Diala, Jour, Volume, 37, 2009

A novel biochemical study on carboxymethyl cellulase (endo-1,4-ß-D-glucanase) produced by *Enterobacter cloacae* isolated from soil

Dr.Sahira N. Muslim and Dr.Neihaya H. Zaki
Dept. of Biology,College of Science / Al-Mustansiriya,Baghdad-Iraq

ABSTRACT

10 *Enterobacter cloacae* isolates were obtained out of 35 soil samples from different locations in the farms of kanat of army / east of Baghdad city .

The carboxymethyl cellulase production condition by *Enterobacter cloacae* were studed ,the Carboxymethyl CM medium was the best medium for production of carboxymethyl cellulose CMCase (endoglucanase) with 5% of bacterial cells /ml ,Ph 7.5 and incubated at 35cº in shaking incubater for 18-24 hrs.

The isolate that gave higher cellulolytic activity was chosen to purify endoglucanase through four stages of purification including (ammonium sulfate precipitation, anion-exchange chromatography by DEAE-Sepharose, gel filtration chromatography by Sephadex G-150 and hydrophobic chromatography by phenyl- Sepharose with 440U/mg specific activity, 29.3-fold purification and 31% recovery.

The purified carboxymethyl cellulase was characterized: the molecular weight was about 38 kDa by gel filtration chromatography. The temperature for maximum activity was 35°C and maximal activity was observed between pH 6.5 and 7.0. The enzyme showed higher activity on carboxymethel cellulose (CMC) with much lower activity on glucan. Cellotriose and cellobiose were not degraded and at least four catiguous glucosyl residues were necessory for degradation by the enzyme.

INTRODUCTION

Enterobacter cloacae is the most frequently isolated Enterobacter species from man and animals and is commonly found on or in plants, insects and many sources in the environment (e.g. water,sewage and soil). It is a gram-negative facultative anaerobic and rod-shaped bacterium (1).

The normal habitat of *Enterobacter cloacae* is probably soil and water (2,3), but this bacterium is occasionally found in the faeces and the respiratory tract of human (2). In agriculture *Enterobacter cloacae* has been found in many insects such as symbiotics or entomopathogenic and in the surface of vegetables (3). In addition to being a human pathogen, *Enterobacter cloacae* is a pathogen of plants such as onion bulb,corn,orchid,apple and elm trees (1).

Cellulose, a bio-organic polymer is known to be the most abundant material on earth produced by terrestrial plants and marine algae (4,5).Cellulose is used as food , by microbes and animals, formed major part of plant structure, forming long rigid microfibrils arranged in parallel fashion joined through D-glucose units linked as β-linkage.The cellulose long chains are embedded in a matrix of hemicellulose and lignin (4). Cellulose

is hydrolysed by a group of enzymes called cellulases, that are produced by many of fungi and bacteria (6), including (i) endoglucanase or 1,4- β -D-glucan-4-glucanohydrolases (ii) exoglucanase or 1,4- β -D-glucanohydrolases (iii) β -glucosidase (4,7).

Several bacteria such as *Clostridium* ,*Bacillus*, *Enterobacter* and *Psudomonas* produce cellulases (5,6,8),these enzymes contribute to suppression of plant diseases by inhibition growth of phytopathogenic fungi such as *Fusarium* and *Pythium*, thus *Enterobacter cloacae* is presently used for biocontrol of post harvest of fruits and vegetables and as a preplant seed treatment for suppression of damping-off (9,10). This enzyme also benefit in digestion of fibers food processing, clarifying and liquefying fruit and vegetables juices, textile processes, paper processing, ethanol fuel production, treatment for phytobezoars (a form of cellulose bezoar found in the human stomach) and animal feeds for improving the nutritional quality and digestibility (11,12,13,14). For these reasons, the aim of this study was to purify CMCase from *Enterobacter cloacae* and to characterize this enzyme by detection the molecular weight for it and the optimum substrate and conditions for its activity. This microbe (*Enterobacter cloacae*) has not been reported for the purification and characterization of cellulase before.

MATERIALS AND METHODS

Samples collection :

Thirty-five soil samples were collected from different locations in a farm in Baghdad city. Top soil was collected by spatula in to clean sterile plastic bags and stored at 4°C prior to use. The soil samples were air-dried (20°C) and passed through a sieve (mesh size, 2mm). Ten gram of sieved soil was suspended in 20 ml of basic salt medium(BSM) (15).

