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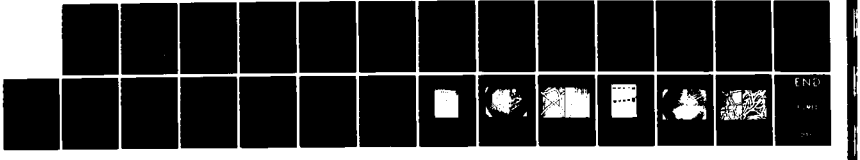

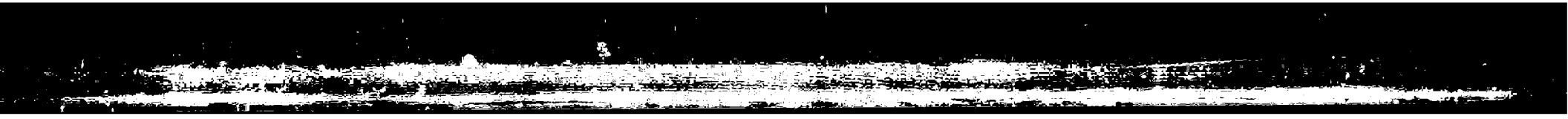

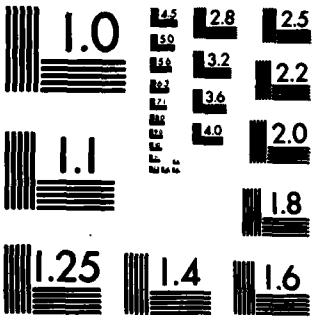

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Demonstration of A Capsule Plasmid in Bacillus anthracis

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Virulent and certain avirulent strains of Bacillus anthracis harbor a plasmid, designated pXO2, which is involved in the synthesis of capsules. Two classes of rough noncapsulated (Cap^-) variants were isolated from the capsule-producing (Cap^+) Pasteur vaccine strain 6602: those which were cured of pXO2 and those which still carried it. Reversion to Cap^+ was demonstrable only in rough variants which had pXO2. By means of a mating system in which plasmid transfer is mediated by a Bacillus thuringiensis fertility plasmid, pXO12, pXO2 was transferred from B. anthracis to Bacillus cereus. B. cereus transcipients which

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ABSTRACT

Virulent and certain avirulent strains of Bacillus anthracis harbor a plasmid, designated pXO2, which is involved in the synthesis of capsules. Two classes of rough noncapsulated (Cap^-) variants were isolated from the capsule-producing (Cap^+) Pasteur vaccine strain 6602: those which were cured of pXO2 and those which still carried it. Reversion to Cap^+ was demonstrable only in rough variants which had pXO2. By means of a mating system in which plasmid transfer is mediated by a Bacillus thuringiensis fertility plasmid, pXO12, pXO2 was transferred from B. anthracis to Bacillus cereus. B. cereus transcipients which acquired pXO2 produced capsules under the same conditions required for capsule synthesis by B. anthracis.

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INTRODUCTION

Bacillus anthracis requires two virulence factors to cause disease. One of these is a toxin composed of three different proteins known as edema factor, lethal factor, and protective antigen (8, 13). Mikesell, et al. (6) and Robillard, et al. (N. J. Robillard, T. M. Koehler, R. Murray, and C. B. Thorne, Abstr. Annu. Meet. Am. Soc. Microbiol. 1983, H54, p.115) demonstrated that a 112 Mdal plasmid, formerly referred to as pBA1, but now designated pXO1, is associated with toxin production; and Vodkin and Leppla (14) showed by cloning experiments that pXO1 carries the structural genes for protective antigen. The other virulence factor is a capsule composed of D-glutamyl polypeptide (5, 9, 12). When virulent strains of B. anthracis are grown on media containing serum and/or bicarbonate, they produce capsules and colonies appear mucoid. In the absence of serum or bicarbonate they fail to produce capsules and colonies appear rough. Thus, mutants which cannot make capsules can be easily distinguished from colonies of the capsulated parental cells and are readily isolated by selecting rough sectors or outgrowth from mucoid colonies grown on medium containing bicarbonate.

In an earlier paper it was reported that the heat-attenuated Pasteur vaccine strains of B. anthracis, which form capsules but which are avirulent because they are unable to produce toxin, were devoid of plasmid DNA (6). With the improvement of plasmid DNA extraction procedures, we have recently shown that the Pasteur vaccine strains do contain a plasmid which we have designated pXO2. We report here that pXO2, which is present in all encapsulated strains of B. anthracis examined, carries information for capsule production.

