0273-2289/91/3102—0159$02.00

Increased Production of Thermostable α-Amylase Enzyme by *Bacillus* sp. TCRDC-25A with Maltodextrins

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Received April 26, 1991; Accepted May 31, 1991

ABSTRACT

Maltodextrins and hydrolysates of rice and corn flour of varying dextrose equivalents (DE) have been used as a carbon source for α-amylase enzyme production by *Bacillus* sp. TCRDC-25A. The rate and total enzyme production was higher in maltodextrin media than in cornstarch. The enzyme production increased with increase in DE up to 45%. The maximum enzyme production of 2390, 2450, and 2510 DUN/mL was obtained in cornstarch maltodextrins, and hydrolysates of corn and rice flours, respectively, in a bench-scale reactor in 40 h.

Index Entries: α-Amylase; maltodextrins; *Bacillus* sp. TCRDC-25A; enzyme production.

INTRODUCTION

The genus *Bacillus* produces a large variety of extracellular enzymes, some of which, such as amylases and proteases, are of significant industrial importance. Among these enzymes, the thermostable variety are more versatile with respect to industrial significance. The enzyme α-amylase, a hydrolytic enzyme, is instrumental in a mild chain hydrolysis of α 1–4 glucosidic bonds of the two prevalent forms of starch amylase and amylopectin to produce maltose, maltotriose, and α-dextrin. This enzyme has

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extensive commercial applications in starch liquefaction, brewing, and desizing in textile industries and paper and detergent manufacturing processes.

In our laboratory, attempts are being made to develop a process expertise for producing thermostable α-amylase enzyme (*1–7*). We have recently reported about a *Bacillus* isolate TCRDC-25A, which appears to have potential as a production strain to produce thermostable α-amylase enzyme (*5*). The enzyme was found to be fairly active up to 95°C, demonstrating optimum activity at 85°C. Preliminary studies in our laboratory have indicated that α-amylase production by *Bacillus* sp. TCRDC-25A can be increased considerably by using maltodextrins as a carbon source in place of starch. Maltodextrins are hydrolysis products of starch having a dextrose equivalent of 20 or more. In this paper, we report the production of α-amylase enzyme from maltodextrins by *Bacillus* sp. TCRDC-25A. This study was aimed at developing a medium containing high enzyme activity per unit volume of the fermentation broth.

MATERIAL AND METHODS

Microorganism and Culture Conditions

Bacillus sp. TCRDC-25A described earlier (*5*) was used in the present study. The strain was maintained at 4°C on 3% agar slants containing medium of the following composition: 1% yeast extract, 0.2% peptone, 0.05% $MgSO_4$, 0.05% KH_2PO_4, 0.15% NaCl, 0.015% $CaCl_2$. The media for enzyme production contained 1–5% (w/v) cornstarch or maltodextrins of cornstarch, hydrolysates of rice or corn flour of varying DE (dextrose equivalent), 2% defatted soya flour (DSF), 0.05% $MgSO_4$, 0.05% KH_2PO_4, 0.15% NaCl, and 0.015% $CaCl_2$. Maltodextrins were prepared by liquefying cornstarch, rice, or corn flour with bacterial α-amylase enzyme (60 U/g of starch) at a temperature of 85°C and at pH 6.0 for different lengths of time.

Enzyme production was carried out by growing the organism in various liquid media. Medium (30 mL) was placed in 250-mL Erlenmeyer flasks and inoculated wth one loopful of culture from a slant. Fermentation was continued at 32°C for 50–70 h in an orbital shaker at 300 rpm. The enzyme production was also studied in a 2.6-L bench-scale reactor. The aeration rate was maintained at 1.5 vvm and the agitation speed was kept constant at 500 rpm. Mineral oil was used to minimize foam formation.

Analytical Procedure

Before enzyme assay, the cells and other solid particles were separated by centrifugation. The clear supernatant was used as crude enzyme preparation. Extracellular α-amylase activity was determined in duplicate by

Table 1
Comparison of α-Amylase Production
in Corn Starch and Corn-Starch Maltodextrin Media[a]

Carbon source	Enzyme activity (DUN/mL) in fermentation time (h)					Cell conc., g dry wt/L	Final pH
	24	40	50	72	84		
Corn starch, 2%	150	302	414	608	608	5.89	8.27
Maltodextrins, 2%	670	810	1050	1050	-	5.76	8.30

[a]The medium contained 2% defatted soya flour, 0.05%, KH_2PO_4, 0.05% $MgSO_4 \cdot 7H_2O$, 0.15% NaCl, 0.01% $CaCl_2$, (pH 7.0), and 2% carbon source.

