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Production and Characterization of Crystalline Cellulose-Degrading Cellulase Components from a Thermophilic and Moderately Alkalophilic Bacterium

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A moderately thermophilic, alkalophlic and powerful crystalline cellulose-digesting bacterium, *Bacillus* K-12, was isolated from filter paper wastes and found to be similar to *Bacillus circulans* or *Bacillus pumilis*, except for its ability to grow at a moderately high pH and temperature. The isolate grew at a pH ranging from 6 to 10 and at a temperature ranging from 35 to 65℃ and produced a large amount of cellulase components containing avicelase, xylanase, CMCase, and FPase when grown in avicel medium for 5 to 7 days at 50℃. The crude enzyme preparation from the culture broth hydrolyzed xylan, raw starch, pullulan and β-1,3 glucan such as laminarin. Furthermore, the enzyme hydrolyzed crystalline cellulose to cellobiose and glucose and had a broad pH activity curve (pH 6~9). The enzyme was stable up to 70℃.

Many microorganisms, mainly fungi and bacteria, have already been found to produce various cellulolytic enzymes (1, 2, 4, 9, 14). Among fungi, *Trichoderma reesei* (13) has been generally regarded as the best producer of cellulases. However, this fungus is mesophilic and the upper limit of the thermostability of the cellulolytic enzymes is below 50℃, and during saccharification of cellulose at this temperature, contamination sometimes occurs by thermotolerant microorganisms belonging to the genus *Bacillus* (11). On the other hand, *C. thermocellum* as bacterium is well endowed, it produces one of the most powerful cellulase systems which are known to be able to saccharify efficiently, whether all forms of cellulase are the most complex as in native, undergraded, and highly crystalline cotton, or the simplest and highly degraded forms (7). Moreover relatively little is known of the nature of the enzymatic components that account for the marked superiority of the bacterial cellulase system. Some of the problems associated with examining isolated components of this system undoubtedly arise from the anaerobic property of the organism. Other problems are related to the multienzyme complex of the cellulase of *C. thermocellum*, as suggested by Ait *et al.* (1). In order to account for the complexity of this cellulase, a cellulose binding factor (CBF), named cellulosome, associated with the cells and also present in the extracellular medium, was proposed by Lamed *et al.* (9). There is thus much still to be learned of this cellulase system of *C. thermocellum* before successful application to biotechnological process.

Cellulolytic enzymes are generally induced as multienzyme systems, composed of five or more different enzymes. Cellulolytic enzymes have been traditionally divided into three classes, endoglucanases (EC 3.2.1.4), exoglucanases or cellobiohydrolases (EC 3.2.1.91) and β-glucosidases (EC 3.2.1.21).

Cellulases all cleave the same chemical bond, the β-1,4 glycosidic bond, but there is variation in the microenvironment of these bonds in natural substrates. These bonds require rigorous distruption of the crystalline state for effective hydrolysis. For industrial utilization of the

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cellulosic materials, the fungal cellulases have been used in large amounts (more than 20 g/l). In bacteria. these enzymes are often produced in small amounts (less than 0.1 g/l). With this background, the present authors tried to find a microorganism that produced large quantities of a cellulolytic enzyme system for the aim of the present research, that is, the strong hydrolysis of the insoluble substrates.

A new, powerful cellulase producing microorganism has been sought in this research with CMCase and crystalline cellulose degrading enzyme productivities as high as those of *T. reesei* and *C. thermocellum* which are the best of cellulase producers, and with cellulolytic enzymes that are stable at high temperatures such as 60℃ or above. As a result of our screening, *Bacillus* sp. K-12, which had been isolated from filter paper wastes submerged in a soil solution, was found to be very suitable for this purpose. This paper deals with the isolation of the cellulolytic enzyme-producing microorganism and examination of the cultural conditions for the bacterial isolate and some properties of its crystalline cellulose-digesting cellulase components.