MATERIALS AND METHODS

Organisms. The organisms used in this study are listed in Table 1.

Media. NBY medium contained 8 g of nutrient broth (Difco Laboratories, Detroit, Mich.) and 3 g of yeast extract (Difco) per liter, pH 6.8. For capsule production, NBY medium was supplemented with $NaHCO_3$ (sterilized by filtration of a 9% solution) at a final concentration of 0.7% (w/v), and with horse serum (GIBCO Laboratories, Grand Island, New York) at a final concentration of 10% (v/v). BHI medium contained 37 g of brain heart infusion (Difco) per liter. L broth contained 10 g of tryptone (Difco), 5 g of yeast extract (Difco), and 10 g of NaCl per liter with the pH adjusted to 7.0. Min IC contained the following (in grams per liter, with the pH adjusted to 7.2 with NaOH): $(NH_4)_2SO_4$, 2; KH_2PO_4, 6; K_2HPO_4, 14; $MgSO_4 \cdot 7H_2O$, 0.2; $FeCl_3 \cdot 6H_2O$, 0.04; $MnSO_4 \cdot H_2O$, 0.0125; trisodium citrate.$2H_2O$, 1; thiamine hydrochloride, 0.01; L-glutamic acid, 2; vitamin-free acid-hydrolyzed casein (Nutritional Biochemicals Corp., Cleveland, Ohio), 5; glucose, 5. R medium has been described previously (7). Phage assay medium contained the following (in grams per liter, with the pH adjusted to 6.0 with HCl): nutrient broth (Difco), 8; $CaCl_2 \cdot 2H_2O$, 0.15; $MgSO_4 \cdot 7H_2O$, 0.2; $MnSO_4 \cdot H_2O$, 0.05; NaCl, 5. For solid medium 15 g of agar was added per liter. Soft agar contained 5 g of agar per liter.

Immunoassay agar plates contained 12 ml of R agar plus 2 ml of antiserum prepared in goats by immunization with viable spores of the Sterne strain of B. anthracis.

Capsule production. The ability of B. anthracis and B. cereus to produce capsules was determined by growing cells on R agar or on NBY agar containing bicarbonate and serum. Plates were incubated in the presence of 5% or 20% CO_2

at 37°C for 24 to 48 h.

Toxin assays. Lethal factor and edema factor activity were assayed as previously described (6).

Detection of plasmid DNA. Plasmid DNA was extracted by a modification (L. Battisti, B. D. Green, and C. B. Thorne, manuscript in preparation) of the method of Kado and Liu (2). Electrophoresis was carried out at 70 volts in 0.7% agarose gels prepared and run in Tris-borate buffer (0.089 M Tris base, 0.089 M boric acid, and 0.0025 M EDTA at pH 8.2 to 8.3).

Plasmid curing. Strains cured of pXO2 were found among spontaneous rough variants (see below) or isolated from cultures treated with novobiocin. For novobiocin treatment, 10 to 100 CFU of B. anthracis were inoculated into 250-ml flasks containing 50 ml of L broth plus novobiocin (1 μg/ml) and incubated three days at 37°C with gentle shaking. Cultures were diluted and 0.1-ml samples were spread on plates of immunoassay agar and incubated at 37°C for 24 h in 5% CO_2. Rough colonies of noncapsulated cells were isolated and tested for loss of pXO2. Strains were cured of pXO1 by serial passage at 43°C (6, N. J. Robillard, T. M. Koehler, B. D. Green, and C. B. Thorne, manuscript in preparation).

Isolation of spontaneous rough mutants of B. anthracis 6602. Cells were plated for single colonies on NBY agar containing bicarbonate and serum and incubated at 37°C in 20% CO_2. After several days rough outgrowth appeared at the edge of some of the mucoid colonies. These were picked and purified by streaking on fresh plates of the same medium.

Propagation and assay of bacteriophage Wα. Bacteriophage Wα (3) was obtained from B. cereus W (ATCC 11950). It was propagated on B. anthracis 6602 R1 in soft overlays of phage assay agar incubated at 37°C for 17 to 20 h. It was assayed against the same strain in soft overlays of phage assay agar incubated at 30°C.

