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NUTRITIONAL AND ENVIRONMENTAL FACTORS AFFECTING CELLULASES ACTIVITY PRODUCED BY HIGH POTENT CELLULOLYTIC BACILLI

Khadiga, A. Abou –Taleb ; W. A. Mashhoor; Sohair, A. Nasr and M. S. Sharaf

Agric. Microbiol. Dept., Fac. of Agric., Ain Shams Univ., P.O.Box 68, Hadayek Shoubra 11241, Cairo, Egypt.

ABSTRACT

Effect of some nutritional and environmental factors on growth and cellulase production by *Bacillus alcalophilus S39 and Bacillus amyloliquefaciens* $C2_3$ was investigated. Results indicated that 1% carboxymethylcellulose (CMC) and 0.7% yeast extract were most effective as the carbon and nitrogen sources respectively. Initial pH 7 and 3% inoculum size (3.5-4.3 $\times 10^6$ CFU/ml) found to be optimal for growth and cellulase production. Incubation temperature at 30ºC and 45ºC achieved the highest activity of cellulase for *Bacillus alcalophilus S39* and *Bacillus amyloliquefaciens* $C2_3$ respectively, and the suitable shaking rate was 150 and 200 rpm.

Keywords: β-glucosidases, FPase, CMCase, *B. alcalophilus*, *B. amyloliquefaciens*.

INTRODUCTION

Cellulose is the most abundant biomass on the earth (Tomme *et al*., 1995). It is the primary product of photosynthesis in terrestrial environments, and the most abundant renewable bioresource produced in the biosphere (100 billion dry tons/year) (Jarvis, 2003 and Zhang & Lynd, 2004). Cellulose is commonly degraded by cellulase which is produced by several microorganisms, commonly by bacteria and fungi (Bahkali, 1996; Mangelli & Forchiassin, 1999; Shin *et al.*, 2000 and Immanuel *et al*., 2006).

Complete enzymatic hydrolysis of enzymes requires synergistic action of 3 types of enzymes, namely cellobiohydrolase, endoglucanase or carboxymethycellulase (CMCase) and β-glucosidases (Bhat, 2000). Cellulases are used in the textile industry for cotton softening and denim finishing; in laundry detergents for color care, cleaning, and anti-deposition; in the food industry for mashing; in the pulp and paper industries for deinking, drainage improvement, and fiber modification and they are even used for pharmaceutical applications (Kirk *et al*., 2002 and Cherry & Fidantsef, 2003).
Bacteria, which has high growth rate as compared to fungi has good potential to be used in cellulase production. However, the application of bacteria in producing cellulase is not widely used. Cellulase yields appear to depend on a complex relationship involving a variety of factors like inoculum size, pH value, temperature, presence of inducers, medium additives, aeration, growth time, etc. (Immanuel *et al*., 2006).

The present work was carried out to optimize the nutritional and environmental parameters for improving cellulase production by the two cellulolytic bacterial strains.

MATERIALS AND METHODS

Bacteria used

The two bacterial strains, *Bacillus alcalophilus S39* and *Bacillus amyloliquefaciens* $C2_3$ used in this study were isolated form soil and compost respectively and were distinguished as potent cellulase producers. The purified bacilli isolates were identified according to their cultural, morphological and biochemical characteristics based on Bergey's Manual of Systematic Bacteriology (Claus and Berkeley, 1986) and Biolog Automated System was used.

Media used

Nutrient agar (Difco Manual, 1984) was used for the maintenance of *Bacillus* strains. Carboxymethyl cellulose medium recommended by Ray *et al*. (2007) was used for the production of cellulase by *Bacillus* sp. It has the following composition (g/l): Carboxymethylcellulose (CMC), 10; Tryptone, 2; KH_2PO_4, 4; Na_2HPO_4, 4; $MgSO_4.7H_2O$, 0.2; $CaCl_2.2H_2O$, 0.001; $FeSO_4.7H_2O$, 0.004 and pH adjusted to 7.

Standard inoculum and fermentation process

For preparation of standard inoculum, both strains were cultured in nutrient broth individually at 30 ºC for 24 h where an average viable count of 3.5 - 4.3 $\times 10^6$ CFU/ml was obtained. Fermentation was carried out in 250 ml plugged Erlenmeyer flasks, each containing 50 ml sterile production medium and inoculated with 3% of standard inoculum (containing about 3.5 $\times 10^6$ and 4.3 $\times 10^6$ CFU/ml for *Bacillus amyloliquefaciens* $C2_3$ and *Bacillus alcalophilus S39*, respectively). The inoculated flasks were incubated at 30 ºC and 45 ºC for *Bacillus alcalophilus S39* and *Bacillus amyloliquefaciens* $C2_3$, respectively on rotary shaker at 150 rpm for 72 h. After incubation, cultures were centrifuged at 1600 g for 15 min at 4°C and supernatants were used as source of crude enzymes. The crude enzyme solution was utilized for determination of enzyme activities (Kotchoni *et al*., 2003).

