

DEOXYRIBONUCLEASES IN
STREPTOMYCES

Sir:

Deoxyribonuclease (DNase) is known to have roles in recombination, replication and repair systems. Generally, DNase requires Mg^{2+} ion for its enzymatic activity¹⁾. Recently, however, ATP-dependent DNases were isolated from *Escherichia coli*, *Bacillus laterosporus*²⁾, *B. subtilis*³⁾, *Streptococcus pneumoniae*⁴⁾ and *Haemophilus influenzae*⁵⁾, which do not require Mg^{2+} ion. In this paper, we report that *Streptomyces* strains released DNase activity into the cultured medium at high frequencies and one of the DNases did not require Mg^{2+} ion and, in addition, showed its enzymatic activity even in the presence of 3.2 mM EDTA.

The mycelium was grown in shaken culture (220 rpm) in a medium consisting of 0.3% caseamino acid; 0.7% glycerol; 2.5% soluble starch; 2.0% potato starch; 0.1% corn steep liquor; 0.1% gluten meal; 0.01% $FeSO_4 \cdot 7H_2O$; and 0.02% silicon oil KM70. The pH was adjusted to 7.0 before autoclaving.

A 4-day cultured broth (50 ml in 500-ml Erlenmeyer flasks) was centrifuged at 3,000 rpm for 20 minutes and the mycelium was removed. The supernatant was assayed for DNase activity. The spectrophotometric assay was carried out in a reaction mixture consisting of 0.25 ml of 0.05 M

phosphate buffer (pH 7.0), 0.1 ml of 0.08 M $MgSO_4$, 10 μ l of the above supernatant, and 0.4 ml of 1.25 mg/ml calf thymus DNA. After incubation for 30 minutes at 30°C, 2 ml of 12% cold perchloric acid was added. Then, it was kept standing for 10 minutes in ice water bath and centrifuged at $22,000 \times g$ for 10 minutes at 0°C. The DNase activity was estimated by measuring the optical absorbance at 260 nm of the supernatant.

Among 496 strains, over 209 strains released DNase activity into the cultured medium, which was indicated by the optical absorbance of over 0.2 at 260 nm in the above assay system. DNase activity was also examined by the electrophoretic method⁶⁾. After sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis containing calf thymus DNA (10 μ g/ml) was carried out and SDS was removed by immersing in 0.04 M Tris-HCl (pH 7.6), the gel was incubated in a buffer consisting of 0.04 M Tris-HCl, pH 7.6; 2 mM $MgCl_2$; 2 mM $CaCl_2$ and 0.02% NaN_3 at 27°C for 2~5 days. DNase activity was detected under the UV-light. In all the broths in which DNase activity was detected by the spectrophotometric method, its activity was found also by the electrophoretic method. However, in some culture broths which were thought to have no DNase activity by the spectrophotometric DNase assay, we detected enzymatic activity by the electrophoretic method. Thus, most *Streptomyces*

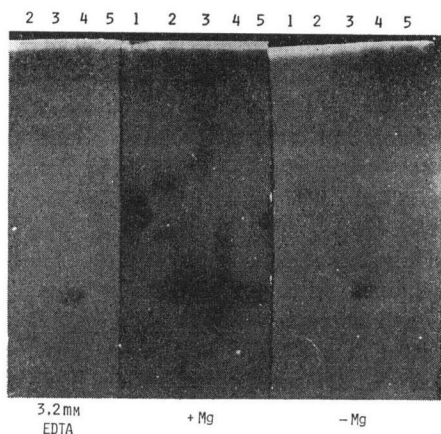
Table 1. Molecular weights of DNases in *Streptomyces* strains.

Strain	Substrate	Broth	Mycelium
Y-4290	Native DNA	20,000 (4~10 days)	20,000 (3~10 days)
		28,000 (4~10 days)	28,000 (3~10 days)
	Denatured DNA	20,000 (2~10 days)	20,000 (2~10 days)
		28,000 (2~10 days)	28,000 (2~10 days)
Y-4098	Native DNA	20,000 (1~10 days)	20,000 (1~10 days)
		---*	32,000 (1~10 days)
		40,000 (1~10 days)	40,000 (1~10 days)
	Denatured DNA	70,000 (1~10 days)	70,000 (1~10 days)
		20,000 (1~10 days)	20,000 (1~10 days)
		40,000 (1~10 days)	40,000 (1~10 days)
		75,000 (1~10 days)	75,000 (1~10 days)
Y-6016	Native DNA	21,000 (1~10 days)	21,000 (2~4 days)
		26,000 (1~10 days)	---*
	Denatured DNA	21,000 (4~5 days)	---*

*---: not detected.

Fig. 1. The effects of EDTA and Mg^{2+} ion on DNase activity.

1: DNase I, 2: Y-6016 strain, 3: Y-6251 strain, 4: Y-4098, and 5: Y-4290 strain.



strains seem to release DNase activity into the culture medium.

The molecular weights of the DNases in the culture broths and the mycelia (supernatant of $12,000 \times g$, 20 minutes at $0^\circ C$ of cells disrupted by the ultrasonic treatment) were measured by the electrophoretic method (Table 1). Most DNases detected in the culture broths showed molecular weights similar to those in the mycelium. However, in some *Streptomyces* strains such as Y-4098, the composition of DNases in the culture broth was somewhat different from that in the mycelium. The effect of EDTA on the enzymatic activity was examined. Almost all the DNases required Mg^{2+} ion for their enzymatic activity as in the case of most other DNases. One of the DNases in *Streptomyces* Y-4098 (molecular weight of 20,000) was an exception. Moreover, this DNase showed enzymatic activity even in the presence of 3.2 mM EDTA when heat denatured DNA was used as a substrate (Fig. 1).

Thus, most *Streptomyces* strains release several kinds of DNases into the medium and their

molecular weights are similar to those in the mycelium. These DNases may have significant detrimental effects in isolating DNA as plasmid DNA from *Streptomyces*.

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