Isolation of capsulated revertants of rough mutants. To demonstrate reversion, approximately 1 x 10^5 spores of a rough mutant were spread with 1 x 10^8 PFU of phage Wα on NBY agar containing bicarbonate and serum. The plates were incubated at 37°C in 20% CO_2 for two days and examined for mucoid colonies.

Transfer of plasmids by mating. Matings were performed as described by Thorne (11).

RESULTS

Plasmid analysis of B. anthracis strains. A number of virulent and avirulent strains of B. anthracis were analyzed for plasmid content. All virulent strains examined, which included NH, Ames, Colorado, Buffalo, and Vollum 1B, carried two plasmids, as demonstrated in Fig. 1, lane 1, for Vollum 1B. In addition to pXO1, they contained a second smaller plasmid which we have designated pXO2. Strains could be cured of pXO2 by growing them in the presence of novobiocin. Three thousand to 5000 colonies each of strains NH, Ames, and Vollum 1B obtained from cultures treated with novobiocin were screened for capsule formation and protective antigen synthesis on immunoassay agar. All colonies produced protective antigen as evidenced by the halos surrounding them. However, approximately 1% of the colonies were rough (nonmucoid), indicating failure to produce capsules. Two rough colonies from each strain were subcultured and examined for plasmid content and the capacity to synthesize toxin and capsules. These strains were designated Vollum 1B VNR-1 and -2, NH NNR-1 and -2, and Ames ANR-1 and -2. They produced biologically active toxin, failed to produce capsules in 5% or 20% CO_2, and contained only one plasmid, pXO1 (Fig. 1, lane 5). They were identical to the toxinogenic noncapsulated

Sterne veterinary vaccine strain with respect to phenotype and plasmid content (Fig. 1, lane 2).

Growth of toxinogenic strains of B. anthracis at 43°C has been shown to result in the elimination of the toxin plasmid, pXO1 (6, 11). After virulent strains, including NH, Ames, and Vollum 1B, were cultured at 43°C, variants were selected on immunoassay agar which were cured of pXO1 but still carried pXO2. Such variants, represented by the Pasteur vaccine strain (ATCC 6602) and Vollum 1B-1 in Fig. 1, lanes 3 and 4, produced no toxin. They synthesized capsules on R agar during growth in 20% CO_2 but not in 5% CO_2.

Isolation of rough (noncapsulated) variants of the Pasteur strain, ATCC 6602. When strains of Cap^+ B. anthracis are grown on agar for several days under conditions conducive to capsule formation, mucoid colonies frequently have areas of rough outgrowth. Such areas of rough growth have been shown to yield reverting and nonreverting noncapsulated mutants (4, 10). To demonstrate further a correlation between capsule formation and the presence of pXO2, we isolated spontaneous rough variants of B. anthracis 6602. Fig. 2 and 3 illustrate the differences in colony and cell morphology between the wild-type mucoid strain and rough variants of strain 6602 grown under conditions required for capsule formation. Strain 6602, like virulent strains of B. anthracis, failed to produce capsules when grown in air (in the absence of bicarbonate and serum) and was indistinguishable in both colony and cell morphology from rough variants grown either in air or CO_2. Upon analysis of several rough variants of strain 6602 for plasmid content, two classes were found. Some of them were cured of pXO2 and contained no detectable plasmid DNA (Fig. 4, lane 2), and others still carried pXO2.

We reasoned that if pXO2 is involved in the formation of capsules, it should be possible to demonstrate reversion to Cap^+ among rough variants which

retain pXO2 but not among rough variants that are cured of the plasmid. Both classes of rough variants were tested for reversion to Cap^+ by exposing them to bacteriophage Wα . The use of this phage, which can lyse noncapsulated cells but not capsulated cells, affords a convenient means of detecting small numbers of Cap^+ revertants in populations of Cap^- cells (4). As predicted, no Cap^+ revertants were found among three independently isolated $(pXO2)^-$ variants, whereas Cap^+ revertants were found in each of three independently isolated rough strains which had retained pXO2.

