ノート

聖徳栄養短期大学紀要No21 (1990)

# Induction of β−1、3−Glucanase System from *Streptomyces* sp. W 19−1

SATORU WATANABE, ISAO KUSAKABE* and KAZUO MURAKAMI*

*Streptomyces* sp. W19-1 produced both β-1, 3-glucanase and β-glucosidase using curdlan as a carbon source. To investigate the induction of the curdlan-degrading enzyme system from *Streptomyces* sp. W19-1, some polysaccharides containing β-1, 3-linkage and β-glucobioses were used. The better inducers of β-1, 3-glucanase were found to be curdlan, laminarin, soluble laminaran and sophorose. *Streptomyces* β-glucosidase was well induced by sophorose, gentiobiose and several polysaccharides. Sophorose was found to be better inducer of *Streptomyces* β-1, 3-glucanase system.

Recently we have reported about two types of β-1, 3-glucanase systems from species belonging to *Streptomyces*.[5,6] *Streptomyces* sp. W19-1 produced a representative enzyme system including β-1, 3-glucanase and β-glucosidase.[7] In the present work, some polysaccharides containing β-1, 3-linkage and β-glucobioses were used to investigate the induction of the curdlan-degrading enzyme system from *Streptomyces* sp. W19-1.

Curdlan and cellobiose were purchased from Wakô Pure Chemical Industries (Osaka, Japan). Laminarin was from Nakarai Chemicals Ltd. (Kyoto, Japan). Soluble and insoluble laminaran were from Tôkyô Kasei Kôgyô Ltd. (Tokyo, Japan). Lichenan from *Cetraria* and *Usnea* were from Sigma Chemical Co. (Missouri, U.S.A). Sophorose (β-1, 2-glucobiose, So) was prepared from a partial acid hydrolysate of stevioside (13-O-β-sophorosyl 19-O-β-glucosyl steviol).[4] Laminaribiose (β-1, 3-glucobiose, La) and gentiobiose (β-1, 6-glucobiose, Ge) were prepared from hydrolysates of curdlan by the β-1, 3-glucanase systems from *Streptomyces* sp. K27-4 and W19-1, respectively[5,6]. All other chemicals were obtained commercially and of analytical grade.

*Streptomyces* sp. W19-1 was grown in the basal medium (25 ml) as described previously,[5,6] containing a carbon source (1.0%) in a shake flask at 35℃ for 4 days on a reciprocal shaker. Effect of carbon compounds on the formation of the enzyme system was investigated. After cultivation, the growth of

*Institute of Applied Biochemistry, University of Tsukuba, Tennoudai, Tsukuba-shi, Ibaraki 305, Japan

Key words: Induction, *Streptomyces*, β-1, 3-Glucanase, β-Glucosidase, Sophorose.

the organism and pH of the culture filtrate were checked, and β-1, 3-glucanase and β-glucosidase activities of the filtrate were determined, according to the method described previously.[7] The final products from curdlan hydrolysis(8 hr reaction) were detected by thin-layer chromatography.[5]

As shown in Table I, *Streptomyces* sp. W19-1 was grown in all cases. But the amount of mycelia was poor when insoluble laminaran was used as a carbon source. The poor amount of mycelia may cause the low enzyme activities. Gentiobiose and glucose brought the pH of medium up above pH 6.6 and the lysis of mycelia were shown. In these casas, the rate of uptake of carbohydrates may be fast and the growth of microorganism may already run through the stationary phase.

The better inducers of β-1, 3-glucanase were found to be curdlan, laminarin, soluble laminaran and So. Several polysaccharides, So and Ge induced β-glucosidase well. Both β-1, 3-glucanase and β-glucosidase were judged as inducible enzymes, because almost no enzyme activities were shown in the case of glucose and none.

In the case of *Streptomyces* β-1, 3-gluca-

**Table 1.** Effect of carbon compounds on enzyme induction.

| Carbon source | Relative activity (%) β-1, 3-Glucanase | Relative activity (%) β-Glucosidase | Growth[a] | pH | Products[b] |
|---|---|---|---|---|---|
| Curdlan | 100 (2.97 units/ml) | 100 (0.21 units/ml) | +++ | 6.20 | $G_1$, Ge |
| Laminarin | 153 | 313 | ++ | 6.42 | $G_1$, Ge |
| Laminaran (soluble) | 110 | 291 | ++ | 6.59 | $G_1$, Ge |
| Laminaran (insoluble) | 3 | 29 | + | 6.41 | ($G_1$, $G_2$, $G_3$) |
| Lichenan *(Cetraria)* | 16 | 145 | +++ | 6.58 | $G_1$, ($G_2$), Ge |
| Lichenan *(Usnea)* | 15 | 107 | ++ | 6.59 | $G_1$, ($G_2$), Ge |
| Sophorose | 95 | 171 | ++ | 6.35 | $G_1$, Ge |
| Laminaribiose | 14 | 68 | ++ | 6.56 | $G_1$, ($G_2$), Ge |
| Cellobiose | 6 | 15 | ++ | 6.46 | ($G_1$, $G_2$, $G_3$) |
| Gentiobiose | 17 | 114 | ++ | 6.80 | $G_1$, (Ge) |
| Glucose | 1 | 5 | ++ | 6.80 | ($G_1$, $G_2$) |
| None | 0 | 3 | + | 7.05 | None |

[a] the relative amount of the mycelium decreases in the following order, +++>++>+.

