse, and C. Nielsen-LeRoux.** 2000. Entomopathogenic bacteria: from laboratory to field application. Kluwer Academic Publishers, Dordrecht, The Netherlands.
3. **Entwistle, P. F., J. S. Cory, M. J. Bailey, and S. Higgs.** 1993. *Bacillus thuringiensis*, an environmental biopesticide: theory and practice. John Wiley & Sons, Inc., New York, N.Y.
4. **Estada, U., and J. Ferré.** 1994. Binding of insecticidal crystal protein of *Bacillus thuringiensis* to the midgut brush border of the cabbage looper, *Trichoplusia ni* (Hübner) (Lepidoptera: Noctuidae), and selection for resistance to one of the crystal proteins. Appl. Environ. Microbiol. **60:**3840–3846.
5. **Ferré, J., M. D. Real, J. Van Rie, S. Jansens, and M. Peferoen.** 1991. Resistance to the *Bacillus thuringiensis* bioinsecticide in a field population of *Plutella xylostella* is due to a change in a midgut membrane receptor. Proc. Natl. Acad. Sci. USA **88:**5119–5123.
6. **Finney, D. J.** 1971. Probit analysis, 3rd ed. Cambridge University, Cambridge, United Kingdom.
7. **Granero, F., V. Ballester, and J. Ferré.** 1996. *Bacillus thuringiensis* crystal proteins Cry1Ab and Cry1Fa share a high affinity binding site in *Plutella xylostella* (L.). Biochem. Biophys. Res. Commun. **224:**779–783.
8. **Herrero, S., B. Oppert, and J. Ferré.** 2001. Different mechanisms of resistance to *Bacillus thuringiensis* toxins in the indianmeal moth. Appl. Environ. Microbiol. **67:**1085–1089.
9. **Iracheta, M. M., B. Pereyra-Alférez, L. Galán-Wong, and J. Ferré.** 2000. Screening for *Bacillus thuringiensis* crystal proteins active against the cabbage looper, *Trichoplusia ni*. J. Invertebr. Pathol. **76:**70–75.
10. **James, C.** 2000. Global status of commercialized transgenic crops: 2000. ISAAA brief no. 21. Preview. International Service for the Acquisition of Agri-Biotech Applications, Ithaca, N.Y.
11. **Jurat-Fuentes, J. L., and M. J. Adang.** 2001. Importance of Cry1 δ-endotoxin domain II loops for binding specificity in *Heliothis virescens* (L.). Appl. Environ. Microbiol. **67:**323–329.
12. **Liu, Y. B., B. E. Tabashnik, W. J. Moar, and R. A. Smith.** 1998. Synergism between *Bacillus thuringiensis* spores and toxins against resistant and susceptible diamondback moths (*Plutella xylostella*). Appl. Environ. Microbiol. **64:**1385–1389.
13. **Liu, Y. B., and B. E. Tabashnik.** 1998. Elimination of a recessive allele conferring resistance to *Bacillus thuringiensis* from a heterogeneous strain of diamondback moth (Lepidoptera: Plutellidae). J. Econ. Entomol. **91:**1032–1037.
14. **Liu, Y. B., B. E. Tabashnik, and M. W. Johnson.** 1995. Larval age affects resistance to *Bacillus thuringiensis* in diamondback moth (Lepidoptera: Plutellidae). J. Econ. Entomol. **88:**788–792.
15. **Liu, Y. B., B. E. Tabashnik, L. Masson, B. Escriche, and J. Ferré.** 2000. Binding and toxicity of *Bacillus thuringiensis* protein Cry1C to susceptible and resistant diamondback moth (Lepidoptera: Plutellidae). J. Econ. Entomol. **93:**1–6.
16. **Liu, Y. B., B. E. Tabashnik, S. K. Meyer, and N. Crickmore.** 2001. Cross-resistance and stability of resistance to *Bacillus thuringiensis* toxin Cry1C in the diamondback moth. Appl. Environ. Microbiol. **67:**3216–3219.
17. **Monnerat, R., L. Masson, R. Brousseau, M. Pusztai-Carey, D. Bordat, and R. Frutos.** 1999. Differential activity and activation of *Bacillus thuringiensis* insecticidal proteins in diamondback moth, *Plutella xylostella*. Curr. Microbiol. **39:**159–162.
18. **Sayyed, A. H., R. Haward, S. Herrero, J. Ferré, and D. J. Wright.** 2000. Genetic and biochemical approach for characterization of resistance to *Bacillus thuringiensis* toxin Cry1Ac in a field population of the diamondback moth. Appl. Environ. Microbiol. **66:**1509–1516.
19. **Schnepf, E., N. Crickmore, J. Van Rie, D. Lereclus, J. Baum, J. Feitelson, D. R. Zeigler, and D. H. Dean.** 1998. *Bacillus thuringiensis* and its pesticidal crystal proteins. Microbiol. Mol. Biol. Rev. **62:**775–806.
20. **Tabashnik, B. E., N. Finson, C. F. Chilcutt, N. L. Cushing, and M. W. Johnson.** 1993. Increasing efficacy of bioassays: evaluating resistance to *Bacillus thuringiensis* in diamondback moth (Lepidoptera, Plutellidae). J. Econ. Entomol. **86:**635–644.
21. **Tabashnik, B. E., N. L. Cushing, N. Finson, and M. W. Johnson.** 1990. Field development of resistance to *Bacillus thuringiensis* in diamondback moth (Lepidoptera: Plutellidae). J. Econ. Entomol. **83:**1671–1676.
22. **Tang, J. D., A. M. Shelton, J. Van Rie, S. de Roeck, W. J. Moar, R. T. Roush, and M. Peferoen.** 1996. Toxicity of *Bacillus thuringiensis* spore and crystal protein to resistant diamondback moth (*Plutella xylostella*). Appl. Environ. Microbiol. **62:**564–569.
23. **Van Rie, J., and J. Ferré.** 2000. Insect resistance to *Bacillus thuringiensis* insecticidal crystal proteins, p. 219–237. *In* J. F. Charles, A. Delecluse, and C. Nielsen-LeRoux (ed.), Entomopathogenic bacteria: from laboratory to field application. Kluwer Academic Publishers, Dordrecht, The Netherlands
24. **Van Rie, J., S. Jansens, H. Höfte, D. Degheele, and H. Van Mellaert.** 1989. Specificity of *Bacillus thuringiensis* δ-endotoxins: importance of specific receptors on the brush border membrane of the mid-gut of target insects. Eur. J. Biochem. **186:**239–247.
25. **Wright, D. J., M. Iqbal, F. Granero, and J. Ferré.** 1997. A change in a single midgut receptor in the diamondback moth (*Plutella xylostella*) is only in part responsible for the field resistance to *Bacillus thuringiensis* subsp. *kurstaki* and *B. thuringiensis* subsp. *aizawai*. Appl. Environ. Microbiol. **63:**1814–1819.