Transfer of pXO2 to B. cereus. To transfer pXO2 to B. cereus, we made use of the Bacillus mating system in which plasmid transfer is mediated by the fertility plasmid, pXO12 (11). A B. anthracis 6602 donor strain carrying pXO2 and pXO12 was constructed by mating B. anthracis M23 cur 1 tr60B-1 $(pXO1)^-(pXO12, pBC16)^+$ Ura^- with strain 6602(pXO2). The mating mixture was plated on Min IC containing 5 μg of tetracycline per ml to select cells of strain 6602 which had acquired the Tc^r plasmid, pBC16. (The absence of uracil prevented growth of the M23 donor strain). The transcipients were then screened by phase microscopy for the presence of parasporal crystals, a phenotypic characteristic of $(pXO12)^+$ cells (11). One such transcipient, B. anthracis 6602 tr172B-2, which was shown to carry pXO2, pXO12, and pBC16 (Fig. 4, lane 3), was mated with B. cereus 569R M20 str-2 Ant^-. The mating mixture was plated on NBY agar plates containing bicarbonate, serum, streptomycin (200 μg/ml), and tetracycline (25 μg/ml) to select B. cereus transcipients that had acquired pBC16. The plates were incubated in 20% CO_2 for 18 h and examined for the presence of mucoid colonies. An average of one Tc^r colony out of 500 was mucoid and produced Cap^+ cells on NBY agar plates containing bicarbonate and serum and incubated in 20% CO_2. The Ant^- and Str^r markers of the original B. cereus recipient were retained, and sporulating cells contained parasporal crystals

characteristic of $(pXO12)^+$ cells. Capsule production by B. cereus$(pXO2)^+$ is demonstrated in Fig. 5 and 6. As with B. anthracis 6602, capsules were produced by B. cereus only when cells carrying pXO2 were grown in CO_2. The two plasmids, pXO2 and pXO12, when present in B. cereus lysates, separated poorly if at all under the conditions used for electrophoresis (Fig. 4, lane 5). However, when $Cap^+Cry^+Tc^r$ B. cereus was used as the donor in matings with B. anthracis M23 cur 1 Ura^- as the recipient, both Cap^+Cry^- and Cap^-Cry^+ cells were found among the Tc^r transcipients.

DISCUSSION

The results presented here demonstrate that the plasmid, pXO2, is involved in the formation of capsules by B. anthracis. All capsule-producing strains, both virulent and avirulent, examined thus far have been shown to harbor pXO2. The recent development of a mating system effective for transferring plasmids among B. anthracis, B. cereus, and B. thuringiensis (11) made it possible to transfer pXO2 from B. anthracis 6602 to B. cereus 569R, which is normally noncapsulogenic. The transfer of pXO2 was mediated by the fertility plasmid, pXO12, which has been shown to be involved in the synthesis of parasporal crystals in B. thuringiensis and strains of B. anthracis and B. cereus to which the plasmid has been transferred (11). B. cereus transcipients were obtained which produced capsules when grown in CO_2 and parasporal crystals upon sporulation, indicating that both pXO2 and pXO12 were present. Interestingly, the two plasmids did not resolve upon gel electrophoresis of B. cereus extracts, although they did resolve when B. anthracis extracts were tested in the same manner. One possible reason for the inability to resolve the two plasmids from

B. cereus is that they were present in the form of a cointegrate or recombinant plasmid. A more likely reason for the failure to see a pXO2 band in electrophoretic gels of B. cereus Cap^+Cry^+ extracts is that pXO2 was present in very small amounts compared to pXO12. Extracts of Cap^+Cry^+ B. anthracis produced a relatively strong band of pXO12 compared to the pXO2 band, which was barely visible.

Novobiocin was determined to selectively cure strains of pXO2 when both pXO2 and pXO1 were present. Approximately 1% of colonies grown from novobiocin-treated cultures were cured of pXO2, but none of several thousand colonies was observed to be cured of the toxin plasmid, pXO1. All strains cured of pXO2 failed to produce capsules under conditions which normally promote capsule synthesis in $(pXO2)^+$ strains.

When spontaneous rough variants were isolated from the avirulent Pasteur strain of B. anthracis, both $(pXO2)^+$ and $(pXO2)^-$ types were found. Rough variants that retained pXO2 were able to revert to Cap^+, but those cured of the plasmid were not revertible. This observation explains earlier reports by Thorne (10) and Meynell (4) that some noncapsulated variants of B. anthracis were stable, while others were able to revert to Cap^+. This also explains why the avirulent Sterne vaccine strain, which produces toxin but not capsules, has not been observed to revert to the capsulated virulent type. The Sterne strain carries pXO1 but not pXO2, and is therefore unable to revert to Cap^+.

