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Isolation of Alkalophilic, Thermophilic *Bacillus* sp. TA-11 and Production of β-Galactosidase

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호알칼리성, 고온성 *Bacillus* sp. TA-11의 분리와 β-galactosidase의 생산

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β-galactosidase를 강력하게 생산하는 호알칼리성, 고온성 세균 TA-11를 부엽토에서 분리하여 *Bacillus* sp. TA-11로 동정 하였다.

Bacillus sp. TA-11에 의한 β-galactosidase 생산은 lactose 1.5%, peptone과 yeast ext. 각각 0.4%, $MgSO_4$ 0.2%, NH_4Cl 0.05% 및 NaCl 0.2% 등을 함유한 배지의 pH를 10.0으로 하여 시험균을 접종한후 50℃에서 2일간 진탕배양 하였을 때 가장 좋았다.

A alkalophilic, thermophilic bacterium TA-11 which produce β-galactosidase highly was isolated from compost and identified to the genus *Bacillus*.

β-Galactosidase production was maximized when it was incubated in synthetic medium containing 1.5% lactose, 0.4% peptone, 0.4% yeast ext., 0.2% $MgSO_4$, 0.05% NH_4Cl and 0.2% NaCl(initial pH:10.0) at 50℃ for 2 days in reciprocal shaker.

Keywords : Alkalophilic, thermophilic *Bacillus* sp. TA-11, β-galactosidase, screening and identification

Introduction

In general, alkalophilic and thermophilic microorganism produce alkaline thermotolerant enzymes, which have some advantages in fermentation, food industry and medical industry to prevent microbial contamination, enzyme inactivation for heat and alkali during the enzymatic hydrolysis process and furthermore reduce one of related process.[1~3]

β-Galactosidase(EC 3.2.1.23) hydrolyse lactose to glucose and galactose and is used widely in dairy and medical industry. The characterization of the enzyme was reported from various sources[4] such as bacteria, yeast and mold. Most of fungal β-galactosidase were more active at lower pH(2.5-5.5) in contrast to enzymes from bacteria and yeast which are most active in neutral pH range(6-7 and 6.5-7.5, respectively), but temperature optima of microbial β-galactosidase was diverse (35-80℃).[5] Although many microbial β-galactosidase have been studied, the enzymes from alkalophilic and thermophilic microorganism have scarcely been reported.

In this study, we performed the isolation and identification of alkalophilic, thermophilic bacteria which produce β-galactosidase highly. Some conditions for the enzyme production have also been examined.

Materials and Methods

1. Media

A screening medium comprised 0.4% polypeptone, 0.4% yeast ext., 0.1% K_2HPO_4 and KH_2PO_4, 0.2% $MgSO_4$, 0.05% NH_4Cl, 0.2% NaCl and 1.0% lactose(pH 9.5 with 20% Na_2CO_3). Na_2CO_3 and lactose were autoclaved separately and added in medium.

2. Isolation and selection of β-galactosidase-producing alkalophilic, thermophilic microorganism

Soils, hotspring waters and composts were inoculated in a 250ml flask containing 100ml of screening medium and incubated at 50℃ for 3 days in reciprocal shaker. 0.5ml of the cultures was transfered to a fresh medium described above and incubated as before and then streaked onto the plates of screening medium. The plates were incubated at 50℃ for 3 days. All of colonies were transfered to slant media and incubated as above.

All of isolated strains were determined for β-galactosidase activity and then isolated strains with high activity were selected.

3. Identification of selected strain

Morphological, biochemical and cultural characteristics of selected strain were investigated according to the Manual of Method for General Bacteriology[6] and Microbiological Method.[7] The identification were performed using Bergey's Manual of Determinative Bacteriology.[8]

4. Enzyme assay

β-Galactosidase activity was estimated spectrophotometrically using o-nitrophenyl β-D-galactoside (ONPG) as the substrate.

The selected strain was incubated at 50℃ for 3 days and harvested the pellets by centrifugation(8,000 rpm for 10 min. at 4℃). The pellets was washed and resuspended in 10mM Z-buffer(100mM Na_2HPO_4, 100mM NaH_2PO_4, 10mM KCl, 1mM $MgSO_4$, 50mM 2-mercaptoethanol, pH 9.5) and disrupted then in ultrasonic disintegrator. After centrifugation (18,000 rpm for 10min. at 4℃), the supernatant was used to the enzyme solution.

The reaction mixture containing 2ml of ONPG in Z-buffer and 0.5ml of the enzyme solution obtained as above was incubated at 50℃ for 10 min..

The enzymatic reaction was stopped by adding 0.5ml of 1.0 M Na_2CO_3. The amount of the product (o-nitrophenol) was determined spectrophotometrically by measuring absorbance at 420nm.

One unit of the enzyme activity was defined as the amount of enzyme hydrolysing 1 μmol. of the substrate per min. and specific activity was expressed as unit per absorbance at 660nm.