MATERIALS AND METHODS

Media

The enrichment medium was formulated as follows: a filter paper strip (13×1 cm^2), 2 g of $(NH_4)_2SO_4$, 14 g of K_2HPO_4, 6 g of KH_2PO_4, 0.2 g of $MgSO_4 \cdot 7H_2O$, 0.5 g of $CaCl_2 \cdot 2H_2O$, 1.0 g of Bacto-peptone, 1 g of yeast extracts and 1 m*l* of a trace element solution in 1 liter of water. The trace element solution contained 20 mg of $(NH_4)_6Mo_7O_{24} \cdot 4H_2O$, 80 mg of $FeCl_3 \cdot 6H_2O$, 60 mg of $CuSO_4 \cdot 5H_2O$, 12 mg of $MnCl_2 \cdot 4H_2O$ and 150 mg of $ZnCl_2$ in 100 m*l* of water. The medium for tube or flask cultures was prepared as follows: the same as above, except for the use of 10 g of carbon source and 50 mM phosphate buffer (pH 7.0) without filter paper strip, in 1 liter.

Isolation of Microorgaism Producing Cyrstalline Cellulose-Digesting Cellulases

The bacterium was isolated from filter paper wastes which had been prepared in soil solution and stored in this laboratory. A soil sample (1.0 g) was suspended in 10 m*l* of saline and treated at 80℃ for 30 min. The thermally treated solution was suitably spread on filter paper wastes, followed by incubation at 40℃ for 5 days without shaking and then filter paper wastes were innoculated into 10 m*l* of the enrichment medium in 50 m*l* test tubes, followed by incubation at 50℃ for 8 days with shaking. The culture broth was diluted and spread usually on potato dextrose agar plates and the colonies were isolated. Thus a bacterium was obtained and used in this study. The stock culture was maintained by periodical transfer to test tube culture with filter paper strips in the medium.

Chemicals

Cellulose powder (Whatman CF11) was obtained from Whatman Co. (Tokyo, Japan). Micro crystalline cellulose (Avicell SF) was purchased from Asahi Kasei Co., Ltd. (Tokyo, Japan). Salin, Laminarin and CMC were obtained from Wako Pure Chemicals Co. (Tokyo, Japan). Soluble starch, pullulan, dextran, raw corn starch and raw potato starch were purchased from Hayashibara Laboratories (Tokyo, Japan). Xylan (from oat spelts) was obtained from Sigma Co., Ltd. (CA, USA). Solka Floc BW-200 was obtained from James River Co., Ltd (USA). Other chemicals were of the purest grade commercially available.

Definition of Enzyme Unit

One unit of enzyme activity is defined as the amount of enzyme which caused the liberation of reducing groups corresponding to one mole of reducing sugar per 60 minutes except CMCase (one micromole per one minute) under the conditions stated above.

Enzyme and Sugar Determination

Soluble protein was determined by the Lowry method (10). Reducing sugars were determined by the dinitrosalicylic acid (DNS) method (3) and the Somogyi-Nelson method (16). TLC was performed by the previous method (8).

Enzyme Assay

Avicelase: Avicelase activity was assayed in 1 m*l* of a reaction mixture containing 0.25 m*l* of 0.2 phosphate buffer (pH 7.0), 0.5 m*l* of 2% avicel SF and 0.25 m*l* of enzyme solution. The mixture was incubated at 60℃. After 30 min incubation, the reducing sugar formed was determined to be glucose by the DNS method or Somogyi-Nelson method.

FPase, CMCase and β-glucosidase: These two enzyme activities were assayed in the same manner as described for the avicelase assay except that 2% CMC and 2% salicin solution were used as the substrates for CMCase and β-glucosidase, respectively. Filter paper-hydrolyzing enzyme (FPase) (12): FPase activity was assayed in 2 m*l* of a reaction mixture containing 0.5 m*l* of 0.2 M phosphate buffer (pH 7.0), 0.5 m*l* of enzyme solution, 1 m*l* of distilled waster and 50 mg of filter paper (Whatman No. 1). The reducing sugar was determined by the same method as mentioned above.

