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BIOTECHNOLOGICAL APPLICATION OF ALKALINE CELLULASE IN FOOD TECHNOLOGY

Ali, S. G.

Botany and Microbiology Department, Faculty of Science, Al-Azhar University, Assiut

ABSTRACT :

The alkalophilic *Bacillus macernas* SM was isolated from polluted *Solanum tuberosum* wastes in Upper Egypt. It produced high level of extracellular alkaline cellulase. The maximum enzyme production induced at a substrate concentration of 0.5%; temperature and pH optima were 40°C and 8 respectively, an optima carbon and nitrogen sources essential for the best yield of enzyme were cellulose and potassium nitrate respectively. On the other hand the best yield of cellulase was obtained after 24h incubation in 1000ml flask volume under static fermentation conditions. The optimal cultural and nutritional conditions for production of maximal alkaline cellulase for application study resulted in: Increasing of maceration yield up to 85.61% of potato pulp followed by increasing a clarification yield 15.27% in guava and filtration yield 70.28 % in tomato juice. The best preservation conditions of enzyme used of both guava clarification and tomato filtration were detected at room temperature. The present cellulase may be of remarkable application in the field of biodetergents and in food processing industry in large-scale.

INTRODUCTION:

Cellulase enzymes are a complex of various endo-B-gluconases, exo-B-glconases, and cellobiase isolated from various sources (Acebal *et al* .1986). The most common pectinase commercially available is a complex preparation produced from *Aspergillus niger* (Petruccioli and Federici, 1992) in which pecteolytic enzymes such as polygalacturonase, pectinesterase etc. as well as hemicellulases, cellulases and proteases are all present (Fogarty and Kelly, 1983; Lambert, 1983 and Ammar *et al.* 1995). Production of the components of the cellulase complex by *Trametes trogii* was investigated under different culture conditions (Levin and Forchiassin ,1997).

The alkaline cellulase produced by alkalophilic *Bacillus sp.* N6-27 was purified to electrophoresis homogeneity by $(NH_4)_2SO_4$ fractionation, The optimum temperature and pH for the enzymatic catalysis were 55 °C and 8.5 respectively. The enzyme activity was stable under 50°C and in the pH range of 6-11. The substrate was carboxymethylcellulose (CMC), the enzyme activity was strongly inhibited by Fe^{2+}, Cu^{2+} and Hg^{2+} (Tian and Wang, 1998). Some authors have reported that as the applied agitation rate increases, the apparent activity of the endoglucanases from *Trichoderma reesei* towards cotton cellulose increases more markedly than does the apparent activity of the cellobiohydrolases (Cortez *et al.* 2001). The effects of commercial pectolytic and cellulolytic

enzyme preparations on the apple cell wall was studied by Dongowski and Sembries (2001).

Dongowski *et al. (*2002) studied the degradation of apple cell wall material by commercial enzyme preparations. Colombatto *et al.*(2003) studied the influence of fibrolytic enzymes on the hydrolysis and fermentation of pure cellulose and xylan by mixed ruminal microorganisms in vitro and concluded that the enzymes enhanced the fermentation of cellulose and xylan by a combination of pre- and postincubation effects. The alkalophilic *Bacillus circulans* D1 was isolated from decayed wood produced high levels of extracellular cellulase-free xylanase (Bocchini *et al* .2003).

MATERIALS AND METHODS:

Media used:

1-Isolation medium: It contains (g/l): cellulose, 10; Beef extract,3; Peptone, 5; NaCl, 5.0 and distilled water up to 1000ml adjusted to pH 8. The ingredients were dissolved by heating in the water bath and sterilized at 121°C for 15 min.

2-Enzyme production medium: It contains (g/l): cellulose, 7.5; KNO_3, 2.5; Beef extract, 1.5;Peptone, 2.5; NaCl,2.5; pH 8 and then inoculation cultures were incubated in 1000 ml flask at 40°C under static conditions.

3-Assay media for cerllulase: This was carried out as in Ali (2000).

Bacterium used:

The bacterial strain under investigation isolated from polluted *Solanum tuberosum* wastes in Upper Egypt and identified as *Bacillus macernas* SM.

Methods:

A-Parameters controlling enzyme production:

1-Substrate concentration: Different concentrations of cellulose (0.25,0.5,1,2,4,5,6 and 8 gram/flask) were applied. At the end of incubation period cellulase activity was determined.

2-pH: Different pH values covering the range of 1-11 were prepared using either NaOH (IN) or HCl(IN) then the cellulase activity has been carried out.

3-Temperature: *Bacillus macernas* SM was allowed to grow on the production medium and incubated at different temperatures ranged from 30-60°C and finally tested for cellulase activity.

