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Selective Isolation of Acidophilic *Streptomyces* Strains for Glucose Isomerase Production

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Approximately 260 *Streptomyces* strains were isolated from neutral pH farmland soil and evaluated for their ability to produce glucose isomerase. The number of acidophilic *Streptomyces* organisms growing at pH 4.0 was low, i.e., 10^3 organisms per g of soil. All of the isolates showed glucose isomerase activity when they were grown in a medium containing D-xylose, an inducer for glucose isomerase. More than half of the strains tested developed heavy growth in 24 h, and many produced high titers of glucose isomerase after 24 h of growth in a medium buffered at pH 5.0.

Glucose isomerase is used commercially to convert glucose into fructose. Since Takasaki (9) first isolated a xylan-assimilating *Streptomyces* species for the industrial production of glucose isomerase, research workers have searched for better *Streptomyces* strains for glucose isomerase production (1, 10).

Recently (4), an attempt was made to isolate glucose isomerase-producing *Streptomyces* strains from different types of soil. It was found that cornfield soil and corn compost were good sources of streptomycetes that produce glucose isomerase.

Most of the *Streptomyces* strains studied grow in the neutral pH range, which is consistent with the optimum pH range of 6.5 to 8.0 for streptomycetes (7).

Acidophilic streptomycetes are, however, widespread in nature. Jensen (3) first isolated a group of closely related acidophilic streptomycetes designated *Streptomyces acidophilus*. Khan and Williams (5) studied many acidophilic streptomycetes isolated from different acidic soil samples. They were able to isolate 174 different strains of acidophilic streptomycetes, distinguished by their color and colony morphology. The approximate numbers of acidophilic streptomycetes in soil ranged from 10^3 to 10^6 organisms per g of dry soil. The acidophilic streptomycetes differed in some physiological properties from the neutrophilic strains. Recently, the wide occurrence of acidophilic streptomycetes in acid soil was demonstrated again by Hagedorn (2).

Although acidophilic streptomycetes occur widely in nature, this group has not been investigated as a source of glucose isomerase. Recently, such an attempt was reported by Bok et al. (S. H. Bok, L. E. Jackson, C. J. Schroedel, and M. Seidman, U.S. patent 4,399,222, August 1983). In the present work, we report the screening and isolation of many acidophilic streptomycetes from soil and the production of glucose isomerase by these soil isolates.

MATERIALS AND METHODS

Media. The medium used for isolation of streptomycetes contained (per liter of distilled water): starch, 10 g; KNO_3, 2 g; K_2HPO_4, 2 g; NaCl, 2 g; $MgSO_4 \cdot 7H_2O$, 0.05 g; $CaCO_3$, 0.02 g; $FeSO_4 \cdot 7H_2O$, 0.01 g; and Bacto-Agar (Difco Laboratories), 20 g. The pH of the medium was adjusted to 4.0 with 1 N HCl. Filter-sterilized nystatin (Sigma Chemical Co.) was added at 100 μg/ml to suppress the growth of fungi.

The medium used for maintenance and sporulation of *Streptomyces* isolates contained (per liter of distilled water): malt extract, 3 g; yeast extract, 4 g; NaCl, 5 g; $MgSO_4 \cdot 7H_2O$, 0.5 g; Bacto-Agar, 15 g; and D-xylose, 10 g. D-Xylose was sterilized separately and added later. The pH of the medium was adjusted to 5.0.

The medium used for the primary and secondary screening for glucose isomerase production contained (per liter of distilled water): corn steep liquor solids, 25 g; $(NH_4)_2SO_4$, 5 g; K_2HPO_4, 5 g; $MgSO_4 \cdot 7H_2O$, 0.5 g; NaCl, 5 g; citric acid, 2 g; and D-xylose, 10 g. D-Xylose was autoclaved separately and added later. The pH of the medium was adjusted to 5.0 with 1 N HCl.

Isolation of *Streptomyces* strains. All *Streptomyces* strains were isolated from cornfield soil in Decatur, Ill. Soil samples were diluted in sterile distilled water and spread on the isolation medium. Powdery-surfaced, leathery colonies with concentric rings were recognized as *Streptomyces* strains after 5 to 7 days of incubation at 30°C. These colonies were picked and purified by restreaking on the same agar medium. Purified cultures were grown on the maintenance medium and kept at 4°C.

Primary screening. To evaluate the potential of individual soil isolates for glucose isomerase production, two loopfuls of spores were inoculated into test tubes (1.8 by 15 cm) containing 5 ml of sterile screening medium. All test tubes were shaken on a Gyrotory shaker (model G-25; New Brunswick Scientific Co.) at 250 rpm and 30°C for 24 h. Each culture broth was then assayed for glucose isomerase activity.

Secondary screening. Several higher-yielding glucose isomerase producers were selected from the primary screening. Spores of these strains were collected from a solid agar plate and introduced into 100 ml of screening medium in 500-ml bottom-baffle Erlenmeyer flasks. All flasks were shaken on a G-24 Gyrotory shaker (New Brunswick Scientific Co.), at 450 rpm and 30°C for 24 h. Cells were then collected by centrifugation for 10 min at 16,000 × *g*. Cell pellets were washed once more in distilled water by centrifugation. Washed cells were freeze-dried and assayed for glucose isomerase activity.