Enzyme assays procedures

Carboxymethyl-cellulase(CMCase) activity

CMCase activity was assayed using a method described by Mandels and Weber (1969). The activity was estimated using 1 % solution of carboxymethlycellulose (CMC) in 0.05 M citrate buffer (pH 4.8) as substrate. The reaction mixture contained 1 ml citrate buffer, 0.5 ml of substrate solution and 0.5 ml of diluted enzyme solution. The reaction was carried out at 50°C for 30 min. The amount of reducing sugar released in the hydrolysis was measured. One unit of CMCase activity was expressed as 1 µ mol of glucose liberated per ml enzyme per minute under the previous circumstance.

Filter-paperase (FPase) activity

The activity of FPase was assayed according to the method explained by Mandels and Weber (1969). This method is similar to the CMCase assay method, but the substrate was Whatman No. 1 filter paper strip (1 x 6 cm) soaked in 1 ml 0.05 M sodium citrate buffer (pH 4.8). The samples were incubated with 0.5 ml enzyme solution at 50°C for 1 h, the reducing sugars liberated during growth were determined. One unit of FPase

activity was determined as 1 µ mol of glucose liberated per ml enzyme per minute under the previous circumstance.

β-Glucosidase activity

One-tenth ml of the culture supernatant was incubated with 0.5 ml of 0.05 M acetate buffer (pH 5) containing 2.5 mg cellobiose. After incubation at 50 ºC for 10 min the glucose released was measured by the glucose oxidase peroxidase method (Zaldívar *et al*., 2001).

Determination of reducing sugars

It was determined by the method recommended by Park and Johnson (1949).

Carbon sources

The appropriate carbon source was selected by replacing the original carbon substrate of the basal medium with equivalent carbon amount of each of the tested carbon sources (Glucose, Carboxymethycellulose, Cellobiose and Cellulose).

Nitrogen sources

To detect the adequate nitrogen source for cellulase production by selected strains, the prescribed nitrogen source of the fermentation medium was replaced by equivalent nitrogen amount of each of the tested nitrogen either organic [Beef extract, Casein, Malt, Peptone, Tryptone, Urea & Yeast extract] or inorganic [KNO_3, $(NH_4)_3PO_4$, $NaNO_3$, NH_4NO_3, NH_4Cl & $(NH_4)_2SO_4$] nitrogen sources.

pH

Seven values of pH ranged between 5.5 and 8.5 were chosen for studying their effects on cellulase enzyme activity.

Incubation temperature

Nine levels of incubation temperature were tested ranged from 5 to 55ºC.

Agitation speed

Erlenmeyer flasks (250 ml) containing production medium were inoculated with the selected bacteria and placed onto a rotary shaker at different rpm ranged between 50 to 200 rpm comparing to static condition to obtain proper aeration for maximal cellulase production.

Inoculum size

The inoculum size was optimized for maximal enzyme production. The fermentation medium was inoculated with 1, 2, 3, 4, 5, 6 and 7 % of standard inoculum.

Statistical analysis

The collected data were statistically analyzed using SPSS Computer Analysis Programs (Foster, 2001).

RESULTS AND DISCUSSION

Effect of different carbon sources

Data presented in Table (1) show that Carboxymethylcellulose (CMC) was most effective as a sole carbon source for cellulase enzyme produced by *Bacillus alcalophilus S39* and *Bacillus amyloliquefaciens* $C2_3$

being 1.81 & 1.88 U/ml of CMCase activity, 0.87 & 0.86 U/ml of FPase activity and 1.31 & 1.41 U/ml of β-glucosidases, respectively.

These results are in agreement with those obtained by Narasimha *et al*., (2006) and Niranjane *et al*., (2007) who found that carboxymethycellulose was the best carbon source followed by cellulose for cellulase production. A higher production of cellulase when CMC served as substrate may be as a result of induction of the enzyme, since cellulose is known to be a universal inducer of cellulase synthesis. Paul and Varma (1993) had reported the induction of endocellulase by CMC.