Other glycan-hydrolyzing enzymes: The procedure for measuring the activities of other glycan-hydrolyzing enzymes was essentially the same as in the case of the cellulose-hydrolyzing enzyme described above.

RESULTS

Characterization of Isolated Bacterium

About 5 hundred microorganisms were isolated from filter paper waste samples as powerful crystalline cellulose-digesting cellulase producers. Most of them were thermophilic fungi and bacteria. Of them, microorganisms which showed morphologically bacteria-like properties were separately cultivated in the medium with avicel as a carbon source. A bacterial strain K-12 showed the highest cellulase productivity among the isloated strains. Morphological and taxonomical characteristics of the isolated bacterium were studied by the methods of Gordon *et al.* (5) and Sneath (15). The isolated bacterium was aerobic, rod-shaped, Gram-positive, motile, and it had terminal endospores in cylindrical sporangia and peritrichous fragella and produced catalase. Therefore, we concluded that this bacterium belonged to the genus *Bacillus*. The results obtained are summarized in Table 1. The outstanding characteristic of this bacterium was that it grew well in the high temperature condition of 60℃. Thus, this bacterium was tentatively called *Bacillus* sp. K-12.

Examination of Culture Conditions and Carbon Sources

Although the selected bacterial strain, *Bacillus* sp. K-12, produced a good composition of cellulolytic enzyme components, FPase, avicelase, xylanase, CMCase and β-glucosidase, the enzyme yields were not as high as expected when it was grown in the screening medium. Media containing different carbon sources were examined to obtain the culture conditions showing the highest avicelase and xylanase activties. Table 2 shows the effect of various soluble sugars and insoluble materials on the production of extracellular CMCase, β-glucosidase, avicelase and xylanase by *Bacillus* sp. K-12. The strain was able to assimilate many kinds of carbon sources. Among the carbon sources examined, however, insoluble materials including cellulose powder, filter paper and avicel were most effective for the production of enzymes (Table 2).

Table 1. Physiological and Biochemical Properties of *Bacillus* sp. K-12

Morphology	
Form	Rod shape
Size (μm)	1.8−2.3×0.5−1.3
Mobility	+
Gram staining	Positive
Spores (μm)	2.1−2.5×0.5−0.8
Biochemical properties	
VP test	+
Formation of indole	−
Reduction of nitrate	−
Utilization of citrate	−
Hydrolysis of raw potato granules	+
Hydrolysis of casein	−
Catalase	+
Anaerobic growth	−
Temperature for growth	25~80℃
pH for growth	7~10℃

Effect of Initial pH and Temperature on Avicelase, FPase, CMCase, Xylanase and β-Glucosidase

The production of avicellase, FPase, CMCase, xylanase and β-glcosidase at various pHs and cultivation temperatures in shown in Fig. 1. The maximum yields of xylanase and β-glucosidase were obtained when the initial pH of the medium was adjusted to pH 6.0, however, for avicelase, FPase and CMCase when it was adjusted to pH 8.0. The temperatures for the optimum production of avicelase, FPase and CMCase were 55~60℃. On the other hand, xylanase and β-glucosidase were maximum at 45 to 50℃. A temperature higher than 60℃

Table 2. Effect of various carbon sources on the production of extracellular cellulase.

Bacillus sp. K-12 was cultivated at 50℃ for 4 days with shaking in 500 ml flasks containing 100 ml of basal medium consisting of 1% (w/w) of a carbon source. Assays of enzyme production were carried out at 50℃ in sodium phosphate buffer (pH 7.5) as mentioned in Materials and Methods