4-Effect of carbon sources: Carbon sources represented by: pectin, lactose, fructose, mannose, galactose, manitol, glucose dextrose, sucrose, and cellulose were introduced into the basal medium at the level of 1%.

5-Static and shaking conditions: Production media were exposed to both static and shaking conditions separately then tested for cellulase activity at the end of incubation time.

6-Nitrogen source: Production medium was supplemented with different nitrogen sources viz. ammonium sulfate, calcium nitrate, urea, peptone, potassium nitrate, glycine and sodium nitrate. The nitrogen sources were added at concentration (0.5%), other conditions were performed under previously determined optimal ones.

7-Incubation time: *Bacillus macernas* SM was allowed to grow on the production medium and incubated at different periods viz. 6, 12, 24, 48 and 62 h. at 40°C . At the end of each incubation interval, cellulase activities were assayed.

8-Flask capacities: The production media was inoculated by *Bacillus macernas* SM using five flasks of various capacities viz. 100,250,500,1000 and 2000ml. At the end of incubation period, cellulase activities were assayed.

B-Application of alkaline cellulase in food industries:

1-Potato maceration: This experiment was performed by mixing equal amounts (1ml) of both citrate-phosphate buffer at different pH values (2 , 3 ,4, 5 ,8 and 9) and alkaline cellulase followed by incubating the reaction mixtures with potato disks, then determining the final weight after 9 h at 30°C. Potato maceration was determined by weighting the left disks of potato after the incubation.

2-Guava clarification: Different cellulase concentrations viz: 62.8, 125.6, 251.20, 1256 and 6280 (units/ml) were added separately to a mixture pulp (2 ml of distilled water plus 2g. of Egyptian guava pulp). The reaction mixture was incubated at 30°C for 9h. Guava clarification was determined by weighing each mixture alone.

3-Tomato filtration: This experiment was designed to determine the minimum concentration of alkaline cellulase to be used in the filtration of tomato juice. The applied concentrations were (units/ml) viz. 125.6, 251.20, 1256 and 6280.

4-Preservation conditions: This experiment was designed to determine the optimal storage temperature for alkaline cellulase in both clarification and filtration processes. Aliquots of enzyme solution were incubated at both room temperature and refrigerator. Samples were assayed after 9h. at 30°C for both clarification and filtration of guava and tomato respectively.

RESULTS:

A-Parameters controlling enzyme production:

1-Carbon sources: Results represented graphically in Fig.(1) showed that the optimum carbon sources introduced into the production medium to obtain the maximum yield of enzyme were cellulose followed by galactose.

2-Nitrogen sources: The results graphically illustrated in Fig. (2) indicated that *Bacillus macernas* SM prefers to utilize glycine as nitrogen source to fulfill the maximum cellulase yield. Potassium nitrate came next to glycine in enhancing enzyme production

3-pH value: Fig. (3) showed that the maximum alkaline cellulase activity was determined at pH 8.

4-Static and shaking conditions: As shown in Fig. (4), both static and shaking cultural conditions are suitable for cellulase production. However, the yield of cellulase was slightly higher at static conditions.

5-Substrate concentration: It could be concluded from the results illustrated in Fig. (5) that the maximum cellulase production was obtained in the presence of 0.5% of cellulose.

6-Temperature: Data illustrated in Fig. (6) showed that the optimal incubation temperature that fulfilled the maximum yield of cellulase production was at 40°C.

7-Incubation time: Data in Fig.(7) showed that *Bacillus macernas* SM was able to grow and produce the highest yield of cellulase at the end of 24h., shorter or longer of incubation periods gave lower yield of cellulase.

8-Flask volume: Data recorded in fig.(8) showed that 1000ml flask containing 100 ml of the medium was more favorable for cellulase production, beyond this particular volume cellulase yield decreased sharply.

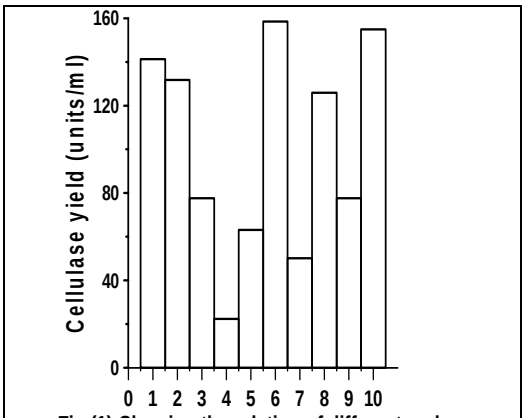

Fig.(1):Showing the relation of different carbon sources to cellulase production by Bacillus macernas. (1-Glucose,2- Maltose,3-Dextrose,4- Fructose,5- Lactose, 6-Cellulose,7- Sucrose,8- Mannitol,9- Starch,10- Galactose)