measuring the decrease in iodine color reaction showing dextrinization of starch (*1,2*). The reaction mixture contained 1 mL of enzyme and 10 mL of 1% starch solution (pH 6.0), which was incubated at 40°C for 10 min. The reaction was stopped by adding 10 mL of 0.1*N* HCl, and 1 mL of this acidified solution was added to 10 mL of 0.1*N* HCl. From this, 1 mL was added to 10 mL iodine solution (0.05% iodine in 0.5% KI). The optical density of the blue solution was determined at 660 nm. The same procedure was repeated using 1 mL of distilled water instead of the enzyme sample in order to measure the optical density without the enzyme. One unit of enzyme activity (DUN) is defined as the quantity of enzyme that causes 1% reduction of blue-color intensity of starch–iodine solution at 40°C in 1 min. The enzyme solution for analysis was diluted appropriately to bring the measured value within the linear range of the optical density. Growth was estimated in terms of dry weight of cells.

RESULTS AND DISCUSSION

Table 1 shows the production of α-amylase enzyme by *Bacillus* sp. TCRDC-25A in media containing 2% cornstarch and 2% cornstarch maltodextrins (DE = 20) as carbon sources. It may be noted that the growth was comparable in both media, but the rate and the total enzyme production was higher in maltodextrin medium as compared to cornstarch medium. About 1050 DUN/mL enzyme was produced in 50 h in maltodextrin medium as compared to 608 DUN/mL in 72 h with cornstarch medium. These data clearly indicate that maltodextrins are more effective than starch in inducing α-amylase production.

Maltodextrins were further studied at concentrations of 1–5% in order to determine their optimum level in the medium. It was found that enzyme production increased continuously with increased maltodextrin concentration in the medium up to 4%. However, at concentrations beyond 4%, there was no appreciable increase in enzyme production. The maximum

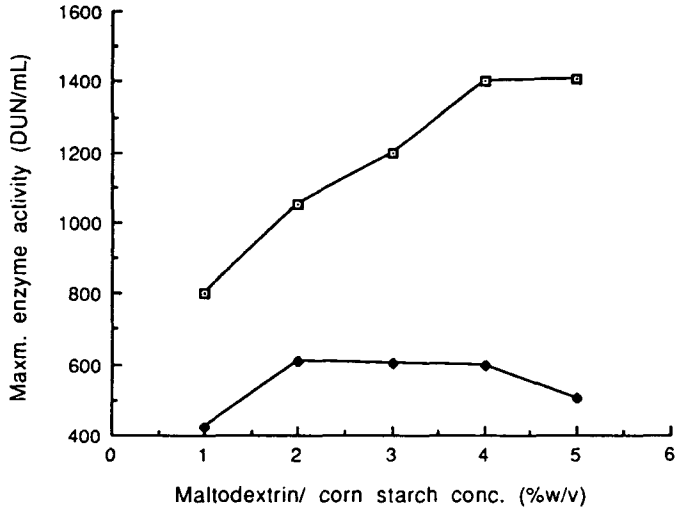

Fig. 1. Effect of substrate (maltodextrins [—⊡—] or corn starch [—◆—]) concentration on maximum enzyme activity.

enzyme activity obtainable with maltodextrins was 1400 DUN/mL (Fig. 1). The enzyme production as a function of cornstarch concentration has also been plotted for comparison in Fig. 1. In this case, 2% cornstarch was found to be the optimum concentration in the medium, which produced about 610 DUN/mL of enzyme.

The DE value of maltodextrins was varied to study its effect on enzyme production in 4% maltodextrin media. The enzyme activity in the fermentation broth increased from 1400 to 1615 DUN/mL with the increase of DE from 20 to 45% (Fig. 2). It is evident from these results that the maltodextrins of higher DE are more effective in α-amylase synthesis.

In place of cornstarch maltodextrins, the crude maltodextrins obtained by hydrolyzing the raw corn and rice flours were also tried as carbon sources for α-amylase production. The results obtained with hydrolysates of 5% rice and corn flours (about 80% starch) of different DE are shown in Fig. 3. The enzyme production was little higher in the media containing hydrolysates of rice and corn flours (HRF and HCF, respectively) than in media with cornstarch maltodextrins. Maximum enzyme activity in the fermentation broth was >1700 DUN/mL. This could be attributable to beneficial effect of proteins and other nutrients present in rice and corn flours.