Table (1): Effect of carbon sources on the activity of cellulase produced by *B. alcalophilus S39* and *B. amyloliquefaciens* $C2_3$

Different carbon sources	*Bacillus alcalophilus S39*				*Bacillus amyloliquefaciens* $C2_3$			
	Biomass g/100 ml	Cellulase Activity (U/ml)			Biomass g/100ml	Cellulase Activity (U/ml)		
		CMCase	FPase	β-glucosidases		CMCase	FPase	β-glucosidases
Glucose	0.501[a]	0.23[e]	0.25[f]	0.05[f]	0.484[b]	0.30[e]	0.10[e]	0.04[f]
Carboxymethycellulose (CMC) (Control)	0.361[f]	1.81[a]	0.87[a]	1.31[b]	0.382[e]	1.88[a]	0.86[a]	1.41[a]
Cellobiose	0.403[c]	0.71[d]	0.19[d]	0.13[e]	0.392[d]	1.32[b]	0.34[c]	0.06[f]
Cellulose	0.157[h]	1.00[c]	0.41[c]	1.27[c]	0.295[g]	1.43[b]	0.51[b]	1.24[d]

Values in the same column followed by the same letter do not significantly differ from each other, according to Duncan's at 5 % level.

Medium containing glucose as the growth carbon source presented the minimum cellulase activity (expressed by CMCase, FPase and β-glucosidase). Muthuvelayudham and Viruthagiri (2006) obtained similar results which showed that the cellulase activity was less when glucose was used as carbon source due to feedback inhibition.

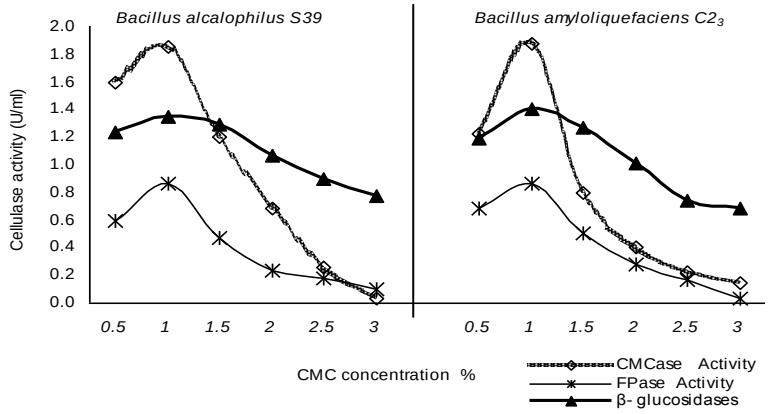

Figure (1): Effect of different concentrations of carboxymethylcellulose (CMC) on the activity of cellulase produced by *B. alcalophilus S39* and *B. amyloliquefaciens* $C2_3$.

Another experiment was carried out to study the effect of different concentrations of carboxymethylcellulose (CMC) which exhibited superiority among other tested carbon sources for *Bacillus* strains. Data in Figure (1) clearly show that 1% carboxymethylcellulose (CMC) gave the highest activity of cellulase being 1.85 & 1.88 U/ml of CMCase ; 0.87 & 0.87 U/ml of FPase and 1.35 & 1.40 U/ml of β-glucosidases by *B. alcalophilus S39* and *B. amyloliquefaciens* $C2_3$, respectively. This is similar with previous investigations (Fukumori *et al*., 1985; Kawai *et al*., 1988 and Shikata *et al*., 1990), where the CMCase activity in *Bacillus* sp. was detected in cultures that contained 1% (w/v) CMC as the growth substrate.

Effect of different nitrogen sources

Data revealed that the supplementation of organic and inorganic nitrogen sources stimulated the cellulase activity. Using of organic N sources responded in the positive cellulase activity more than the inorganic ones. Among the tested complex N sources, the effectiveness in supporting cellulolytic activity by both tested bacilli significantly decreased in the following order : yeast extract > peptone > beef extract > NH_4Cl. Results recorded in Table (2) clearly show that yeast extract was the best nitrogen source for cellulose activity being 2.07 & 2.17 U/ml of CMCase, 0.99 & 1.01 U/ml of FPase and 2.18 & 2.55 U/ml of β-glucosidases for *Bacillus alcalophilus S39* and *Bacillus amyloliquefaciens* $C2_3$, respectively. Data are in accordance with the results of Ray *et al*., (2007) who reported that organic nitrogen sources were found to be more suitable for optimizing cellulase production by *B*acillus *subtilis* and *Bacillus circulans* than inorganic sources.