Carbon source	Degree of growth (A_{660})	CMCase (U/ml)	β-Glucosidase (U/ml)	Avicelase (U/ml)	Xylanase (U/ml)
None	3.4	0.9	0.1	0.0	0.1
Cellulose powder[b]	NM[a]	7.4	1.5	0.9	2.3
Filter paper[c]	NM	6.7	1.3	1.0	2.1
Avicel	NM	7.0	1.4	0.9	2.2
NM300	NM	6.8	1.4	0.9	2.1
Xylan	NM	5.6	1.3	0.8	3.6
CMC	3.7	3.2	0.9	0.4	1.8
Soluble starch	3.9	1.8	0.3	0.1	0.8
Cellobiose	3.8	3.2	1.2	0.2	1.5
Glucose	5.5	1.2	0.1	0.0	0.3
Lactose	3.2	1.0	0.0	0.0	0.2
Fructose	5.5	1.1	0.0	0.0	0.2
Sucrose	4.2	1.3	0.1	0.0	0.4
Xylose	5.1	5.7	0.2	0.4	0.3

[a] Not measurable because of its insoluble character.
[b] Whatman CF11
[c] Whatman No. 1

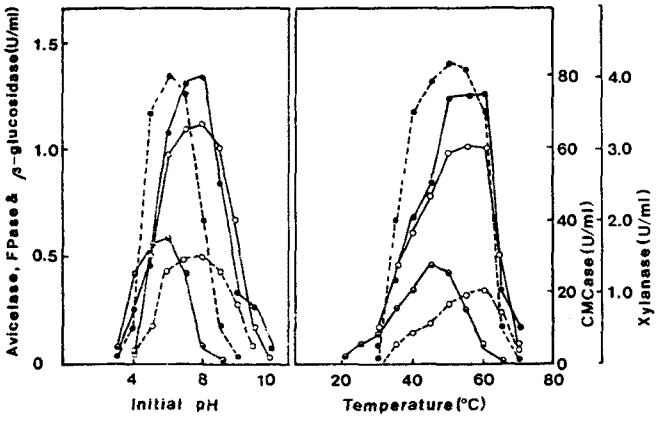

Fig. 1. Effects of cultivation temperature and initial pH on cellulytic enzyme production by *Bacillus* sp. K-12.

In order to investigate the effect of pH, various initial pHs of the medium were obtained, within the range of 3.5 to 10.0, by adding 1 N HCl or NaOH. To elucidate the effect of temperature, cultivations were carried out using a temperature gradient cultivation system at temperatures between 20 and 70℃ for 8 days. ●—●, Avicelase; ○—○, FPase; ○---○, CMCase; ●---●, Xylanase; ◒—◒, β-Glucosidase.

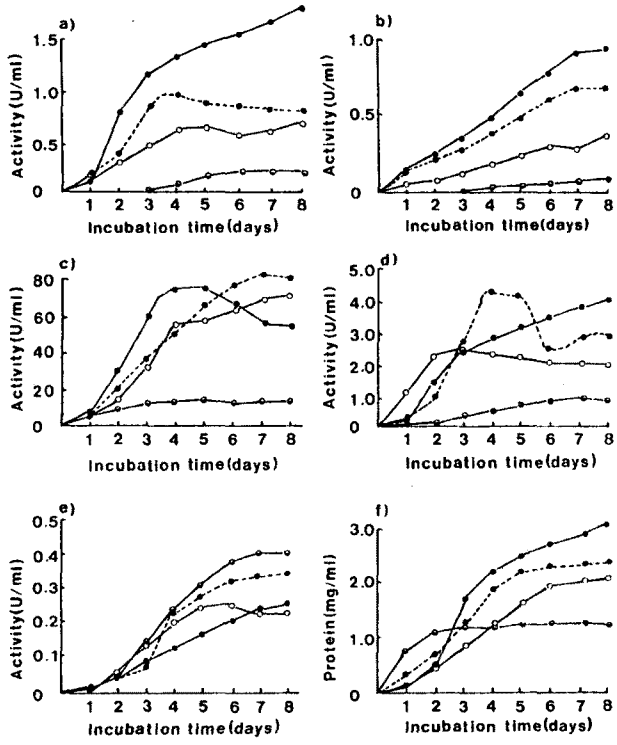

Fig. 2. Time course of production of cellulolytic enzymes by *Bacillus* sp. K-12.

A 500 ml sakaguchi flask culture (working volume 200 ml) with 2% carbon source after inoculation with 20 ml of a 3 day flask culture of the bacterium was carried out for 8 days at 50℃.