Results and Discussion

1. Screening for β-galactosidase-producing alkalophilic, thermophilic bacteria

About 280 strains of alkalophilic, thermophilic bacteria were isolated from 25 samples in Chungnam province. Among the collection of them, the crude enzyme of isolated strain No.TA-11 exhibited an extremely high β-galactosidase activity. Therefore, we selected the strain for further studies.

2. Identification of TA-11 strain

Morphological, biochemical and cultural characteristics of the TA-11 strain are summarized in Table 1.

The selected strain TA-11 was cylindrical rod shaped bacterium of (0.6-0.8)×(4.0-6.0)μm, gram positive, motile and also it was Voges-Proskauer test(−), oxidase and urease test(−), indole test(+), and hydrolysed casein and produced acid from glucose well.

As the results were analyzed using Bergey's manual, TA-11 strain should belong to the genus *Bacillus*.

Bacillus sp. TA-11 grew in the pH range of 7.5 to 11.5 and the optimum pH was seen at 9.5. The strain grew well in the temperature range of 30 to 70℃ and the optimum temperature was at 50℃. Both data demonstrated the strain was alkalophilic and also thermophilic.

Meanwhile, *Bacillus* sp. TA-11 were resistant to ampicillin, penicillin and chloramphenicol, not to tetracycline and streptomycin.

3. Production of the β-galactosidase from *Bacillus* sp. TA-11

1) Initial pH of medium

Effect of initial pH of medium on production of the β-galactosidase were examined in enzyme-producing medium(same as screening medium) of various pH.

As shown in Fig.1, maximum activities were observed in cultures from medium of initial pH 10.0. It was similar to optimum growth pH, which indicates *Bacillus* sp. TA-11 may produce alkaline β-galactosidase specifically.

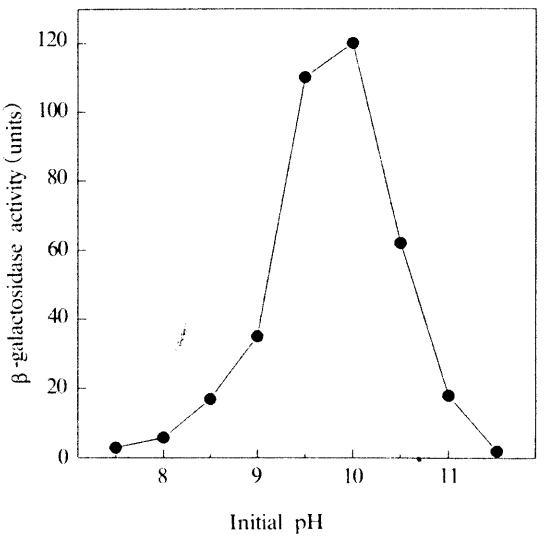

Fig 1. Effect of initial pH of medium on production of the β-galactosidase by *Bacillus* sp. TA-11.

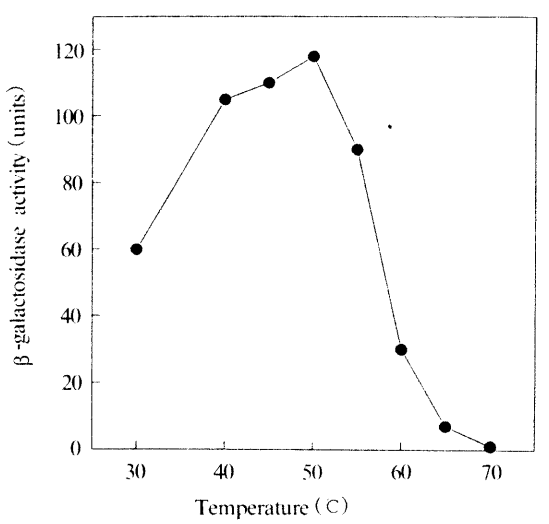

Fig 2. Effect of incubation temperature on production of the β-galactosidase by *Bacillus* sp. TA-11.

2) Incubation temperature

Optimal temperature for enzyme production was 50℃(Fig. 2). It was lower than 60℃ of *Thermus* sp.[9], but higher than 30-40℃ of mesophilic enzyme.[4]