The results of enzyme production in a bench-scale reactor with 4% cornstarch maltodextrins and hydrolysates of rice and corn flours (5% each) are compared in Fig. 4. As can be seen, the enzyme production rate in the reactor was higher than that in shake flasks. Also, the enzyme activities were much higher. This may be attributed to better aeration and agitation controls in the reactor. Maximum enzyme production was achieved in 40 h when the activities with cornstarch maltodextrins and hydrolysates of

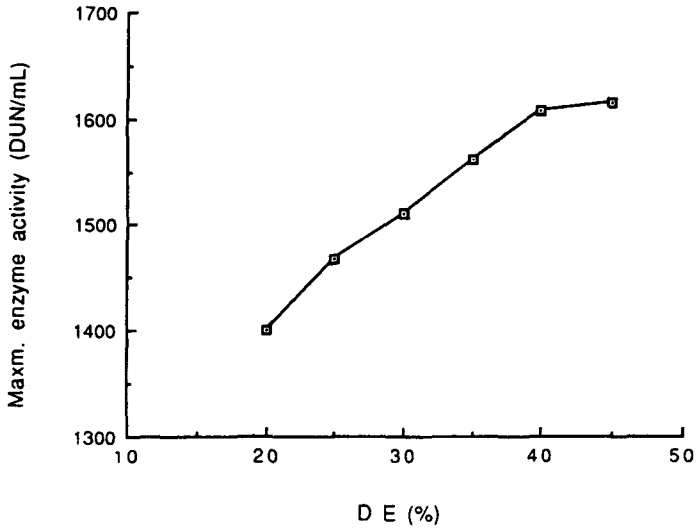

Fig. 2. Effect of DE on maltodextrins on maximum enzyme activity.

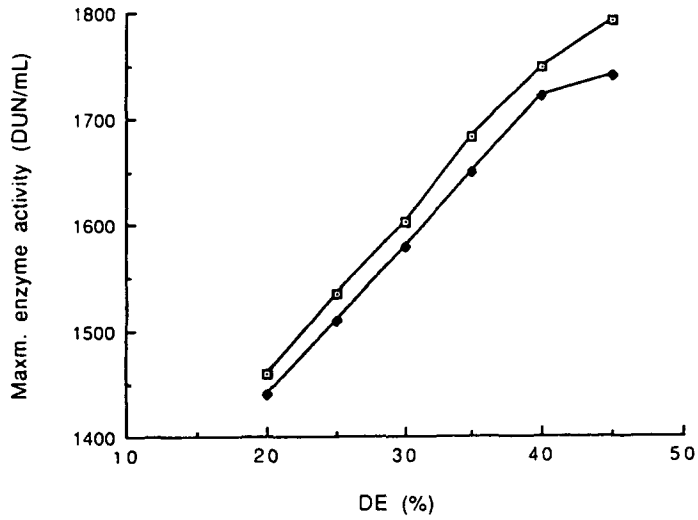

Fig. 3. Maximum enzyme activity as a function of DE in hydrolyzed rice-flour (HRF, —⊡—) and hydrolyzled corn flour (HCF, —◆—).

rice and corn flour media were 2390, 2510, and 2450 DUN/mL, respectively. These enzyme activities are equivalent to 67,090, 70,460, and 68,770 U/mL in the modified method of Fuwa (*8*), and are about twofold higher than that obtained with other media (*5*). However, a consideration of enzyme units by different laboratories does not always yield absolute comparison.

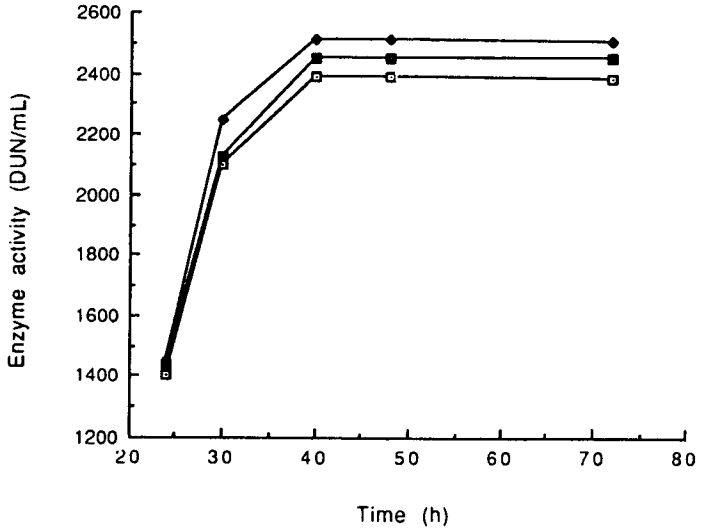

Fig. 4. α-amylase production from hydrolyzed corn starch (HCS or maltodextrins, —⊡—), HRF (—◆—), and HCF (—■—) in laboratory fermenter.

ACKNOWLEDGMENTS

The authors are grateful to the Director, Thapar Corporate R & D Centre, Patiala, India for permission to publish these results. The valuable assistance provided by M/S. Ravi Dutt and Karm Raj in carrying out the experiments is gratefully acknowledged.

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