●—●, Avicel medium; ●---●, Xylan medium; ○—○, CMC medium; ◒—◒, Glucose medium: a) Avicelase, b) FPase, c) CMCase, d) Xylanase, e) β-Glucosidase and f) Soluble starch.

caused a marked decrease in both the cellulase components and bacterial growth.

Production of Cellulolytic Enzyme with Avicel, Xylan, CMC and Glucose in Optimum Medium

Fig. 2 shows the time course of a shaking culture of *Bacillus* sp. K-12 and the cellulolytic enzyme production under the optimum condition. Each culture was carried out with 2% avicel, xylan, CMC or glucose as a carbon source, the pH being controlled at 7.5 by the addition of 2 N NH_4OH. In the avicel medium, avicelase and FPase in the culture broth increased rapidly, reaching maximum levels on the 8th day of cultivation. In the xylan medium, they increased more slowly than in the avicel medium. On the contrary, CMCase and xylanase in the xylan medium were more than in the media with other carbon sources, reaching maximum levels on the 7th day and 4th day for CMCase and xylanase, respectively. Whole celluloytic activities produced by *Bacillus* sp. K-12 were detected in the culture supernatant.

Compared with the cellulolytic enzyme production by other *Bacillus* sp., the enzyme production by *Bacillus* sp. K-12 has several advantages:

1) The time needed for the maximum production of cellulolytic enzymes is only 4~7 days.

2) With the bacterial growth of *Bacillus* sp. K-12, the pH of the culture broth remained in the moderate alkaline range of pH 6.0 to 8.5.

3) The risk of contamination by other microorganisms is expected to decrease because the optimum temperature is 50~65℃, which is considerably higher than those for other *Bacillus* sp. and fungi.

Some Properties of the Crude Enzyme Preparation

Fig. 3 shows that the optimum pH of each component of the cellulolytic enzyme was around 6.5 for xylanase and β-glucosidase and 8.0 for avicelase, FPase and CMCase, and indicates that they were stable in the pH ranges from 5.0 to 7.0 and from 6.5 to 9.0. As shown in Fig. 4, the optimum temperatures of avicelase, FPase and CMCase production were all about 65℃, their activities being stable even after treatment at 70℃ for 30 minutes. As a contrast, the optimum temperatures of xylanase and β-glucosidase were 45℃ with stability up to 60℃. After heat treatment at 80℃ for 30 minutes, however, their activities could no longer be observed.

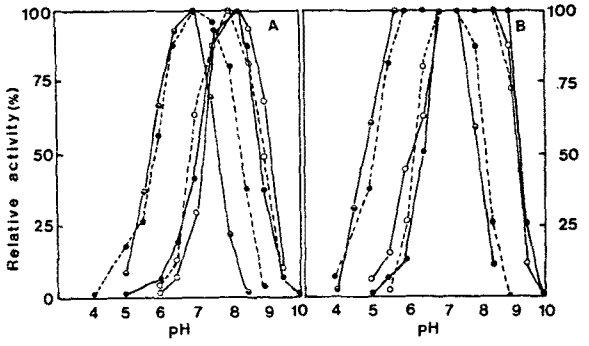

Fig. 3. Optimum pH and pH stability of the cellulolytic enzyme components produced by *Bacillus* sp. K-12.
After incubation of each enzyme component solution at 50℃ for 30 min with the following buffers, the optimum pH was examined. Acetate buffer (0.1 M) was used over the pH range of 4.0 to 7.0. Phosphate buffer (0.1 M) was used in range of 6.9 to 7.0 and Tris HCl buffer (0.1 M) was used above pH 7.5. The pH stability of each enzyme component was examined by measuring its remaining activity after incubation for 1 hr at 4℃ in 0.1 M buffer, within a pH range of 4.0 to 10.0. The symbols are the same as Fig. 1. A, optimum pH; B, pH stability.