In view of the demonstration that rough variants which still carry pXO2 are able to revert to Cap^+, it will be interesting to determine whether the mutations that engender the rough phenotype are chromosomal- or plasmid-borne. We plan to test this by taking advantage of the B. anthracis mating system to move pXO2 from rough $(pXO2)^+$ variants to strains that have been cured of the plasmid.

ACKNOWLEDGMENTS

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LITERATURE CITED

1. Beall, F. A., M. J. Taylor, and C. B. Thorne. 1962. Rapid lethal effect in rats of a third component found upon fractionating the toxin of Bacillus anthracis. J. Bacteriol. 83:1274-1280.
2. Kado, C. I., and S.-T. Liu. 1981. Rapid procedure for detection and isolation of large and small plasmids. J. Bacteriol. 145:1365-1373.
3. McCloy, E. W. 1951. Studies on a lysogenic Bacillus strain. I. A bacteriophage specific for Bacillus anthracis. J. Hyg. Camb. 49:114-125.
4. Meynell, E. W. 1963. Reverting and non-reverting rough variants of Bacillus anthracis. J. Gen. Microbiol. 32:55-60.
5. Meynell, G. G., and E. Meynell. 1966. The biosynthesis of poly D-glutamic acid, the capsular material of Bacillus anthracis. J. Gen. Microbiol. 43:119-138.
6. Mikesell, P., B. E. Ivins, J. D. Ristroph, and T. M. Dreier. 1983. Evidence for plasmid-mediated toxin production in Bacillus anthracis. Infect. Immun. 39:371-376.
7. Ristroph, J. D., and B. E. Ivins. 1983. Elaboration of Bacillus anthracis antigens in a new defined culture medium. Infect. Immun. 39:483-486.
8. Stanley, J. L., K. Sargeant, and H. Smith. 1960. Purification of anthrax toxin factors I and II of the anthrax toxin produced in vivo. J. Gen. Microbiol. 22:206-218.
9. Thorne, C. B. 1956. Capsule formation and glutamyl polypeptide synthesis by Bacillus anthracis and Bacillus subtilis. Symp. Soc. Gen. Microbiol. 6:68-80.

10. Thorne, C. B. 1960. Biochemical properties of virulent and avirulent strains of Bacillus anthracis. Ann. N. Y. Acad. Sci. 88:1024-1033.

11. Thorne, C. B. 1985. Genetics of Bacillus anthracis. In Microbiology 1985. In press.

12. Thorne, C. B., C. G. Gomez, and R. D. Housewright. 1952. Synthesis of glutamic acid and glutamyl polypeptide by Bacillus anthracis. II. The effect of carbon dioxide on peptide production on solid media. J. Bacteriol. 63:363-368.

13. Thorne, C. B., D. M. Molnar, and R. E. Strange. 1960. Production of toxin in vitro by Bacillus anthracis and its separation into two components. J. Bacteriol. 79:450-455.

14. Vodkin, M. H., and S. H. Leppla. 1983. Cloning of the protective antigen gene of Bacillus anthracis. Cell 34:693-697.

LEGENDS TO FIGURES

FIG. 1. Agarose gel electrophoresis of plasmid DNA from strains of B. anthracis. Lane 1, Vollum 1B (virulent); Lane 2, Sterne (avirulent); Lane 3, Pasteur vaccine strain, ATCC 6602 (avirulent); Lane 4, Vollum 1B-1 (avirulent); Lane 5, Vollum VNR-1 (avirulent). Plasmids are labeled as follows: (a) pXO1; (c) pXO2. The large diffuse band in all lanes is chromosomal DNA.

FIG. 2. Colonies of B. anthracis Pasteur strain grown on bicarbonate agar in 20% CO_2. Left, strain 6602 wild type. Right, 6602 R1 (rough variant of 6602).

FIG. 3. Phase-contrast photomicrograph of cells of B. anthracis Pasteur strain grown on bicarbonate agar in 20% CO_2. Left, strain 6602 wild type. Right, 6602 R1 (rough variant of 6602). Bar, 2.0 μm.

