Note

Cyclodextrin Glucanotransferase
from Alkalophilic Bacteria
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Studies on cyclodextrin glucanotransferase (CGTase) [EC. 2.4.1.19] from alkalophilic bacteria isolated in Japan were well summarized by Horikoshi *et al.* in "Alkalophilic Microorganisms."¹⁾ Recently, from some soil in Taipei, the authors succeeded in isolating a strain of alkalophilic bacterium capable of producing a sufficient amount of CGTase in the culture medium of high pH.

Of a collection of 300 strains of alkalophilic bacteria which form clear, transparent zones around colonies on modified Horikoshi medium-II²⁾ agar plates containing 2% corn starch (pH 10.3), one strain, No. HA3-3-2, was found to exhibit sufficient productivity of CGTase in the screening test. As shown in Table I, when compared as to the activity producing cyclodextrin (CD), the CGTase-productivity of strain No. HA3-3-2 was almost equal to that of *Bacillus* No. 17-1, one of the best CGTase-producing strains isolated by Horikoshi *et al.*³⁾

In this screening test, the CD-forming activity was directly determined as follows: The shaking-culture fluid (96 hr, 37°C) was centrifuged and the supernatant was diluted successively twice with 25 mM Borax buffer (pH 8.5) so as to obtain dilution stages of 2¹, 2², 2³, ..., 2ⁿ. Each 1 ml of the diluted enzyme solution was mixed with 5 ml of 2% soluble starch solution prepared in the same buffer and the mixture was kept at 55°C for the CD-forming reaction. After 48 hr, 2.5 ml of trichloroethylene (TCE) was added to the reaction mixture with violent shaking and the resulting suspension was kept at room temperature overnight. Then, abundant precipitates of CD-TCE complex were observed to have settled onto the bottom of the test tubes until some dilution stage, followed by a very poor yield of precipitates at the next stage. The difference of yields between the two neighboring stages was so obviously distinguishable that it appears easy to set limits for CGTase activity capable of producing sufficient amounts

of CD. Under the above conditions, the CD-forming activity can be expressed by the limit dilution stage (2ⁿ) and this method is called the "CD-TCE method" in this paper. Although the values obtained are semi-quantitative, the CD-TCE method is considered to be not only easy and simple but also direct and efficient for the screening of CGTase-producing bacteria. The starch-dextrinizing activity of CGTase was also determined according to the iodine method of Nakamura *et al.*⁴⁾; one unit of activity was defined as the activity producing a 10% reduction in the intensity of the blue color (700 nm) on 10 min reaction at 50°C and pH 8.5.

TABLE I. COMPARISON OF PRODUCTIVITY OF CYCLODEXTRIN GLUCANOTRANSFERASE BETWEEN *Bacillus* No. 17-1 AND No. HA3-3-2

Strain	Assayed by	
	CD-TCE method ^b	Iodine method ^c
<i>Bacillus</i> No. 17-1 ^a	2 ⁷ ~ 2 ⁸	1480
<i>Bacillus</i> No. HA3-3-2	2 ⁸	1060

^a Horikoshi's strain capable of producing a sufficient amount of CGTase.

^b Cyclodextrin-forming activity.

^c Starch-dextrinizing activity.

FIG. 1. Activity of CGTase from *Bacillus* No. HA3-3-2 at Different pHs.

Buffer system: Sodium citrate pH 3.8~5.8, sodium phosphate pH 5.8~7.0, Tris-HCl pH 7.0~9.0, sodium carbonate pH 9.0~10.7. Assay method: Iodine method (○—○, 50°C, 10 min), CD-TCE method (●---●, 55°C, 48 hr).

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FIG. 2. Stability of CGTase from *Bacillus* No. HA3-3-2 at Different pHs.

Buffer system: The same as in Fig. 1. The enzyme solution was kept at 25°C for 48 hr, at different pHs. After adjusting the pH to 8.5, the remaining activity was assayed by the iodine method (○—○) and the CD-TCE method (●---●). In the case of the iodine method, the initial activity was set as 100%. In the case of the CD-TCE method, the remaining activity is expressed as the dilution stage ($n: \ln 2^n$).

The strain, No. HA3-3-2, was an aerobic, spore-forming, gram prevailing positive, motile, rod-shaped bacterium, possessing peritrichous flagella, and considered to belong to the genus *Bacillus*. The CD produced by this strain was mostly the β -form.

The properties of the enzyme were also preliminarily examined, using the crude enzyme prepared as follows: The cell-free enzyme solution was mixed with ammonium sulfate to achieve 60% saturation and the resulting precipitates were collected by centrifugation, dissolved into 0.01 M Tris buffer (pH 8.5) and then dialyzed for 24 hr

against the same buffer. The yield of activity was almost 60% with the CD-TCE method.

The effect of pH on the enzyme activity is shown in Fig. 1. The crude enzyme displayed the CD-forming activity in a wide pH range of 5.5~9.5 and the optimum pH was around 6, when assayed by the CD-TCE method. The pH-activity curve obtained by the iodine method showed apparently a different figure from those of Horikoshi's CGTases.⁵⁾ The CGTase of *Bacillus* No. HA3-3-2 was observed to be fairly stable between pH 6~11 (Fig. 2). The optimum temperature was 55°C and the activity was stable below 60°C.

In conclusion, *Bacillus* No. HA3-3-2, an alkalophilic bacterium isolated from some soil in Taiwan, seems to be useful for the practical production of CGTase. This CGTase was found to be so active and stable in a wide pH range that it will also be available for industrial purposes.

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