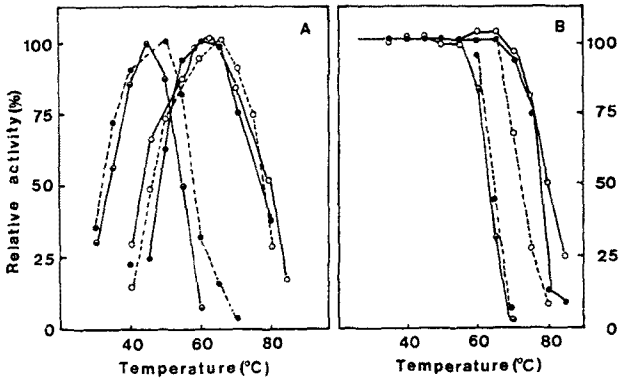

Fig. 4. Optimum temperature and thermostability of the cellulolytic components produced by *Bacillus* sp. K-12.
After incubation of each enzyme components at the indicated temperature for 30 min, the optimum temperature was tested. Aliquents of each enzyme components in 0.1 M Tris HCl buffer (pH 7.0 for xylanase and β-glucosidase, pH 8.0 for avicelase, CMCase and FPase) were incubated at 0℃ and the indicated temperature for 30 min, cooled immediately to 0℃ and then assyed for the remaining activites. The symbols are the same as in Fig. 1. A, optimum temperature; B, thermostability.

Digestion of Insoluble Cellulose by Culture Broth of *Bacillus* sp. K-12

The ability of the extracellular cellulase produced by *Bacillus* sp. K-12 to digest various insoluble cellulose sources was examined and the results are given in Fig. 5. Several kinds of insoluble cellulose were added to the crude enzyme solution to final concentrations of 4%, and saccharification was carried out at 60℃. Avicel, KC-flock and xylan were almost completely converted to soluble sugar after 60 h of incubation. Some 80% of the filter paper and 55% of the cotton were hydrolyzed despite of their resistance to enzymatic degradation due to their high crystallinity. Although avicel and xylan were digested more rapidly than filter paper, the relative rate of digestion of filter paper was higher with our cellulase than with cellulases of other bacteria (2, 4, 14). Furthermore, when the amount of cellulase added to the reaction mixture was increased from 5.20 U to 15.0 U, 76% of the substrate was digested in 4 days. These results indicate that *Bacillus* sp. K-12 produces an extracellular cellulase which can digest insoluble cellulose at a substantial rate. The major hydrolysis product of these substrates was glucose, with small amounts of cellobiose and unknown sugars (Fig. 6). With these results, it was reasonably concluded that *Bacillus* sp. K-12 is an advantageous cellulase source capable of hydrolyzing cellulose to glucose rapidly.

Table 3. Hydrolysis of various substrates by the culture broth of *Bacillus* sp. K-12

Substrate	Linkage	Hydrolysis (%)[c]
Soluble starch	α-1,4 and 1,6 G[a]	100
Raw corn starch	α-1,4 and 1,6 G	68
Raw potato starch	α-1,4 and 1,6 G	56
Pullulan	α-1,4 and 1,6 G	45
Xylan	β-1,4 X[b]	100
Schizophyllan	β-1,3 and 1,6 G	98
Laminarin	β-1,3 G	100
Avicel	β-1,4 G	64
MN300	β-1,4 G	62
CMC	β-1,4 G	58
Soka floc BW-200	β-1,4 G	92
Cellobiose	β-1,4 G	100
Gentiobiose	β-1,6 G	100
Filter paper	β-1,4 G	40
Cotton	β-1,4 G	34

Each reaction mixture, consisting of 50 μl of enzyme solution (Culture broth contaning 0.074 units of avicelase, 3.9 units of CMCase, 0.015 units of β-glucosidase and 110 μg protein), 0.5 ml of 1% substrate, 0.4 ml of 0.1 M phosphate buffer (pH 7.0) and 50 μl of toluene was incubated for 0.5 h at 60℃.
[a] Glucose
[b] Xylose
[c] {Reduced sugar (glucose/total sugar)} × 100