Table (2): Effect of nitrogen sources on the activity of cellulase by produced by *B. alcalophilus S39* and *B. amyloliquefaciens* $C2_3$

Nitrogen sources	*Bacillus alcalophilus S39*				*Bacillus amyloliquefaciens* $C2_3$			
	Biomass g/100ml	Cellulase Activity (U/ml)			Biomass g/100ml	Cellulase Activity (U/ml)		
		CMCase	FPase	β-glucosidases		CMCase	FPase	β-glucosidases
Beef extract	0.417^{c}	2.05^{b}	0.98^{c}	2.04^{c}	0.420^{b}	1.99^{c}	0.99^{c}	2.25^{b}
Casein	0.395^{gh}	0.41^{l}	0.04^{n}	0.67^{lmn}	0.413^{d}	0.03^{n}	0.01^{o}	0.64^{mn}
Malt	0.356^{p}	1.18^{j}	0.01^{o}	0.73^{lm}	0.380^{k}	0.31^{m}	0.04^{mn}	0.68^{lmn}
Peptone	0.407^{e}	2.05^{b}	0.99^{c}	2.16^{b}	0.381^{k}	2.14^{a}	1.00^{b}	2.47^{a}
Tryptone (Control)	0.360^{o}	1.83^{f}	0.87^{i}	1.34^{h}	0.387^{i}	1.88^{ef}	0.89^{i}	1.41^{gh}
Urea	0.375^{lm}	1.50^{h}	0.47^{k}	0.70^{lmn}	0.385^{ij}	1.64^{g}	0.64^{j}	0.75^{kl}
Yeast extract	0.404^{ef}	2.07^{b}	0.99^{c}	2.18^{b}	0.417^{c}	2.17^{a}	1.01^{a}	2.55^{a}
KNO_3	0.407^{e}	1.24^{i}	0.06^{l}	1.06^{j}	0.420^{b}	0.81^{k}	0.05^{m}	0.81^{k}
$(NH_4)_3PO_4$	0.378^{l}	0.41^{l}	0.05^{m}	0.64^{n}	0.395^{gh}	1.14^{j}	0.02^{o}	0.98^{j}
$NaNO_3$	0.374^{lm}	1.88^{ef}	0.89^{h}	1.62^{e}	0.370^{lmn}	1.90^{ef}	0.90^{g}	1.10^{i}
NH_4NO_3	0.397^{g}	1.88^{ef}	0.90^{gh}	1.42^{fg}	0.371^{lmn}	1.89^{ef}	0.92^{f}	1.55^{f}
NH_4Cl	0.359^{o}	1.97^{cd}	0.96^{e}	1.77^{d}	0.361^{o}	1.93^{cde}	0.98^{c}	1.80^{d}
$(NH_4)_2SO_4$	0.424^{a}	1.91^{de}	0.92^{f}	1.49^{f}	0.417^{c}	1.91^{de}	0.97^{d}	1.55^{f}

Values in the same column followed by the same letter do not significantly differ from each other, according to Duncan's at 5 % level.

Data illustrated in Figure (2) obviously indicates that suitable concentration of yeast extract was found to be 0.7% which gave the highest CMCase being 2.35 & 2.30 U/ml; FPase being 1.15 & 1.19 U/ml of and β-glucosidases being 3.56 & 3.49 U/ml of *B. alcalophilus S39* and *B. amyloliquefaciens* $C2_3$, respectively. It is notable at all experiments to state that there was no statistically relationship between the production of cellulase enzyme and biomass yield.

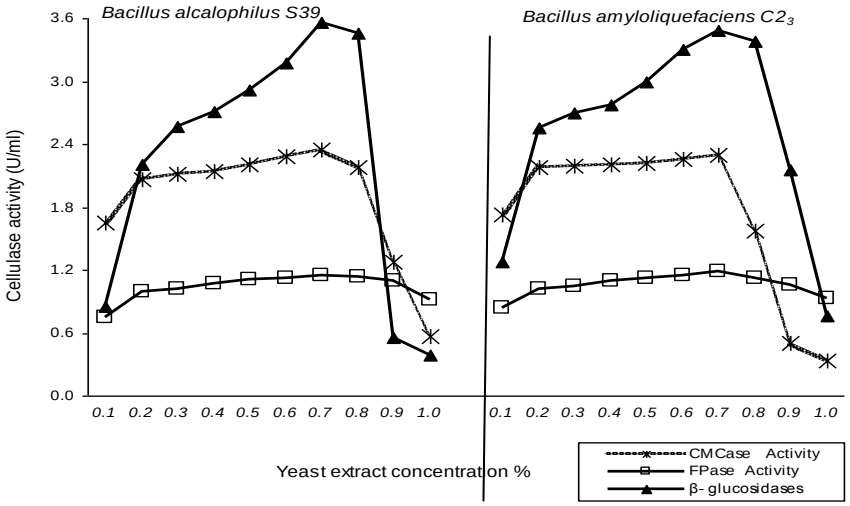

Figure (2): Effect of different concentrations of yeast extract on the activity of cellulase produced by *B. alcalophilus S39* and *B. amyloliquefaciens* $C2_3$.