Isolation and characterization of *Enterobacter cloacae* :

One loopfull of suspension soil samples was plated on blood agar and MacConkeys agar, then incubated tests at 30°C for 18-

24 h. Several biochemical tests were done to differentiate *Enterobacter cloacae* from the other species. These include the following tests : inability to ferment lactose, a negative indole test, a negative urease test,and ability to decarboxylate arginine, but not lysine (16,17,18). In addition to these biochemical tests, API 20E identification was used to differentiate *Enterobacter cloacae* from the other types.

Cellulolytic activity :

10 µL of *Enterobacter cloacae* cultures were placed into wells (5 mm in diameter) in carboxy methyl cellulose (CMC) agar medium that contained 0.5g carboxy methyl cellulose; 0.1g $NaNO_3$; 0.1g K_2HPO_4 ; 0.1g KCL; 0.05g $MgSO_4$; 0.05g yeast extract; 0.1g glucose and 1.7% w/w agar in 100 ml of water and incubated at 30°C. After 18-24 h the plates were stained with congo red, destained with 1M NaCl solution. CMCase activity was indicated by the formation of clear halos around the wells (19,20).

Effect of different media on CMCase production :

Enterobacter cloacae was grown on different media. These media were including SC medium [containing (g/L): Avicel or CMC 5g, peptone 1g, $Ca(NO_3)_2.4H_2O$ 5.5g, KH_2PO_4 1.3g, $MgSO_4.7H_2O$ 0.5g, plus 1ml nutrient solution], JP medium containing Avicel (17g/L) and peptone 20g/L (21), and CM medium containing 10g/L trypton, 5g/L yeast extract, 5g/L NaCl and 2 ml of 3% CMC (4). Cells were cultured at 30 °C and 200 rpm for 20, 24, 48, 72, 96, and 120h. The supernatant was obtained by centrifugation at 10,000 rpm for 10 min and carboxy methyl cellulase activity was assayed.

Carboxymethyl cellulase assay :

Carboxymethyl cellulase activity was assayed by mixing 2ml of 3% CMC solution with 1ml of enzyme sample in 0.1M citrate buffer solution. The mixture was incubated at 30C° for 2h. after incubation, 3ml dinitrosalicylic acid (DNSA) reagent was added and the solution was boiled for 5min to stop the reaction. The absorbance was measured at 540nm and the glucose content was obtained by using calibration curve relating glucose concentration (0-3mM) to *A*540. One unit of CMCase

activity was defined as amount of enzyme that released 1μM of reducing sugar (glucose) from CMC per minute (4,21).

Protein estimation :

Analysis for protein was determined by method (22) by spectrophotometric assay at 600nm in each stage of carboxy methyl cellulase purification.

Purification of carboxymethyl cellulase :

Enterobacter cloacae carboxymethyl cellulase (CMCase) was purified by amodification of the method (23). Cells were grown in CM medium, that previously described, and incubated at 30°C in shaking incubator for 18-24h. Supernatant were carefully removed after centrifugation at 10000xg for 30min at 4°C and carboxymethyl cellulase activity in supernatant was assayed.

The supernatant was resuspended in 50mM Tris-HCL buffer (pH=7.4) and precipitated with ammonium sulfate 40 to 60% saturation for 1h with gentle stirring. The precipitated proteins were recovered by centrifugation of 10,000xg for 30min, and were dialyzed against 10mM Tris-HCl buffer (pH=7.4) supplemented with 0.15M NaCl. The insoluble residue was removed after dialysis by centrifugation at 10,000xg for 30min, then to the supernatant CMCase activity was assayed.

A three step: Chromatographic procedure was employed to purify CMCase. For the first - mentional step, The supernatant was loaded on to a DEAE-Sepharose, Fast Flow anion - exchange column (1.6 by 17.5cm ;1ml min^{-1}) previously equilibrated in 50mM Tris-HCl buffer (pH=7.4). The CMCase was eluted in 50mM Tris-HCL buffer(pH=7.4) with a 0.1-0.5M NaCl gradient. The fractions (5ml) containing the highest CMCase activity were pooled, dialyzed against 50mM Tris-HCL buffer (pH=7.4) and used in gel filtration step. Gel filtration was carried out in sephadex G-150 column (2.5×40cm) which had been equilibrated and washed with 25mM Tris-HCL buffer (pH=7.4) and the elution done by the same buffer. The fractions(5ml) containing the highest CMCase activity were pooled and used in hydrophobic chromatography step. This step was carried out in phenyl – sepharose 6 column

(1.6 by 11.5cm;1ml min^{-1}) previously equilibrated in 50mM Tris-HCl buffer (pH=7.4) supplemented with 40% (w/v) ammonium sulfate. CMCase was eluted first via 50 mM Tris-HCl buffer (pH=7.4) supplemented with 40% (w/v) ammonium sulfate and then with a 30 to 0% ammonium sulfate gradient. The fractions (5ml) were collected and assayed for CMCase activity.

