Actinomycetales from Corn

A. J. LYONS, T. G. PRIDHAM, AND R. F. ROGERS
Northern Regional Research Laboratory, U.S. Department of Agriculture, Peoria, Illinois 61604

Received for publication 29 October 1974

Mesophilic Actinomycetales were isolated from whole corn, brewer’s grits, and break flour received from three different mills. In addition, strains were isolated from high-moisture (27%) field corn; high-moisture, silo-stored corn (untreated); and high-moisture corn treated with ammonia, ammonium isobutyrate, or propionic-acetic acid. According to standard techniques, 139 strains were extensively characterized and 207 additional strains were partially characterized. On the basis of these characterizations, the streptomycete strains were identified by both the systems of Pridham et al. and Hütter because these systems are rapid and accurate. In general, only Streptomyces griseus (Krainsky) Waksman and Henrici was isolated from high-moisture whole corn (treated or untreated) except from grain exposed to ammonium isobutyrate. Strains isolated from high-moisture corn subjected to that treatment represented both S. griseus and S. albus (Rossi Doria) Waksman and Henrici. The strains isolated from corn and corn products from the three mills were identified with a number of streptomycete species. Of all Actinomycetales isolated, only three were not streptomycetes—two from brewer’s grits and one from break flour.

Mesophilic Actinomycetales are present in agricultural grains, residues, and commodities. Streptomyces albus (Rossi Doria) Waksman and Henrici, the type strain for the genus Streptomyces Waksman and Henrici, reportedly was isolated from straw in Prague about 1900 (4). This species also was isolated by Graves et al. (2) along with S. griseus (Krainsky) Waksman and Henrici from wheat and wheat flour. Mehrotra (7) reported isolating more than a thousand streptomycetes per gram of wheat grains. He found that S. albus (probably S. griseus) by the Pridham et al. (11) classification was in about 47% of all samples tested. He also reported finding only a few Actinomycetales in other grains (rice, barley, and millet). So far as we know, there have been no reports on the identity of Actinomycetales isolated from corn.

Bothast and co-workers (1) have studied the microbiology of corn products. Total aerobic bacterial, mold, and Actinomycetales populations were determined for samples of whole corn, brewer’s grits, and break flour supplied by three different mills. During their study, 190 strains of mesophilic Actinomycetales were selected and were isolated by dilution plating with Difco plate count agar, containing 100 ppm of cycloheximide (Actidione), and then by incubating at 28°C for 14 days. In addition to these strains, one of us (R.F.R.) later isolated 155 more from high-moisture corn, Harvestore silo corn (untreated), and corn treated with ammonia, ammonium isobutyrate, or propionic-acetic acid.

Because corn is an economically important crop in the United States and its production, harvesting, storage, and processing involve many aspects of applied microbiology, it was believed that information on the identities of the 345 strains of Actinomycetales would be of interest. The purpose of this study, then, was to determine the identities of these isolates using the most recent acceptable criteria for characterization and identification. All 345 strains were characterized and identifications were made according to the Pridham et al. (10, 11) and Hütter (3) systems and keys (Table 1), because these two keys are the most practical ones extant and can lead to rapid identifications.

(This paper was presented at the Annual Meeting of the American Society for Microbiology, 12 to 17 May, Chicago, Illinois.)

MATERIALS AND METHODS

Strains. The Actinomycetales studied were isolated and purified from treated and untreated corn and corn fractions. All are maintained at the Northern Regional Research Laboratory as yeast extract-agar slant cultures (8) and as lyophilized preparations. Liquid inocula for all media needed for charac-
terizations were prepared with tryptone-glucose-liver extract-yeast extract broth as described by Lyons and Pridham (5).

Characterization. Strains were characterized according to methods outlined by Pridham et al. (5, 6, 9, 10). These methods allow use of both the Pridham et al. and Hütter keys.

Strains isolated and purified from samples from mill I were extensively characterized by the following criteria: spore-wall ornamentation; spore-chain morphology; ability to produce melanin pigments and to darken peptone-iron agar; color of aerial mycelium; ability to utilize D-glucose, D-arabinose, and L-rhamnose; amount of growth on Czapek's sucrose agar; ability to produce antibiotics; and the identity of the isomer of diaminopimelic acid (DAP) in whole cell hydrolysates. Also, strains isolated from the 1972 high-moisture field corn crop (untreated), Harvestore silo corn, and ammonia-treated corn were characterized according to these same criteria. All strains other than those from mill I were characterized only to the extent that identities could be made using the Pridham et al. key and the Hütter key. Criteria for these characterizations included only the first five mentioned above.