Effect of initial pH

Results illustrated by Figure (3) clearly show that cellulase activity, gradually increased as the pH values increased from 6.5 to 7.5 and reached its maximum at initial pH of 7 being 2.41 & 2.40 U/ml of CMCase, 1.19 & 1.19 U/ml of FPase and 3.55 and 3.49 U/ml of β-glucosidases by *B. alcalophilus S39* and *B. amyloliquefaciens* $C2_3$, respectively. The cellulase activity was less in other tested pH levels, where enzyme activity was minimal at pH 5.5 and it indicates a marginal increase at pH 6.5 and 7. Further, this activity was greatly reduced to reach the lowest at pH 8.5 (where 2.06 & 2.07 U/ml of CMCase; 1.07 & 1.04 U/ml of FPase and 0.72 and 0.56 U/ml of β-glucosidases was obtained by *B. alcalophilus S39* and *B. amyloliquefaciens* $C2_3$, respectively.

Obtained data confirmed the findings reported by Ray *et al*. (2007) who mentioned that pH 7 – 7.5 more suitable for optimization of cellulase production by *Bacillus subtilis* and *B. circulans*. Furthermore, the cellulolytic enzyme, endogluconase obtained from *Cellulomonas*, *Bacillus*, and *Micrococcus* spp. hydrolyzed substrate in the pH range of 4.0 to 9.0, with maximum activity transpiring at pH 7 (Immanuel *et al*., 2006).

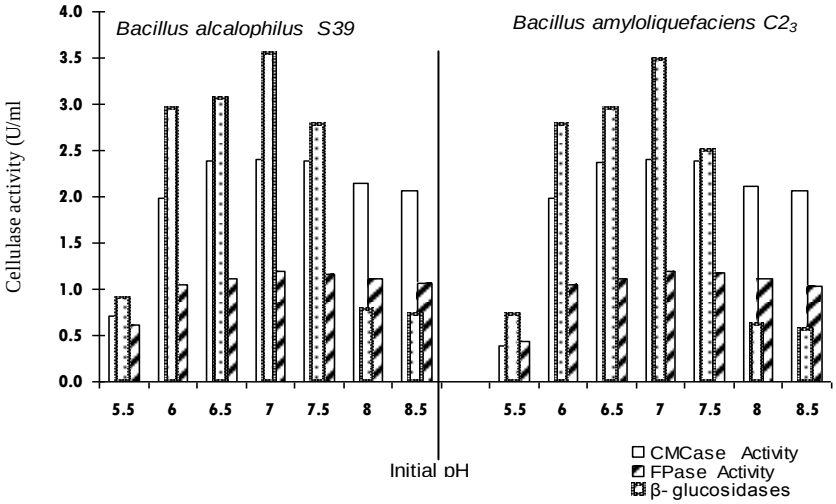

Figure (3): Effect of initial pH on the activity of cellulase produced by *B. alcalophilus S39* and *B. amyloliquefaciens* $C2_3$.

Incubation temperature

It is obvious from Figure (4) that the highest cellulase activity was obtained at temperatures 30 to 45ºC for *B. alcalophilus S39* and *B. amyloliquefaciens* $C2_3$ respectively, whereas it was less at other tested degrees for each strain.

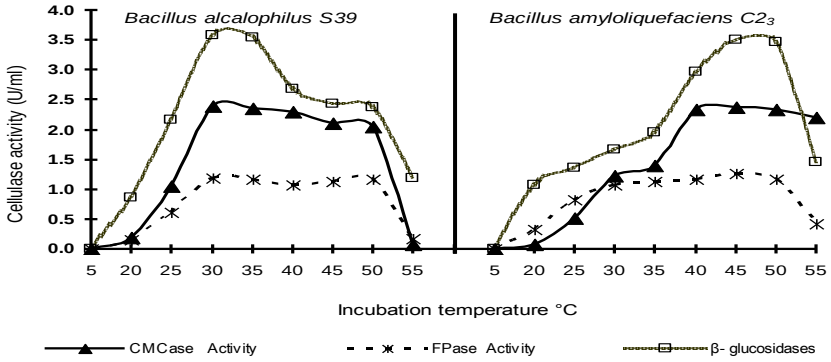

Figure (4): Effect of incubation temperature on the activity of cellulase produced by *B. alcalophilus S39* and *B. amyloliquefaciens* $C2_3$.

