Note

Preservation of Bacterial β -Amylase

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In the previous paper,¹⁾ the author reported that *Bacillus cereus* var. *mycoides* produces β -amylase and a kind of pullulanase. The β -amylase and pullulanase were purified to be homogeneous by disk electrophoresis, by starch adsorption, by ammonium sulfate fractionation and by Sephadex G-100 column chromatography.²⁾

Purified β -amylase lost activity remarkably during storage. As it was supposed that this loss was attributable to removal of a certain factor during purification, the effect of adding reagents was examined on preservation of enzyme activity during storage.

The cultivation of *Bacillus cereus* var. *mycoides* was carried out by the same method described in the previous paper.²⁾ The precipitate formed by the addition of two volumes of cold acetone to one volume of culture filtrate of bacterium was collected by centrifugation and dried. The activities of β -amylase and pullulanase of this enzyme preparation were 1.4×10^5 units and 1.0×10^4 units per *g*, respectively. This enzyme preparation maintained enzyme activities for at least two years.

One gram of this enzyme preparation was dissolved in 200 ml of distilled water and was centrifuged. Fifty grams of corn starch were added to the supernatant obtained and stirred overnight in a refrigerator. The starch was collected by filtration and washed with distilled water. β -Amylase adsorbed on starch was eluted with 10% maltose solution and dialyzed against distilled water. The β -amylase thus prepared (270 units/mg protein) was used in this experiment.

The β -amylase solution in 2×10^{-2} M phosphate buffer (pH 7.0) was kept for one to two months at 5°C in the presence of various metal ions or carbohydrates. (Phosphate buffer does not show inhibition against β -amylase reaction, while tris buffer shows a little inhibition. Therefore, although phosphate forms a precipitate with metal ions in storage, phosphate buffer was used throughout the experiment.) The activity of β -amylase was analyzed by using the procedure described in the previous paper.¹⁾

TABLE I. EFFECTS OF METAL IONS AND CARBOHYDRATES ON PRESERVATION OF β -AMYLASE OF *Bacillus* sp.

Material added	Concentration	Remaining β -amylase activity (%) ^a
None		9.3
Maltose	1%	2.4
Dextrin	1%	6.5
CaCl ₂	1×10^{-2} M	13.6
SrCl ₂	1×10^{-2} M	13.6
BaCl ₂	1×10^{-2} M	14.5
LiSO ₄	1×10^{-2} M	14.5
MgSO ₄	1×10^{-2} M	11.8
FeSO ₄	1×10^{-2} M	12.1
NiSO ₄	1×10^{-2} M	79.1
CoCl ₂	1×10^{-2} M	71.1
MnCl ₂	1×10^{-2} M	18.0
ZnSO ₄	1×10^{-2} M	67.0

^a $\frac{\text{ β -Amylase activity remaining after one month}}{\text{Initial β -amylase activity}} \times 100$

Four milliliters of enzyme solution containing about 260 units of β -amylase and 2×10^{-2} M phosphate buffer (pH 7.0) was kept for one month at 5°C.

TABLE II. EFFECTS OF CONCENTRATION OF Ni²⁺, Co²⁺, Mn²⁺ AND Zn²⁺ ON PRESERVATION OF β -AMYLASE OF *Bacillus* sp.

Metal salt	Concentration (M)	Remaining β -amylase activity (%)
None		2.5
CoCl ₂ {	5×10^{-3}	76.5
	1×10^{-2}	89.7
	2×10^{-2}	82.0
NiSO ₄ {	5×10^{-3}	85.2
	1×10^{-2}	80.3
	2×10^{-2}	81.4
ZnSO ₄ {	5×10^{-3}	54.1
	1×10^{-2}	66.4
	2×10^{-2}	49.9
MnSO ₄ {	5×10^{-3}	8.5
	1×10^{-2}	10.4
	2×10^{-2}	98.3

Four milliliters of enzyme solution containing about 230 units of β -amylase and 2×10^{-2} M phosphate buffer (pH 7.0) was kept for two months at 5°C.

Table I shows the effect of metal ion, maltose and dextrin on preservation of bacterial β -amylase. The activity of the enzyme decreased to 9.3% of initial activity after one month. However, it was found that when metal ions, such as Ni²⁺, Co²⁺ or Zn²⁺ exist in enzyme solution, the enzyme activity was effectively preserved. Maltose, dextrin and other metal ions

tested were not effective for preserving the enzyme.

Table II shows the effect of the concentration of Ni^{2+} , Co^{2+} , Zn^{2+} and Mn^{2+} on the preservation of bacterial β -amylase. Ni^{2+} and Co^{2+} were more effective in preserving the enzyme compared with Zn^{2+} . Mn^{2+} was also effective for preserving the enzyme at a concentration of 2×10^{-2} M. It is not made clear yet why the β -amylase is stabilized by presence of

these metal ions.

REFERENCES

- 1) Y. Takasaki, *Agric. Biol. Chem.*, **40**, 1515 (1976).
 - 2) Y. Takasaki, *ibid.*, **40**, 1523 (1976).
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