DAP analyses. Whole cell hydrolysates were analyzed for DAP isomer by the methods described by Pridham and Lyons (10), except that the 72-h broth cultures were autoclaved for 25 min at 121°C before the whole cells were separated by filtering through Whatman no. 54 filter paper with a Buchner funnel. The cells were washed on the funnel with distilled water and then dried with 96% ethanol. They then were scraped from the filter paper and air-dried on a watch glass. The resulting products were stored at −20°C until analyzed.

Identification of streptomycetes. All strains having L-DAP were identified by both the Pridham et al. key (10) and the Hütter key (3). Strains having meso-DAP were not identified because they are not considered to be streptomycetes.

RESULTS

The 15 different species of streptomycetes identified in this study were: *Streptomyces albicus*, *S. collinus* Lindenbein, *S. diastaticus* (Krainsky) Waksman and Henrici, *S. exfoliatus* (Waksman and Curtis) Waksman and Henrici, *S. flavofuscus* (Waksman) Waksman and Henrici, *S. flavidovirens* (Kudrina) Pridham et al., *S. fradiae* (Waksman and Curtis) Waksman and Henrici, *S. griseoflavus* (Krainsky) Waksman and Henrici, *S. griseus*, *S. longisporoflavus* Waksman in Waksman and Lechevalier, *S. michiganensis* Corbaz et al., *S. ramulosus* Ettinger et al., *S. violaceus* (Rossi Doria) Waksman in Waksman and Lechevalier, *S. violaceus-niger* (Waksman and Curtis) Pridham et al.,
and S. violaceus-ruber (Waksman and Curtis) Pridham.

The identities and extent of occurrence of the strains isolated from corn (whole and milled) from the mills appear in Table 1. The identities of strains from high-moisture field corn and the extent of their occurrence are given in Table 2.

About 35% of the 345 strains exhibited the formation of curious bodies (small clumps of cells) which we termed “soft sclerotia” in the

Table 2. Identification and incidence of Streptomyces isolated from high-moisture corn from sources other than mills

| Source                        | No. of strains | No. of strains identified as* | No. of strains identified as† | S. griseus | S. albus | S. griseus | S. albus | S. exfoliatus | S. violaceus | S. colli | S. mitchiganensis |
|-------------------------------|----------------|--------------------------------|--------------------------------|------------|---------|------------|---------|---------------|-------------|---------|---------------------|
| Untreated field corn (27% moisture) | 19             | 19                             | 19                             |            |         |            |         |               |             |         |                     |
| Barrel stored corn†           | 5              | 5                              | 3                              | 2          |         |            |         |               |             |         |                     |
| Harvested corn                | 10             | 10                             | 10                             |            |         |            |         |               |             |         |                     |
| Ammonia-treated corn          | 49             | 46                             | 46                             | 1          | 1       | 1          |        |               |             |         |                     |
| Ammonium isobutyrate-treated corn | 67             | 42                             | 25                             | 41         | 25      | 1          | 1       |               |             |         |                     |
| Propionic-acetic acid-treated corn | 5              | 5                              | 5                              |            |         |            |         |               |             |         |                     |

* Identification made according to taxonomic key of Pridham et al. (10, 11).
† Identification made according to taxonomic key of Hütter (3).
‡ Whole field corn (high moisture) stored in a barrel (untreated).

![FIG. 1. Soft sclerotia formed by S. griseus isolated from corn after 14 days at 28 C on a yeast extract agar petri dish culture (ca. 300×).](image-url)
aerial mycelium formed on yeast extract agar and glycerol-asparagine agar (Fig. 1). The soft sclerotia also seem to combine as larger masses over the entire mycelium of more heavily growing areas and sometimes resemble the exudate that forms in hygroscopic cultures. Although this phenomenon usually was associated with Rectiflexibles type cultures, we did find three strains of the Spiralis type that also exhibited this characteristic.

Only 197 of the 345 strains were tested for antibiotic activity; 95% of these strains showed activity. Although some of this activity was only minimal, it was readily detected by a simple spectrum-dish test (6). Cross-antagonism studies on the basis of a similar technique and a streptomycin-producing strain were carried out with 62 strains. The results suggest that none of the 62 produce streptomycin.

Only four strains of the 345 tested darkened peptone-iron agar, i.e., positive chromogenicity. Of that many strains randomly selected, 80 to 90 would be expected to be chromogenic (25 to 30%) based on our experience. Of the 345 strains, only one had spiny sporewalls and one had hairy walls; the rest were smooth walled. Again, based on the random selection techniques used, at least 5% of the strains would be expected to be other than smooth.