These results are closed to those obtained by Bakare *et al*. (2005) who found that the cellulase enzyme produced by *Pseudomonas fluorescence* was activated at 30 to 35 ºC showing the optimum temperature

at 35 ºC. Ray *et al*. (2007) reported that minimum cellulase yield was observed when fermentation was carried out at 45°C, while maximum yield was obtained at 40°C by *Bacillus subtilis* and *Bacillus circulans*. Immanuel *et al*. (2006) also recorded maximum endoglucanase activity in *Cellulomonas*, *Bacillus* and *Micrococcus* sp. at 40°C and neutral pH.

Effect of shaking rate

It was found from the current data (Fig. 5) that the maximum activity was obtained at the range of shaking rate of 150-200 rpm for *B. alcalophilus S39* and *B. amyloliquefaciens* $C2_3$. No significantly different was noticed in enzyme activity produced at rate of 150 and 200 rpm.

Similar data was found by Bin Amwarali Khan and Husaini (2006) who noticed a remarkable increase of cellulase activity in fermentation medium under shaking condition compared to static condition. It was observed more than 2 fold higher cellulase enzyme activity in shaking condition (2.97 IU/ml) compare to non shaking condition (1.38 IU/ml) for *Bacillus amyloliquefaciens* UMAS 1002 strain. They also reported that the highest cellulase enzyme production by *Bacillus amyloliquefaciens* UMAS 1002 strain were 2.97 and 2.89 IU/ml at agitation speed of 100 and 200 rpm, respectively.

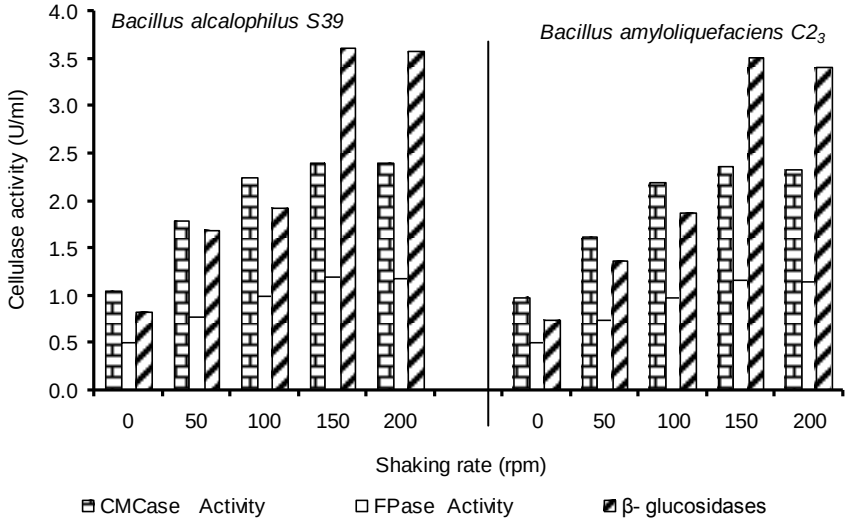

Figure (5): Effect of agitation speeds (rpm) compared to static condition on cellulase activity produced by *B. alcalophilus S39* and *B. amyloliquefaciens* $C2_3$.

Inoculum size:

The illustrated data in Fig. (6) reveal that inoculation with 3.0 % ($3.5X4.3\ 10^6$ CFU/ml) was enhanced cellulase activity being 2.40 & 2.39 U/ml of CMCase; 1.20 & 1.18 U/ml of FPase and 3.61 & 3.53 U/ml of β-

glucosidases by *B. alcalophilus S39* and *B. amyloliquefaciens* $C2_3$, respectively.

These results were almost similar with findings collected by Ray *et al*. (2007) elucidated the enzyme production increased gradually up to 3% inoculum size, but decreased thereafter. The enzyme production by both strains *Bacillus subtilis* and *Bacillus circulans* in 3% inoculum size was not significantly different ($P < 0.05$) from that in 2% inoculum size.