Table 4. Comparison of the enzyme productivity of *Bacillus* sp. K-12 with that of *C. thermocellum*

Organism	Carbon source	Enzyme activity (Units/ml of CB[a])				
		Avicel	β-Glucosidase	CMCase	FPase/protein	Xylanase mg/ml CB
C. th[c]	5% Cellulose powder	0.8	1.2	10.2	0.4	1.2
	5% Avicel	0.7	1.5	9.4	0.5	1.0
	FP[b]	0.7	1.0	10.7	0.5	0.8
B. K-12[d]	Cellulose powder	0.9	1.5	7.4	0.8	2.3
	Avicel	0.9	1.4	7.0	0.8	2.2
	FP	1.0	1.3	6.7	0.6	2.1

[a] Culture broth, The present strain was grown under its optimum culture condition using the medium shown in Materials and Methods.
[b] Filter paper-hydrolyzing units (F.P.U)
[c] *C. thermocellum*
[d] *Bacillus* sp. K-12

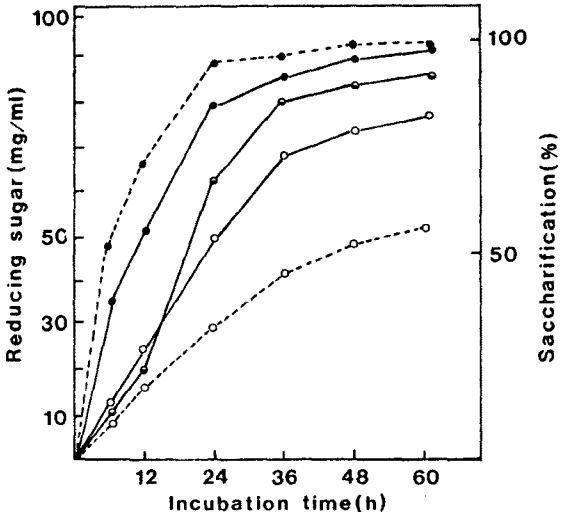

Fig. 5. Hydrolysis of insoluble cellulose by crude enzyme of *Bacillus* sp. K-12.
Enzyme preparation with 3.2 mg of protein and 5.2 units of avicelase per ml was used. Symbols: ●—●, Avicel SF; ●---●, Xylan; ○—○, Filter paper (Whatman No. 1); ◒—◒, KC-flock W-100; ○---○, absorbant cotton. Results indicate that various kinds of enzymes that hydrolyze glycans were produced by *Bacillus* sp. K-12 in the culture broth.

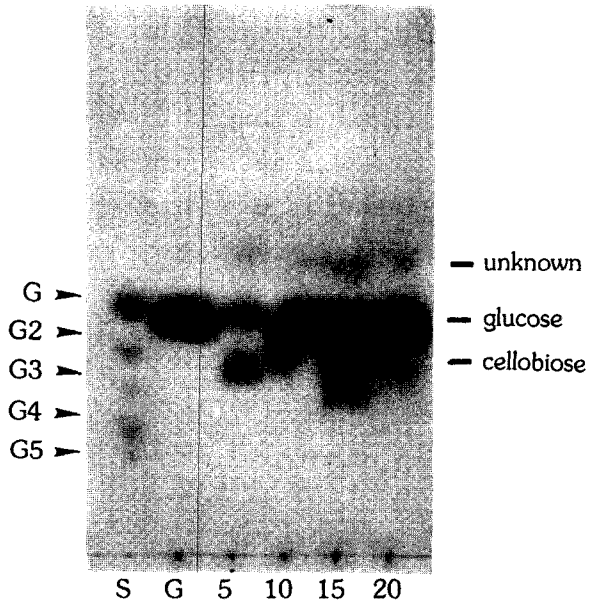

Fig. 6. Hydrolysis of avicel SF by the crude enzyme solution.
The reaction mixture containing of 0.2 ml of 0.1% avicel suspension in 0.05 M sodium phosphate buffer (pH 7.5) and 0.1 ml of enzyme solution (80 μg of protein) was incubated at 60℃. At time intervals (min), the reaction mixture was sampled and analyzed by thin layer chromatography. G, glucose; G2, cellobiose; G3, cellotriose; G4, cellotetrose; G5, cellopentose. "S" denotes standard mixture containing a series of cellooligosaccharides. "Unknown" denotes "unknown compounds" in this analytical system.