DISCUSSION

A variety of species of streptomycetes were isolated from corn and corn fractions supplied by three different mills. In contrast, Streptomyces isolated from whole high-moisture field corn (not grain from the mills) stored treated and untreated varied little. The results suggest that floristic changes occur sometime after storage and during processing. Strains isolated from mill samples showed in common: non-chromogenic, smooth spore-wall ornamentation, the soft sclerotia, and antibiotic activity. They differed in spore-chain morphologies and color of the aerial mycelium. Almost all the strains isolated from the various treatments of the high-moisture field corn, with the exception of the ammonium isobutyrate treatment, were identified as S. griseus with the Pridham et al. system (10, 11). In addition to the strains of S. griseus, strains of S. albus also were isolated from the ammonium isobutyrate-treated, whole high-moisture field corn. These were not isolated, however, until 24 days after treatment. Only strains of S. griseus were isolated from this sample for the first 12 days; but then that species was no longer detected, possibly because of the ammonium isobutyrate treatment. After that time only strains of S. albus were detected. None of the other treatments had this effect on high-moisture whole corn samples.

Although 95% of the 197 strains tested produced antibiotics in laboratory tests, we know of no studies wherein whole corn or milled fractions have been tested for the presence of antibiotics produced as a consequence of the metabolic activities of Actinomycetales in corn or its milled products. Our interest in antibiotic production is concerned with its use in more precise characterization of strains through identification of the antibiotics produced.

ACKNOWLEDGMENTS

We are indebted to B. Phrompatima, an Agency for International Development scholarship trainee, Department of Health, Bangkok, Thailand, for technical assistance during this study, and to C. P. Kurtzmann (Northern Regional Research Laboratory) for the photomicrograph.

LITERATURE CITED

1. Bothast, R. J., R. F. Rogers, and C. W. Hesseltine. 1973. Microbial survey of corn in 1970-71. Cereal Sci. Today 18:22-24.
2. Graves, R. R., R. F. Rogers, A. J. Lyons, Jr., and C. W. Hesseltine. 1967. Bacterial and actinomycete flora of Kansas-Nebraska and Pacific Northwest wheat and wheat flour. Cereal Chem. 44:288-290.
3. Hütt, R. 1967. Systematik der Streptomyzeten unter berücksichtigung der von ihnen gebildeten Antibiotica. In Bibliotheca Microbiologica, Fasc. 6. S. Karger, Basel, Switzerland.
4. Lyons, A. J., Jr., and T. G. Pridham. 1962. Proposal to designate strain ATCC 3004 (IMRU 3004) as the neotype strain of Streptomyces albus (Rossi-Doria) Waksamian and Henrici. J. Bacteriol. 83:370-380.
5. Lyons, A. J., and T. G. Pridham. 1965. Colorimetric determination of color of aerial mycelium of streptomycetes. J. Bacteriol. 89:159-169.
6. Lyons, A. J., and T. G. Pridham. 1971. Streptomycetes torulosus sp. n., an unusual knobby-spored taxon. Appl. Microbiol. 22:190-193.
7. Mehrotra, B. S. 1972. Investigations of selected microorganisms in cereal flours of India, to provide basic information related to the utilization of cereal grains in foods and feedstuffs. In 3rd annual report, U.S. Department of Agriculture, PL-480 Project UR-AT-140-179. Grant no. FG-In-410.
8. Pridham, T. G., P. Anderson, C. Foley, L. A. Lindfelsen, C. W. Hesseltine, and R. G. Benedict. 1957. A selection of media for maintenance and taxonomic study of Streptomyces. Antibiot. Annu. 1956-57:947-963.
9. Pridham, T. G., C. W. Hesseltine, and R. G. Benedict. 1958. A guide for classification of streptomycetes according to selected groups. Placement of strains in morphological sections. Appl. Microbiol. 6:52-79.
10. Pridham, T. G., and A. J. Lyons. 1969. Progress in clarification of the taxonomic and nomenclatural status of some problem actinomycetes. Develop. Ind. Microbiol. 10:183-221.
11. Pridham, T. G., A. J. Lyons, Jr., and H. L. Seckinger. 1965. Comparison of some dried holotype and neotype specimens of streptomycetes with their living counterparts. Int. Bull. Bacteriol. Nomencl. Taxon. 18(4):191-237.