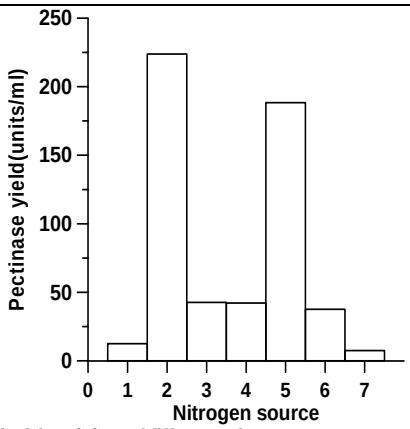

Fig.(2):Relaion of fifferent nitrogen sources to pectinase production by Bacillus macernas.(1-Amm.sulphate, 2-Glycine, 3-Urea,4-Peptone,5-Potassium nitrate. 6-Calcium nitrate,7-Amm.chloride)

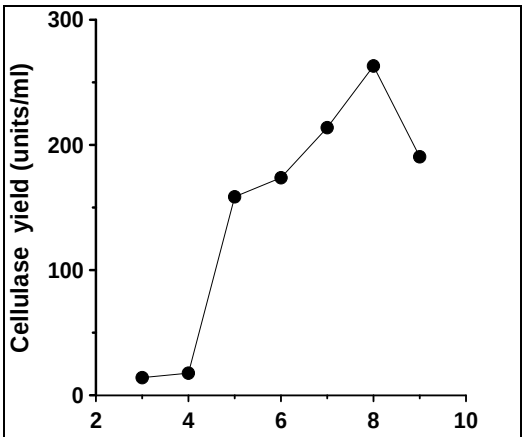

Fig.(3):Relation of various pH values to cellulase production by B.macernas.

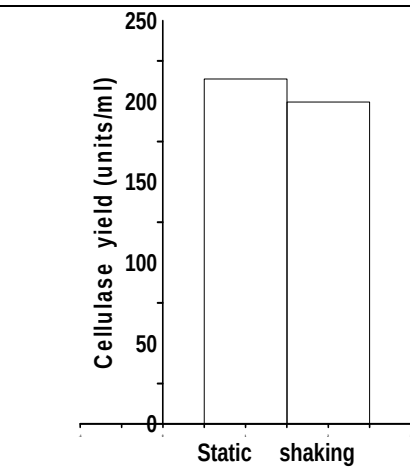

Fig.(4):Relation of both static and shaking conditions to cellulase production by Bacillus macernas.

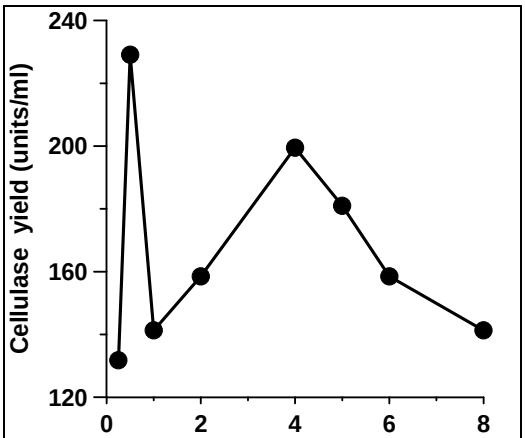

Fig.(5): Relation of different substrate concentration to cellulase production.

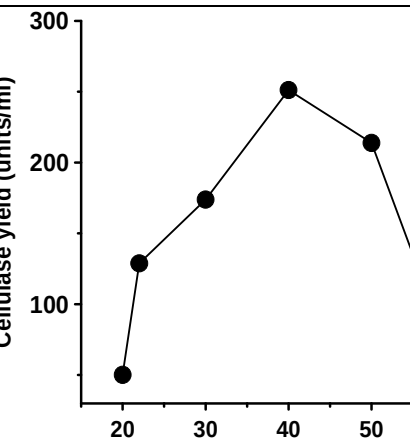

Fig.(6): Relation of incubation temperatures to cellulase production.