Table 1. Microbiolgical characteristics of the selected strain TA-11

Test		Results
Morphological	Characteristics	
	Shape and size	Cylindrical rod (0.6~0.8) × (4.0~6.0) μm
	Gram staining	Postive
	Motility	+
	Flagella	+
Biochemical	Characteristics	
	Methyl red test	−
	Voges-Proskauer test	−
	Catalase test	+
	Oxidase test	−
	Utilization of citrate	−
	Indole test	+
	Hydrolysis of casein	+
	gelatin	−
	starch	−
	Formation of ammonia from peptone medium	−
	Urease test	−
	β-Galactosidase activity	+
	Amylase activity	−
	Protease activity	+
	Production of acid from glucose, sucrose and maltose	+
	galactose, lactose and xylose	+
	glycerol and ribose	−
Cultural	Charcateristics	
	Growth on nutrient agar (pH 7.0)	−
	(pH 10.0)	+
	Growth on 1% glucose nutrient agar (pH 7.0)	−
	(pH 10.0)	+
	Growth on 7% NaCl nutrient agar (pH 10.0)	−
	Temperature for growth	Up to 70℃ (optimum temp.: 50℃)
	pH for growth	7.5~11.5 (optimum pH: 9.5)
	Oxygen requirement	Facultative anaerobic
Antibiotic	resistance	
	Penicillin(200μg/ml), Ampicllin (100μg/ml) and Chloramphenicol(20μg/ml)	+
	Tetracycline(12μg/ml) and Streptomycin (300μg/ml)	−

3) Modes and periods of incubation

Effect of incubation modes and periods on the enzyme production were determined by varying the incubation modes(shaking and stationary) and periods(1-5 days) at initial pH 10.0 of medium and 50℃.

As shown in Fig.3, β-galactosidase production has reached maximum level at 48 hours of cultivation time in shaking culture and 108 hours in stationary culture. Growth and absolute activity were about 2 times higher in shaking culture than in stationary culture, but specific activity was almost same.

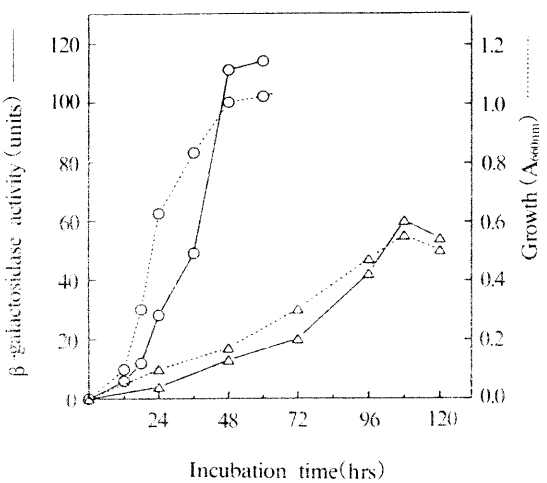

Fig 3. Effect of incubation modes and period on production of the β-galactosidase by *Bacillus* sp. TA-11.
○—○:shaking culture
△—△:stationary culture

4) Effect of sugars

Effect of sugars on production of the enzyme were investigated by adding 30mM glucose, galactose, fructose, lactose, sucrose, maltose, ribose, xylose and glycerol in enzyme-producing medium.

Among them, lactose was very proper for the enzyme production(Table. 2). This results indicated β-galactosidase of *Bacillus* sp. TA-11 was not constitutive enzyme, but induced by lactose. Meanwhile, its induction efficiency was better than chemical inducer such as IPTG.(not data)

Table 2. Effect of sugars on production of the β-galactosidase by *Bacillus* sp. TA-11

Sugars (30mM)	Cell yield (A_{660nm})	β-Galactosidase activity (U)
Glucose	1.50	59
Galactose	1.09	61
Fructose	1.10	35
Lactose	1.36	121
Sucrose	1.00	55.5
Maltose	1.32	36
Ribose	1.05	69.5
Xylose	1.32	44
Glycerol	1.07	69
Control	0.13	0.12

5) Concentration of lactose

As shown in Fig. 4, addition of 1.5% lactose in enzyme-producing medium was observed high galactosidase productivity.

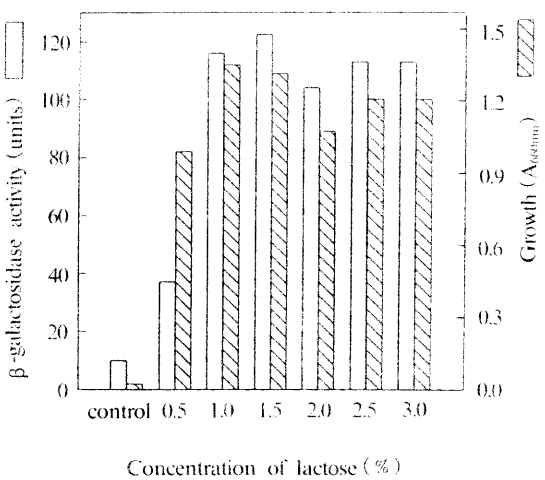

Fig 4. Effect of lactose concentration on production of the β-galactosidase by *Bacillus* sp. TA-11.

Consequently, we obtained alkalophilic, thermophilic *Bacillus* sp. TA-11 which produce resonable amounts of a β-galactosidase and examined some

conditions for enzyme production.

The biosynthetic regulation of the β-galactosidase (induction and repression system) will be studied further.

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