Enzyme assay. For primary screening, 0.5 ml of culture broth containing cells were transferred into 5 ml of 1% glucose-maleate buffer-salts solution (maleic acid, 11.6 g;

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TABLE 1. Glucose isomerase production by acidophilic soil *Streptomyces* isolates in the primary screening test

Glucose isomerase activity (optical density at 480 nm/0.5 ml of broth)	Total no. of soil isolates
0.1–0.2	26
0.2–0.3	66
0.3–0.4	90
0.4–0.5	46
0.5–0.6	20
0.6–0.7	8

$MgSO_4 \cdot 7H_2O$, 0.5 g; $CoCl_2 \cdot 6H_2O$, 0.03 g; and 0.2 M NaOH, 1,000 ml; pH adjusted to 6.60) and incubated for 60 min in a 65°C water bath. The mixture was then cooled in a cold water bath, and 0.2 ml was transferred into test tubes containing 1.8 ml of water. The amount of fructose which had been produced was determined by the method of Kulka (6) with a Spectronic 70 colorimeter (Bausch & Lomb, Inc.). The enzyme activity was expressed as the optical density at 480 nm per 0.5 ml of culture broth.

For secondary screening, 25 mg of dry cells was incubated with 5 ml of 30% glucose-maleate buffer-salts solution for 60 min in a 65°C water bath. The reaction was terminated by cooling in a cold water bath and by adding 0.1 ml of 4 N HCl. The sample was boiled for 5 min, cooled, desalted with ion-exchange resins, and centrifuged. The supernatant solution was passed through a computer-programmed liquid chromatograph (8) (Waters Associates) to determine the fructose concentration. One unit of glucose isomerase activity was defined as that amount of enzyme which produces 1 μmol of fructose per min at pH 6.60 and 65°C.

RESULTS

The primary screening assay data (Table 1) show that all of the acidophilic *Streptomyces* strains produce glucose isomerase.

The growth rate of acidophilic *Streptomyces* strains in the screening medium was relatively high. More than half of the isolates grew heavily (5 to 15 g of dry cells per liter) in 24 h at pH 5.0 in the screening medium. After growth the pH reached ca. 7.0. At pH 4.0, they grew well on solid agar medium, but they did not grow rapidly in the screening medium.

Some of the strains that grew rapidly and produced high titers of glucose isomerase in the primary screening test were evaluated further in shake flask fermentations. The dry cell weights ranged between 9 and 14 g/liter. All of the strains tested produced 3,000 to 5,000 U of glucose isomerase per liter (Table 2). Soil isolate no. 93, which was one of the best strains in the primary screening assay, produced the highest glucose isomerase titer, ca. 5,000 U/liter, and the highest specific glucose isomerase activity, 530 U/g of dry cells.

TABLE 2. Glucose isomerase production in shake flask fermentation by acidophilic *Streptomyces* isolates in the secondary screening test

Soil isolate strain no.	Dry cell wt (g/liter)	Volumetric glucose isomerase production (U/liter)	Specific glucose isomerase production (U/g)
36	13.6	4,600	340
59	9.5	3,900	410
74	10.1	4,100	400
93[a]	9.5	5,000	530
102	11.0	4,800	430
103	11.9	3,600	310
135	9.5	4,200	450
143	12.2	4,200	340

[a] Deposited as NRRL 11496 (Bok et al., U.S. patent 4,399,222, August 1983).

DISCUSSION

The present study was undertaken to isolate acidophilic *Streptomyces* strains and to evaluate their ability to produce glucose isomerase. Neutral farmland soil was used to isolate acidophilic *Streptomyces* strains because acidic soils were not available readily in Decatur, Ill. The number of such organisms in neutral pH soil was low, i.e., 10^3 organisms per g of soil. Although the wide occurrence of acidophilic actinomycetes in acidic soil is well known (2, 5, 11), their occurrence in neutral pH soil has not been reported. The present work indicates that acidophilic *Streptomyces* strains occur in neutral pH soil as well as in acidic soil.

Many acidophilic *Streptomyces* strains can grow rapidly in an acidic growth medium, producing a high cell density. We found some strains to produce 12 to 14 g of dry cell mass per liter of medium in 24 h. This is a very desirable characteristic for industrial glucose isomerase production, since the enzyme is intracellular and therefore high cell mass usually is equivalent to high enzyme yield. An acidic fermentation process may have some advantage, in that contaminants are less likely to grow.

Our secondary screening data show that acidophilic *Streptomyces* strains can produce high specific glucose isomerase titers in wild-type cells. Some of the strains produced up to 5,000 U of glucose isomerase per liter at pH 6.60 and 65°C (equivalent to ca. 10,000 U/liter at pH 7.0 and 75°C).

Commercial glucose isomerase titers have been reported to be around 35,000 U/liter at pH 7.0 and 75°C (1). However, these commercial titers were obtained only after years of strain improvement and fermentation process optimization. Our study indicates that acidophilic *Streptomyces* strains have great potential as sources of glucose isomerase.

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