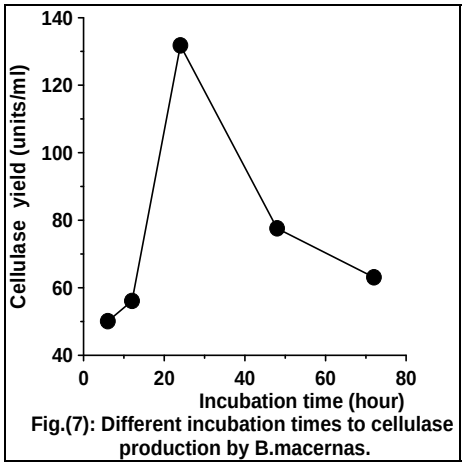

Fig.(7): Different incubation times to cellulase production by B.macernas.

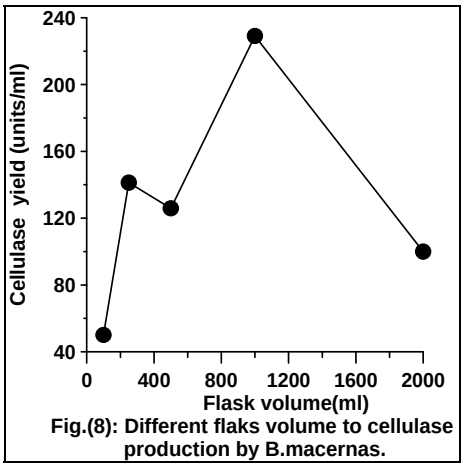

Fig.(8): Different flaks volume to cellulase production by B.macernas.

B-Application of alkaline cellulase in food industries:

1-Potato maceration: Data recorded in table (1) showed that a pH range of 3-5 was suitable for maceration of potato disks and the highest maceration yield (85.61%) was obtained at pH 4 comparable to control (46.03%).

Table(1): Effect of pH values on the maceration of potato pulp.

pH	Maceration yield (%)
2	34.09
3	81.02
4	85.61
5	80.57
8	58.28
9	46.65
Control (no enzyme)	46.03

2-Guava clarification: As shown in table (2) clarification of guafa pulp increased proportionally with the increase in enzyme concentration and the best clarification yield (15.27%) was obtained at a concentrations of 6280 units/ml.

Table (2): Different concentration of cellulase to clarification yield of guava pulp.

Enzyme concentration (units/ml)	Clarification yield (%)
62.8	4.48
125.6	5.71
251.20	7.09
1256	7.71
6280	15.27
Control (no enzyme)	9.00

3-Tomato filtration: Results in table (3) indicated that the minimum concentration of cellulase enzyme to be used for the filtration of tomato juice was 251.20 units/ml corresponding to 70.28% filtration yield.

Table (3): Different concentration of cellulase to clarification yield of Tomato filtration juice.

Enzyme concentration (Units/ml)	Filtration yield (%)
125.6	54.14
251.20	70.28
1256	50.83
6280	66.42
Control (no enzyme)	36.61

4-Preservation conditions: As shown in table(4), the optimal incubation temperature for preservation of both guava clarification and tomato filtration were detected at room temperature.

Table (4): Different condition for preservation of clarification and filtration processes.

Preservation conditions	Yield (%)	
	Guava clarification	Tomato filtration
Room temperature (30°C)	22.55	25.87
Refrigerator (10°C)	15.65	33.18

DISCUSSION:

The alkalophilic *Bacillus macernas* SM was isolated from polluted *Solanum tuberosum* wastes in Upper Egypt, produced high levels of extracellular alkaline cellulase. Some environ-

mental and nutritional parameters controlling the productivity of alkaline cellulase under bench scale fermentation conditions has been undertaken. Similarly, the alkaline cellulase produced by alkalophilic *Bacillus* sp. N6-27 was purified to electrophoresis homogeneity by $(NH_4)_2SO_4$ fractionation (Tian and Wang,1998).

Data of the present work showed that the best substrate concentration that fulfill the highest yield of alkaline cellulase was 0.5%. Irwin *et al.*(2003) tested corn fiber hydrolysis by *Thermobifida fusca* extracellular enzymes and reported that the activity assays on a variety of synthetic and natural substrates showed major differences in the concentrated extracellular enzyme activities.

The best carbon and nitrogen sources essential for the highest yield of cellulase enzyme were cellulose and glycine respectively. On the other hand the best yield of cellulase was obtained after 24h incubation in 1000 ml flask volume under static conditions. Working with the wood decaying fungus *Trametes trogii*, Levin and Forchiassin (1997) found that endoglucanase, exoglucanase and beta-glucosidase were produced under shaken and stationary conditions using crystalline cellulose as the only carbon source. They also reported that, in shaken cultures beta-glucosidase values doubled those obtained in stationary conditions. Eight apple pomace liquefaction juices were produced to characterize soluble cell wall material released by the action of pectolytic and celluolytic enzyme preparations. Very high colloid amount from 9.7 to 19.6 g/l were recovered from the juices by ethanol precipitation. The crude polysaccharides consisted mainly of galacturonic acid (49-64 mol %), arabinose (14-23), galactose(6-15) and minor amounts of rhaminose, xylose and glucose (Mehrlander *et al* . 2002).