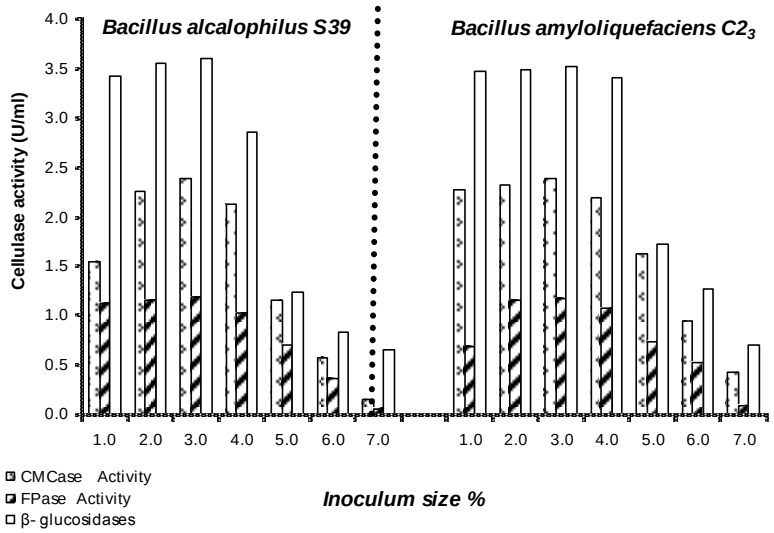

Figure (6): Effect of inoculum size (%) on the production of cellulase activity of *B. alcalophilus S39* and *B. amyloliquefaciens* $C2_3$.

In the present study, it could be concluded that carbon and nitrogen sources, pH value, temperature, inoculum size and aeration play a most crucial role in cellulase production by *B. alcalophilus* and *B. amyloliquefaciens*.

REFERENCES

Bahkali, A. H. (1996). Influence of various carbohydrates on xylanase production by *V. tricorpus*. *Bioresource Technol.,* 33: 265 - 268.

Bakare, M. K.; I. O. Adewale; A. Ajayi and O.O.Shonukan (2005). Purification and characterization of cellulase from the wild-type and two improved mutants of *Pseudomonas fluorescens*. Afr. J. Biotechnol., 4:898-904.

Bhat, M.K. (2000). Cellulases and related enzymes in biotechnology. Biotechnol. Adv., 18: 355-383.

Bin Amwarali Khan, F. A. and A. A. S. A. Husaini (2006). Enhancing α–amylase and cellulase in vivo enzyme expressions on sago pith residue using *Bacillus amyloliquefaciens* UMAS 1002. Biotechnology. 5: 391- 403.

Cherry, J. R. and A.L. Fidantsef (2003). Directed evolution of industrial enzymes: an update. Curr. Opin. Biotechnol., 14: 438 - 443.

Claus, D. & R. C. W. Berkeley, (1986). Genus *Bacillus*. In Bergey's Manual of Systematic Bacteriology, vol. 2, pp. 1105-1139. Sneath, P. H.; N. Mair; M. E. Sharpe & J. G. Holt (eds.). Williams & Wilkins Co., Baltimore, USA.

DIFCO Manual (1984). Dehydrated culture Media and reagents for Microbiology. 10th Ed. Difco Laboratories, Detroit, M. (ed.) U.S.A.

Foster, J. J. (2001). Data analysis using SPSS for Windows Versions 8 –10. Foster, J.J. (ed.), Sage publication Ltd. London.

Fukumori, F.; T. Kudo and K. Horikoshi (1985). Purification and properties of a cellulase from alkalophilic *Bacillus* sp. No. 1139. J. Gen. Microbiol., 131: 129-135.

Immanuel, G.; R. Dhanusa; P. Prema and A. Palavesam (2006). Effect of different growth parameters on endoglucanase enzyme activity by bacteria isolated from coir retting effluents of estuarine environment. Int. J. Environ. Sci.Tech., 3: 25-34.

Jarvis, M. (2003). Cellulose stacks up. Nature. 426: 611- 612.

Kawai, S.; H. Okoshi; K. Ozaki; S. Shikata; K. Ara and S. Ito. (1988). Neutrophilic *Bacillus* strain, KSM-522, that produces an alkaline carboxymethyl cellulase. Agric. Biol. Chem., 52: 1425-1431.

Kirk, O.; T. V. Borchert and C. C. Fuglsang (2002). Industrial enzyme applications. Curr. Opin. Biotechnol., 13: 345-351.

Kotchoni, O.S.; O.O. Shonukan and W.E. Gachomo (2003). *Bacillus pumilus* BpCRI 6, a promising candidate for cellulase production under conditions of catabolite repression. Afr. J. Biotechnol., 2:140-146.

Mandels, M. and J. Weber (1969). The production of celluases. In: Cellulases and their applications. Hajny, G.J. and E.T. Resse (ed.), Amer. Chem. Soc. Adv. Ser., 95:391- 414.