FIG. 4. Agarose gel electrophoresis of plasmid DNA from B. anthracis and B. cereus. Lane 1, B. anthracis 6602 wild type; Lane 2, B. anthracis 6602 R1 (rough variant); Lane 3, B. anthracis 6602 tr172B-2, a transcipient carrying pXO2, pXO12, and pBC16; Lane 4, B. cereus 569R M20; Lane 5, B. cereus 569R M20 tr49G-4, a transcipient carrying pXO2, pXO12, and pBC16. Plasmids are labeled as follows: (b) pXO12; (c) pXO2; (d) pBC16. The extra bands in some lanes are alternative forms of pBC16.

FIG. 5. Colonies of B. cereus grown on bicarbonate agar in 20% CO_2. Left, B. cereus 569R M20. Right, B. cereus 569R M20 tr49G-4, a Cap^+ transcipient carrying pXO2.

FIG. 6. Phase-contrast photomicrograph of cells of B. cereus grown on bicarbonate agar in 20% CO_2. Left, B. cereus 569R M20 Cap^-. Right, B. cereus 569R M20 tr49G-4, a Cap^+ transcipient carrying pXO2. Bar, 2.0 μm.

TABLE 1. Strains used in this study[1]

Strain	Relevant Characteristics	Plasmids	Origin/reference
B. anthracis			
Ames	Virulent, Cap^+Tox^+	pXO1, pXO2	USDA[2]
Buffalo	Virulent, Cap^+Tox^+	pXO1, pXO2	R. A. Packer
Colorado	Virulent, Cap^+Tox^+	pXO1, pXO2	A. McChesney
NH	Virulent, Cap^+Tox^+	pXO1, pXO2	USAMRIID[3]
Pasteur 6602	Avirulent, Cap^+Tox^-	pXO2	ATCC[4]
Pasteur 6602 R1	Avirulent, Cap^-Tox^-	None	This study
Pasteur 6602 tr172B-2	Avirulent, $Cap^+Tox^-Cry^+Tc^r$	pXO2, pXO12, pBC16	This study
Sterne (Weybridge)	Avirulent, Cap^-Tox^+	pXO1	MRE[5]
Vollum 1B	Virulent, Cap^+Tox^+	pXO1, pXO2	USAMRIID
Vollum 1B-1	Avirulent, Cap^+Tox^-	pXO2	This study
Vollum 1B VNR-1	Avirulent, Cap^-Tox^+	pXO1	This study
Weybridge A M23 cur 1	Avirulent, Ura^- Cured of pXO1	None	C. B. Thorne
Weybridge A M23 cur 1 tr60B-1	Avirulent, $Ura^-Cry^+Tc^r$	pXO12, pBC16	This study

TABLE 1. (Continued)

B. cereus			
569R M20	Ant$^-$ *str-2*	One unnamed	C. B. Thorne
569R M20 tr49G-4	Ant$^-$ *str-2* Cry$^+$Cap$^+$Tc^r	pXO2, pXO12, pBC16	This study
11950	Carries bacteriophage Wα		ATCC

[1]Abbreviations: Cap, capsule; Tox, toxin; Cry, parasporal crystal; tr, transcipient; Ura, uracil; Ant, anthranilic acid; Tc^r, plasmid-encoded tetracycline resistance.

[2]U. S. Department of Agriculture, Ames, Iowa.

[3]U. S. Army Medical Research Institute of Infectious Diseases.

[4]American Type Culture Collection.

[5]Microbiological Research Establishment, Porton, England.

