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SOURCES OF AMYLASE-PRODUCING BACTERIA[1]

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In the search for bacteria possessing high amylolytic activity, some thousand or more isolates were screened on soluble starch agar and the extent of their activities determined qualitatively by the loss of medium response to Lugol's iodine solution. The selected isolates were then grown on a liquid wheat bran medium and the resulting starch-dextrinizing activity measured quantitatively. No attempt was made to determine the properties of the various amylases. The primary purpose of this report is to point out the original sources from which the isolates were made and to correlate the nature of the substrates with the extent of amylase production by the bacteria.

METHODS

Preliminary testing on starch agar. A loopful of a 24-hour-old peptone beef extract broth suspension of each isolate was spotted at each of three points on the surface of a soluble starch agar plate (2 g Lintner soluble starch per liter of peptone beef extract agar). After 48 hours' incubation at 30 C the plates were flooded with Lugol's iodine solution (1 g iodine and 2 g potassium iodide made up to a volume of 300 ml with water), the excess poured off after several minutes, and the diameter of the cleared zone around the bacterial colony noted. All isolates showing a wide, cleared zone (approximately 10 to 15 mm) were retained for further culturing in a wheat bran medium.

Preparation and inoculation of wheat bran medium. Experience with a variety of media indicated that the evaluation of amylase activity was most comparable and consistent on a wheat bran medium prepared as follows: 50 grams of wheat bran per liter of distilled water was autoclaved (30 minutes at 121 C) and strained through a fine cheesecloth. To one liter of the strained bran extract were added 10 grams peptone (bacto) and 2 ml of phosphate buffer (15 g KH_2PO_4 and 35 g $K_2HO_4 \cdot 3H_2O$ per 100 ml distilled water). This medium was dispensed in 200-ml amounts into glass-covered preparation dishes (diameter 100 mm, depth 50 mm) so that the liquid depth was 2.5 cm (Beckord, Peltier, and Kneen, 1945) and autoclaved for 20 minutes at 121 C. All stock cultures were carried on wheat bran agar slants made by adding 20 grams of agar per liter of wheat bran medium.

Each dish was inoculated with a loopful from a 24-hour wheat bran agar slant and allowed to incubate (5 days at 35 C) quiescently in order to facilitate pellicle formation (Beckord, Peltier, and Kneen, 1945). The amylase-producing capacity of each isolate was desired in this investigation. Accordingly, dextrinizing activity, which can be determined by a convenient method in the laboratory,

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was selected as the criterion by which this particular property was to be ascertained.

Evaluation of amylase activity. After incubation, a 10-ml aliquot of the medium was withdrawn from below the pellicle and tested directly for dextrinizing activity by a method described previously (Beckord, Kneen, and Lewis, 1945). Amylase activity is expressed in terms of "dextrinization time," namely, the time in minutes required by 10 ml of wheat bran medium to convert 20 ml of

TABLE 1

Dextrinizing activity of liquid wheat bran cultures of 265 bacterial isolates hydrolyzing starch agar

Incubation: 35 C, 5 days

SOURCE OF ISOLATION	ISOLATES HYDROLYZING STARCH AGAR NO.	"DEXTRINIZATION TIME" OF WHEAT BRAN CULTURE MEDIUM				
		1–10 min	11–20 min	21–30 min	31–60 min	60+ min
Manures*	42	1	0	0	0	41
Composts	26	0	0	0	0	26
Sludges	16	0	0	0	0	16
Soils	17	1	0	1	1	14
Fish meal	2	1	1	0	0	0
Soybeans	8	1	3	0	0	4
Peanuts	7	1	3	1	0	2
Beet pulp	2	0	0	0	0	2
Flours	7	3	0	1	0	3
Starches	7	5	0	0	0	2
Thin stillage	8	2	5	0	0	1
Bread (ropy)	17	17	0	0	0	0
Laboratory contaminants	2	1	1	0	0	0
Air (dust)†	60	4	11	2	3	40
Insects‡	44	0	1	0	0	43
Totals	265	37	25	5	4	194

* Organisms isolated from the first 13 substrates by Lillian Wind, Mildred Penner, and Irma Tingelhoff, Dept. of Bacteriology, University of Nebraska.