Mangelli, P. and F. Forchiassin (1999). Regulation of the cellulase complex production by *Saccobolus saccaboloides*, induction and repression by carbohydrates. Mycologia. 91: 359 - 364.

Muthuvelayudham, R. and T. Viruthagiri (2006). Fermentative production and kinetics of cellulase protein on *Trichoderma reesei* using sugarcane bagasse and rice straw. Afr. J. Biotechnol., 5: 1873-1881.

Narasimha G.; A. Sridevi; B. Viswanath; S. M. Chandra and R. B. Reddy (2006). Nutrient effects on production of cellulolytic enzymes by *Aspergillus niger*. Afr. J. Biotechnol., 5: 472- 476.

Niranjane A. P.; P. Madhou and T. W. Stevenson (2007). The effect of carbohydrate carbon sources on the production of cellulase by *Phlebia gigantea*. Enzyme Microbial Technol., 40: 1464-1468.

Park, J.T. and M.J. Johnson (1949). A submicro-determination of glucose. J. Biol. Chem., 181: 149-151.

Paul, J.; A. K. Varma (1993). Hydrolytic enzymes production in *Micrococcus roseus* growing n different cellulosic substrates. Lett. Appl. Microbiol., 16:167- 169.

Ray, A. K.; A. Bairagi; K. S. Ghosh and S. K. Sen (2007). Optimization of fermentation conditions for cellulase production by *Bacillus subtilis* CY5 and *Bacillus circulans* TP3 isolated from fish gut. Acta Ichthyologica ET Piscatoria. 37: 47- 53.

Shikata, S.; K. Saeki; H. Okoshi; T. Yoshimatsu; K. Ozaki; S. Kawai and S. Ito (1990). Alkaline cellulase for laundry detergents: production by alkalophilic strains of *Bacillus* and some properties of crude enzymes. Agric. Biol. Chem., 52: 91-96.

Shin, C.S.; J.P. Lee; P.S. Lee and S.C. Park (2000). Enzyme production of *Trichoderma reesi* Rut C-30 on a various lingocellulosic substrates. Appl. Biochem. Biotechnol., 86: 237-245.

Tomme, P., R. A. Warren and N. R. Gilkes (1995). Cellulose hydrolysis by bacteria and fungi. Adv. Microb. Physiol., 37: 1- 81.

Zaldívar, M.; J. C. Velásquez; I. Contreras and L. M. Pérez (2001). *Trichoderma aureoviride* 7-121, a mutant with enhanced production of lytic enzymes: its potential use in waste cellulose degradation and/or biocontrol. EJB Electronic J. Biotechnol., 4:1-9. http://www.ejb.org/content/vol4/issue3/full/7

Zhang, Y-HP. and L.R. Lynd (2004). Toward an aggregated understanding of enzymatic hydrolysis of cellulose: noncomplexed cellulose systems. Biotechnol. Bioeng., 88: 797- 824.

العوامل الغذائية والبيئية المؤثرة علي نشاط السليوليز المنتج بواسطة سلالتين من العصويات ذات الكفاءة العالية المحللة للسليلوز

خديجة أحمد أبوطالب ، وجدى عبد المنعم مشهور، سهير أحمد نصر و محمد سعيد شرف

قسم الميكروبيولوجيا الزراعية- كلية الزراعة- جامعة عين شمس- شبرا الخيمة- القاهرة- مصر.

تم إجراء هذا البحث لدراسة تأثير بعض العوامل الغذائية والبيئية على النمو ونشاط انزيم السليوليز بواسطة *Bacillus amyloliquefaciens* $C2_3$ و *Bacillus alcalophilus* S39 . وأشارت النتائج الى ان كربوكسى ميثيل السليلوز كان أفضل مصدر كربون وأن مستخلص الخميرة هو أفضل مصدر نيتروجينى بتركيز 1٪ ، و0,7٪ علي الترتيب فى بيئة الإنتاج لإعطاء أقصى نشاط لإنزيم السليوليز.

كما وجد أن أنسب درجة من الـ pH الأولَي هي 7 ، وأفضل كمية لقاح هي 3 ٪ (4,3-3,5 × 10^6 مستعمرة/مل) للنمو وإنتاج أنزيم السليوليز، وأن أنسب درجة حرارة تحضين هي 30، 45° م لميكروبي*Bacillus alcalophilus* S39 و*Bacillus amyloliquefaciens* $C2_3$ علي الترتيب للحصول علي أعلي نشاط لإنزيم السليوليز مع استخدام معدل رج 150-200 لفة/ دقيقة.