FIG. 1. Green, Battisti, Thorne, and Ivins

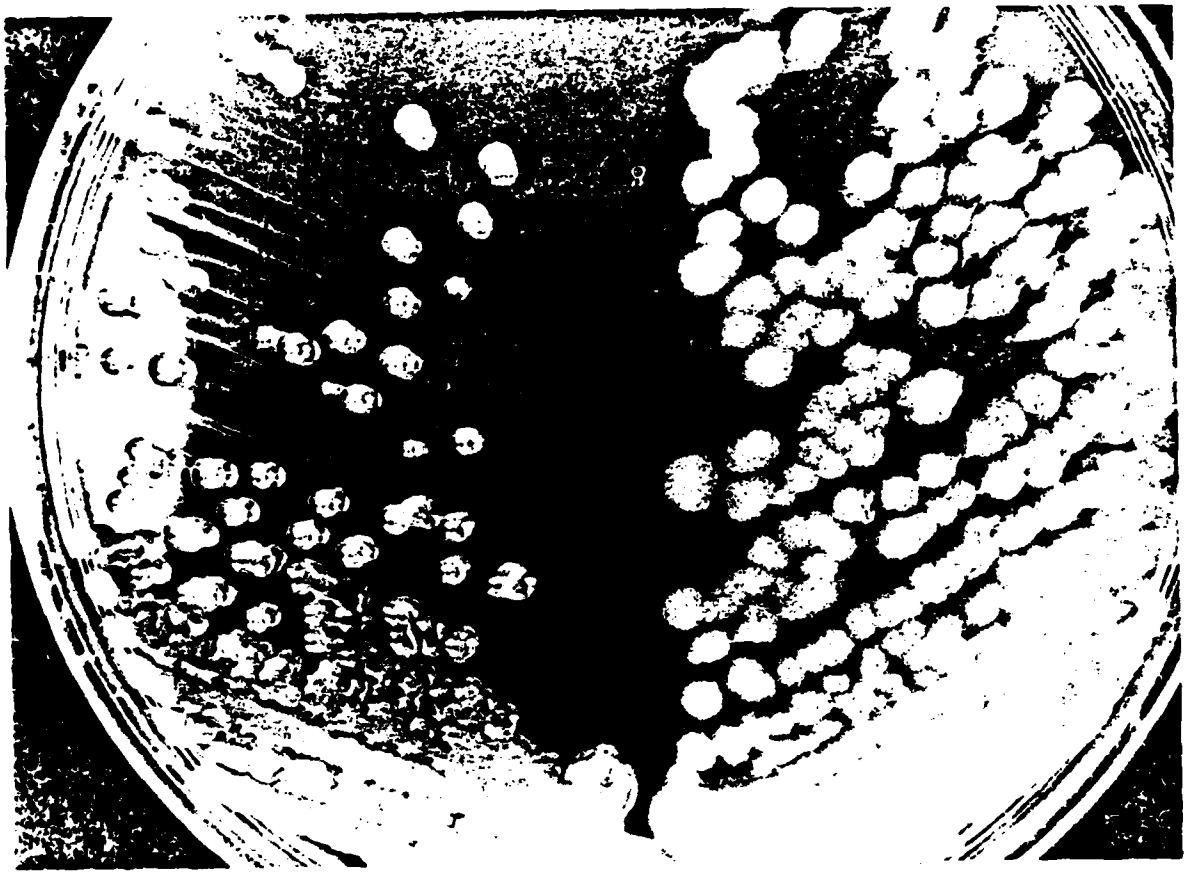

FIG. 2. Green, Battisti, Thorne, and Ivins

FIG. 3. Green, Battisti, Thorne, and Ivins

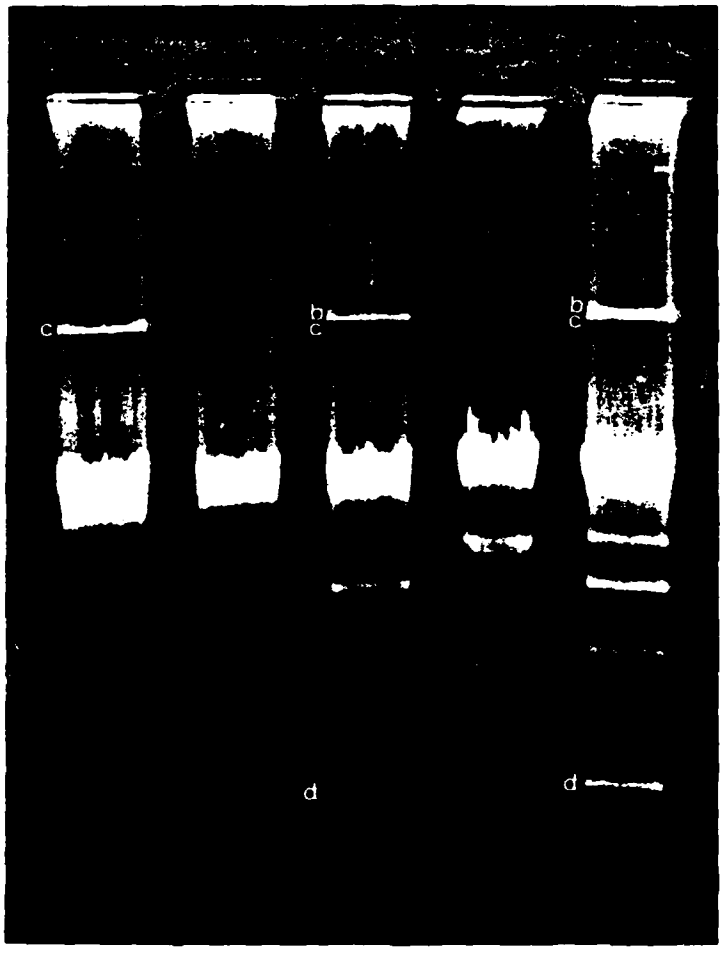