Hydrolysis of Various Glycans by the Culture Broth

As shown in Table 3, the enzyme system produced by *Bacillus* sp. K-12 was capable of hydrolyzing various kinds of glycans. These results indicate that various kinds of enzymes needed to hydrolyze glycans were produced by *Bacillus* sp. K-12 in the culture broth. Also this enzyme preparation may be able to digest other insoluble substrates such as hemicellulose and laminarin because of its high concentration of xylanase and laminarinase as shown in Table 3.

Comparison of Cellulase

Table 4 compares the typical contents of this cellulase components produced by *Bacillus* sp. K-12 with those of the cellulase components produced by *C. thermocellum* (17). As can be seen in Table 4, the composition

ratio of FPase: avicelase: CMCase: β-glucosidase of the cellulolytic enzyme system produced by strain *Bacillus* sp. K-12 was 1.0:1.1:8.7:1.7, while in the case of the enzyme system produced by *C. thermocellum*, the ratio was 1.0:1.4:20.0:3.0. These results indicate that the enzyme system found in strain K-12 has a remarkably high avicelase and FPase content.

DISCUSSION

For several decades, almost all cellulase studies have involved the searches for sources of the enzymes from fungi, mostly from the cultivation of biomass resources, however, cellulolytic enzymes produced by bacteria have not found in industrial use as yet. Recently, in order to use to cellulase systems of bacteria, cellulases of *C. thermocellum* were exclusively studied, however, this bacterium also could not be used for practical utilization of biomass (see introduction). As an alternative, alkalophilic *Bacillus* sp. were isolated and characterized. Alkaline cellulases found so far have been very few. Most cellulases of microbial origin are known to have pH optima in the acidic or neutral range, therefore alkaline cellulase is useful because of the alkalinity of detergent compositions. In the present study, we have described the isolate of slightly alkalophilic *Bacillus* sp. K-12, which secretes an inducible, slightly alkaline and thermotolerent cellulase.

The cellulase system produced by the strain, K-12, exhibits remarkably higher β-glucosidase, avicelase and FPase activities, which are very important for the enzymatic conversion of crystalline raw cellulose into oligosaccharides, and finally glucose (Fig. 6), than in the case of the conventionally known cellulase systems produced by several strains of *C. thermocellum, T. reesei*, etc. Furthermore, since the cellulolytic enzyme system produced by strain K-12 is aerobic, comparatively thermostable and moderately alkalophilic, it permits the saccharification of cellulose at the higher temperatures than those at which the saccharification has been carried out using fungal cellulase, and can protect the culture systems against some bacterial and fungal contamination (6).

The CMCase, and β-glucosidase activities reached the maximum after 4 days of incubation at 60℃ with high activites of avicelase, FPase and xylanase. These cellulase activities were detected in the culture broth during the incubation period. This bacterial cellulase system has a comparatively higher activity than that of *C. thermocellum*, one of the best cellulase producer among bacterial species.

The culture broth of *Bacillus* sp. K-12 strongly degraded filter paper strips at 60℃ for 6 h and hydrolyzed 95% and 90% of CMC and avicel to glucose. Moreover, outstanding production of xylanase marked this cellulase system to be effective. The crude enzyme was optimally active at 60℃ and pH 8.5. In addition, K-12 also produced xylanase, β-glucosidase and so on. From this experimental data, it was proposed that K-12 might become an advantageous source of various enzymes reuqired for utilization of the biomass. Details of the other properties of the novel type of cellulase from *Bacillus* sp. K-12 and molecular cloning of the genes encoding the cellulase will be reported in a subsequent paper.

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