† From the collection of Floyd Schroeder, Preparator, Dept. of Bacteriology, University of Nebraska.

‡ From the collection of Dr. Edward A. Steinhaus, Rocky Mountain Laboratory, Hamilton, Montana.

1 per cent boiled starch (at 30 C and pH 6) to the point where the "red-brown" color is given with iodine. Occasionally certain cultures produced so much amylase that it was necessary to use an aliquot smaller than 10 ml. Since the time required for dextrinization is inversely proportional to the amount of enzyme present, the dextrinization time for a 10-ml aliquot could be calculated. If the "end point" was not reached by one hour's action of a 10-ml aliquot, the activity was recorded as 60^+ minutes.

RESULTS

The results are presented in the data of table 1. Of the 1,000 or more organisms isolated, 265 hydrolyzed starch agar and were cultured further in the wheat

bran medium. Thirty-seven of the 265 isolates had dextrinization times of 10 minutes or less and were considered highly active. The majority of the cultures were considered inactive since 194 gave a dextrinization time of more than 60 minutes.

Manures, composts, sludges, soils, and insects were poor natural sources. Of 145 organisms isolated from these materials, 2 were active (1 to 10 minutes), 1 was fairly active (11 to 20 minutes), and the remainder were inactive. Ropy bread consistently yielded high amylase producers. From 4 different samples of ropy bread 17 isolations were made, all of which were very active. In fact, some of these isolates gave activities of less than 1 minute. Since one-fourth of the 60 organisms from the air (isolated during July and August) were either active or fairly active, air was one of the relatively good sources.

In many instances, only a small number of isolations were made, so that the data were not considered sufficient to draw any definite conclusions concerning a particular source, but they do give an indication of the potentialities of the source. It would appear that fish meal, soybeans, peanuts, flours, starches, and thin stillage were good sources since a relatively high percentage of the isolates from these were amylase producers. Neither of the 2 isolates from beet pulp were active.

From the data in table 1, the inference is gained that some sources yield amylase-producing bacteria in greater numbers than others. The sources investigated may be rated in decreasing activity as follows: (1) ropy bread, (2) starches and flours, (3) thin stillage, (4) soybeans and peanuts, and (5) air. Since there was apparently a lack of amylase-producing organisms in manures, composts, sludges, and soils, the active bacteria from the air may have had their origin, principally, from plant materials rather than decomposed plant and animal residues in the soil.

Isolates showing superior amylase production gave the following preliminary characterization: gram-positive sporeforming bacilli, approximately one micron in length, with central spores usually smaller than the cell. Agar colonies were either small, smooth, glistening, and round, or intermediate in size, flat, rough, and irregular, or sometimes rather large, spreading, and mucoid. The great majority of isolates fermented glucose and sucrose with the production of acid. Lactose was not fermented. Most of them liquefied gelatin completely within 72 hours. Provisionally, they all appear to fit into the *Bacillus subtilis* group.

SUMMARY

The members of a recent collection of some 1,000 or more bacterial isolates from a variety of different natural sources were grown on starch agar plates and the zones of starch hydrolysis noted.

Of this number, 265 hydrolyzed starch and were further cultured in a liquid wheat bran medium. The amount of amylase produced was determined from the dextrinizing activity of the culture medium.

Of the 265 selected isolates grown in the wheat bran medium, 194 gave a dextrinization time of 60^+ minutes, whereas thirty-seven yielded the dextrini-

zation time of 1 to 10 minutes; twenty-five, 11 to 20 minutes; five, 21 to 30 minutes; and four, 31 to 60 minutes, respectively.

Manures, composts, sludges, soils, and insects were extremely poor sources. Ropy bread, flour, starches, and other plant materials were excellent to fair sources. Exposure of plates to the air was fairly effective in picking up amylase-producing bacteria.

A preliminary characterization of the high-amylase-producing isolates placed them in the *Bacillus subtilis* group.

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