FIG. 4. Green, Battisti, Thorne, and Ivins

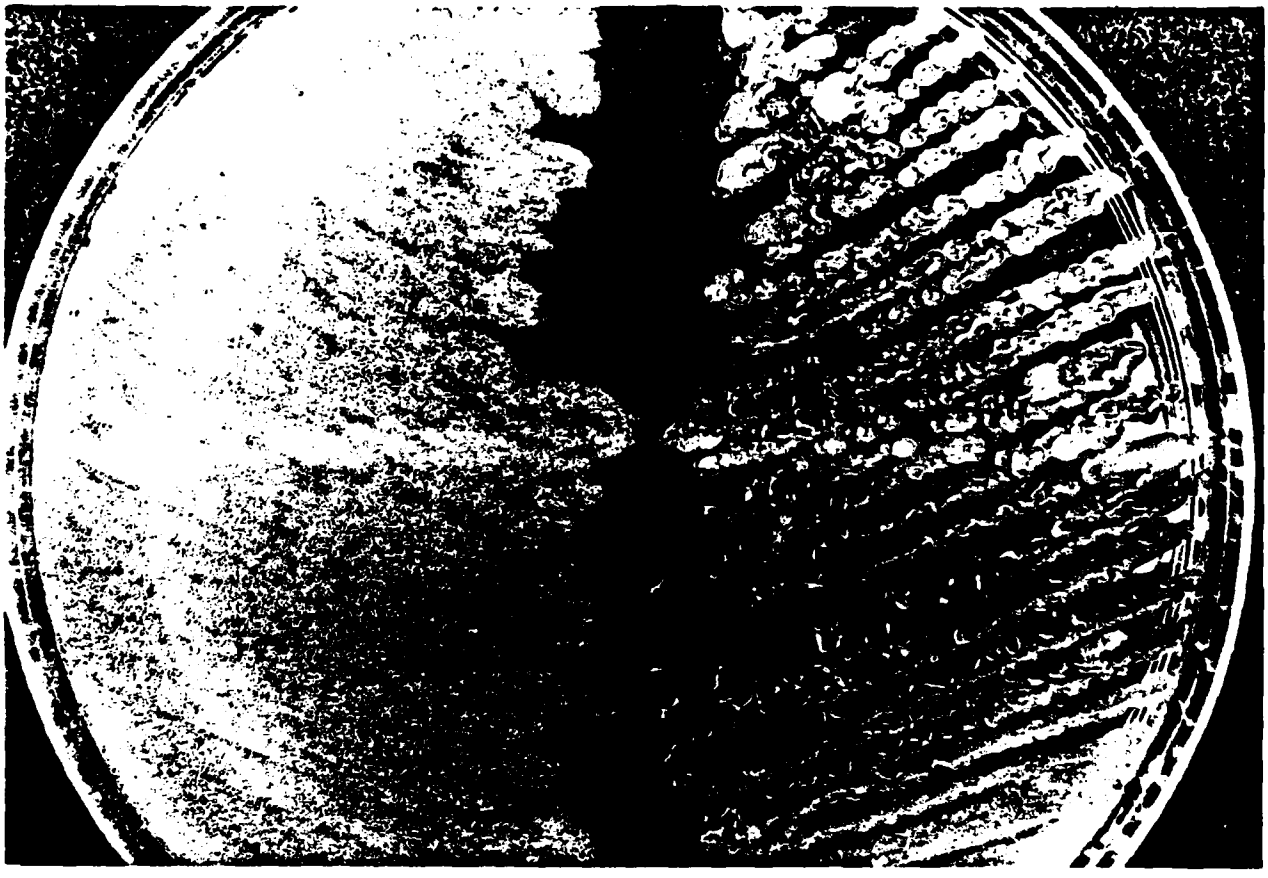

FIG. 5. Green, Battisti, Thorne, and Ivins

FIG. 6. Green, Battisti, Thorne, and Ivins

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