[b] products of curdlan hydrolysis, $G_1$, glucose ; $G_2$, laminaribiose ; $G_3$, laminaritriose ; Ge, gentiobiose. Parentheses mean small amount of product.

nase, So is better inducer than La, cellobiose(Ce) or Ge. This indicates that induction of β-1, 3-glucanase by So is similar to that of other microorganism's β-1, 4-glucanase by So, which is a more efficient inducer of cellulase(EC 3.2.1.4) than Ce.[9~12] Therefore, better inducers than the natural products or substrates are sometimes their positional isomers.

*Streptomyces* β-glucosidase was well induced by So, Ge and several polysaccharides(Table I). Induction of β-glucosidase by polysaccharides was seemed to be related with that of β-1, 3-glucanase, because β-glucosicase activity was high when β-1, 3-glucanase was well induced.

The activity of β-glucosidase induced was as follows; So>Ge>La>Ce. The degree of induction of the β-glucosidase was not related with the order of the relative rate of hydrolysis(La≫So>Ge>Ce).[8] As to β-glucosidases, the positional isomer can also serve as a better inducer. Allolactose, a positional isomer of lactose, is known to be excellent inducer of β-galactosidase(EC 3.2.1.23) in *Escherichia coli*.[2,3] In the xylan-degrading enzyme system of *Cryptococcus albidus*, β-1, 2-xylobiose induced the production of both β-xylanase(EC 3.2.1.8) and β-xylosidase (EC 3.2.1.37).[1]

*Streptomyces* β-glucosidase has also a transfer activity, which is easy to produce Ge from other β-linked disaccharides.[8] If such transfer activity occurs *in vivo* during the growth, Ge might be a naturally occurring inducer of the β-glucosidase. Induction of the β-glucosidase by the β-linked disaccharides might be due to the ability of production of Ge from their disaccharides.

The amount of the final products was large when β-1, 3-glucanase activity was high. And the high activity of the β-glucosidase brought about the large amount of glucose and Ge. The final products of curdlan hydrolysis by the enzyme system, which were induced by curdlan, laminarin, soluble laminaran and So, were similar one another. And in the cases of two types of lichenan and La, the final products were similar.

Some of the remained problems should be pointed out here. That is, the structures and sugar compositions of the polysaccharides were not elucidate. It is possible that another enzyme system might be induced when carbon compound was changed. It will be also neccessary to investigate the effect of concentration of inducers and time course of enzyme production. Further study will be done in future.

## REFERENCES


1) BIELY, P. and PETRAKOVA, E.: J. Bacteriol., **160**, 408(1984).

2) BOOS, W., SCHAEDEL, P. and WALLENFELS, K.: Eur. J. Biochem., 1, 328(1967).

3) JOBE, A. and BOURGEOIS: J. Mol. Biol., **69**, 394(1972).

4) KUSAKABE, I., KUSAMA, S. and MURAKAMI, K.: Agric. Biol. Chem., **51**, 2255 (1987).

5) KUSAMA, S., KUSAKABE, I. and MURAKAMI, K.: Agric. Biol. Chem., **48**, 1433 (1984).

6) KUSAMA, S., KUSAKABE, I. and MURAKAMI, K.: Agric. Biol. Chem., **48**, 2655 (1984).

7) KUSAMA, S., KUSAKABE, I. and MURAKAMI, K.: Agric. Biol. Chem., **49**, 2055 (1985).

8) KUSAMA, S., KUSAKABE, I. and MURAKAMI, K.: Agric. Biol. Chem., **50**, 2891 (1986).

9) LOEWENBERG, J. R. and CHAMPMAN, C.

M.: Arch. Microbiol., **113**, 61(1977).
10) MANDELS, M., PARRISH, F. W. and REESE, E. T.: J. Bacteriol., **83**, 400(1962).
11) NISIZAWA, K., SUZUKI, H., NAKAYAMA, M. and NISIZAWA, K.: J. Biochem., **70**, 375(1971).
12) STERNBERG, D. and MANDELS, G. R.: J. Bacteriol., **139**, 761(1979).

**放線菌のβ－1、3－グルカナーゼ系の誘導について**

—渡邉　悟　日下部　功　村上　和雄—

β－1，3－グルカンであるカードランによって放線菌β－1，3－グルカナーゼ系が誘導されるが、β－1、3結合を含むいくつかの多糖とβ－グルコ二糖について、放線菌β－1、3－グルカナーゼ系の誘導能を調べた。カードランの他、ラミナリン、水溶性ラミナラン、ソホロースがβ－1、3－グルカナーゼをよく誘導した。また、不溶性ラミナラン以外の多糖とソホロース、ゲンチオビオースがβ－グルコシターゼをよく誘導した。β－グルコ二糖のうち、セルラーゼの誘導物質として知られるソホロースが、放線菌β－1，3－グルカナーゼ系の誘導に適していることが判明した。