Studying the effect of various incubation temperatures and pH values indicated that temperature and pH optima were 40°C and 8 respectively.

Optimization of cultural and nutritional conditions of maximal production of alkaline cellulase were used in application study and resulted in: increasing of potato maceration yield up to 85.61% followed by increasing in yield of both clarification and filtration up to 15.27% and 70.28% for guava and tomato respectively; the optimum preservation temperature for both clarification and filtration of guava and tomato was 30°C (room temperature). In relation to these results, Irwin *et al* .(2003) found that saccharification was enhanced by the addition of beta-glucosidase or by the addition of a crude xylanase preparation from *Aureobasidium* sp.

Bocchini *et al.* (2003) mentioned that cellulase-free xylanase preparation was thermally stable up to 60 °C, with an optimal hydrolysis temperature of 70 °C. It was stable over a wide pH range (5.5-10.5), with an optimum pH at 5.5 and 80% of its activity at pH 9.0. This cellulase-free xylanase preparation was used to biobleach kraft pulp, enzymatic treatment of kraft pulp decreased chlorine dioxide use by 23 and 37% to obtain the same kappa number (kappa number) and brightness, respectively.

According to Essa and Salama (2002) pectinase and cellulase enzymes were used to investigate efficacy for improving juice yield, stability and quality from prickly pear fruit, pectinase improved the yield, stable color, color-assayed as release of anthocyanins or carotenoids and clarity of juice. McHan (1986) improved ensiling and fiber digestion of coastel bermudagrass with an alkalizing agent and a cellulase enzyme complex.

In conclusion, the present enzymes may be of remarkable application in both practical and laboratory fields with regard to its action and activities at room temperature.

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التطبيقات البيوتكنولوجية لإنزيمات السيلوليز القلوية في تكنولوجيا صناعة الغذاء

صلاح الدين جمال الدين على

قسم النبات والميكروبيولوجى – كلية العلوم (بنين)– جامعة الأزهر – أسيوط

يهتم هذا البحث باستخدام نوع من البكتريا المعزولة من بعض المخلفات الغذائية لتحفيز إنتاج إنزيمات السيلوليز القلوية واستخدامها في تكنولوجيا صناعة الغذاء. وقد تم استخدام أقوى العزلات البكترية قدرة على إنتاج إنزيمات السيلوليز القلوية *Bacillus macernas* SM. لدراسة الظروف المثلى لإنتاج الإنزيمات، وأفضل زمن تحضين كان لمدة ٢٤ ساعة عند ٤٠°م، وأنسب مصدر كربوني هو السليولوز، والتحضين عند ظروف مزارع ثابتة. كما كان أنسب تركيز لمادة التفاعل ٠,٥%، وأفضل مصدر نيتروجيني كان الجليسين، وأنسب درجة حموضة (pH) كانت عند ٨، وحجم المخمر كان (١٠٠٠ مل).

تمت العمليات التطبيقية بتجميع كل هذه الظروف المناسبة للحصول على أعلى معدل من إنتاج إنزيمات السيلوليز وتطبيقها في مجالات مختلفة وكانت النتائج المتحصل عليها كالتالي: زيادة معدل النقع في لب البطاطس إلى ٨٥,٦١%، زيادة معدل الترويق في عصير الجوافة إلى ١٥,٢٧%. ازداد أيضا معدل الترشيح في عصير الطماطم إلى ٧٠,٢٨%. وقد مثلت الدراسات التطبيقية أن أنسب ظروف للتخزين باستخدام إنزيمات السيلوليز الحالية في عمليات الترويق والترشيح في عصائر الجوافة والطماطم على التوالي كانت عند درجة حرارة الغرفة.

وبناء على هذا يمكن تطبيق هذه الإنزيمات على المستوى المعملي والتجريبي أو على المستوى الصناعي والتجاري نظرا لكفاءتها الملحوظة في العمل والنشاط عند درجة حرارة الغرفة وخصوصا في مجالات ترويق وترشيح العصائر وأشباهها، كما يمكن التركيز على إمكانية استخدامها في مجالات الصناعة والغذاء نظرا للنتائج التي تم التوصل إليها في مجال التطبيق بالإضافة إلى قدرة الإنزيمات الحالية بالعمل والنشاط عند ظروف ثابتة مما يوفر عديد من الآلات والأموال في تكنولوجيا صناعة الغذاء.