Characterization of CMCase :

1- Evaluation of the molecular weight :

The molecular weight was evaluated by gel filtration according to the principles described by (24). Gel filtration was carried out in Sephadex G-150 column. This column was equilibrated in 25mM Tris-HCL buffer. The void volume (Vo) was determined by using blue dextran. Elution volumes (Ve) of proteins of Known molecular mass (Bovine serum albumin [66 kDa], ovalbumin [45kDa], chemotrypsinogen A [25kDa] and ribonuclease A [13kDa] dissolved in 25 mM Tris-HCl buffer) were measured and used as reference standards in CMCase native molecular mass determination. The relationship between (Ve/Vo) and log molecular weight for standared protiens was plotted to obtain the standard curve. The molecular weight for CMCase was evaluated from incidence (Ve/Vo) value for CMCase on the standard curve.

2- Effect of temperature on CMCase activity :

The temperature optimum of the purified CMCase was evaluated at temperatures ranging from 20 to 70°C under standard assay conditions at 50mM phosphate buffer (pH=6.5).

3- Effect of pH on CMCase activity :

The influence of pH on the enzyme activity was determined by measuring the enzyme activity at varying pH values ranging from 4 to 8 at 30°C using different suitable buffers, 50mM sodium acetate (pH 4,4.5,5,5.5 and 6), 50mM sodium phosphate (pH 6.5,7,7.5 and 8) respectively.

4- Substrate specificity :

Substrate specificity of the purified enzyme was determined by performing the CMCase assay with different substrates: carboxymethyl-cellulose (CMC), cellobiose, glucan, avicel

(microcrystalline cellulose), xylan, cellotriose, cellohexaose, cellopentaose, lichenan and cellotetraose.

RESULTS AND DISCUSSION

Isolation and characterization of *E.cloacae* :

The results revealed that 10(28%) isolates of *Enterobacter cloacae* were obtained out of 35 soil samples. *Enterobacter cloacae* was isolated from green house of cucumbers by streaking on an agar-meat infusion (AMI) at 32°C with in 72h (25). The complex mixtures of carbohydrates,amino acids,organic acids,and other nutrients released from seeds and roots are thought to support the growth of beneficial bacteria in the spermosphere and rhizosphere (26). *Enterobacter cloacae* was used as a growth regulator in green house cucumbers because of their ability to produce extracellular polysaccharides, serving as growth regulators,that stimulate the growth and yield in cucumbers(25). (27)reported that the growth conditions of *Enterobacter cloacae* isolated from rice stems and roots included the optimum time of culture was 36h, growth temperature was ranged from 27-30°C and the optimum pH value of media was ranged from 6-7. In a study done by (28), in the soil of dumping sites of Bereg country, found that the genera of *Bacillus*, *Enterobacter*, *Psudomonas* and *Actinomyces* were isolated as microflora. *Enterobacter* spp., *Klebsiella* spp., *Bacillus* spp. And *Azospirillum* spp. Were branded as nitrogen fixing organisms per gram of soil (29).

Cellulolytic activity :

10 *E.cloacae* isolates were tested for measuring the CMCase activity by detection the diameter of clear zone of lysis in carboxymethyl cellulose agar medium (Figure-1).

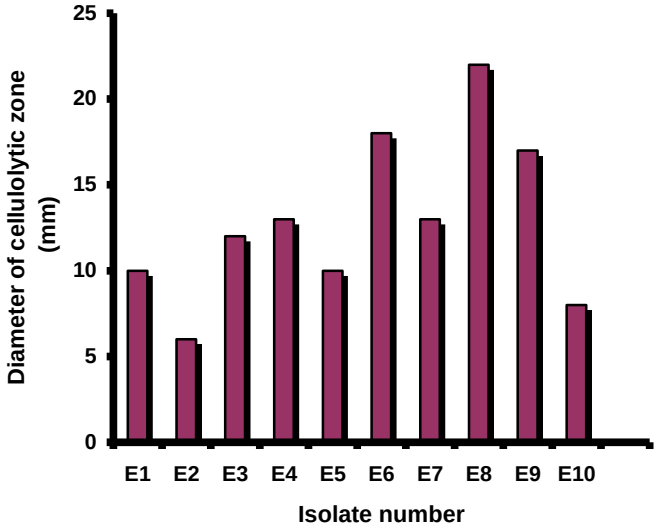

Figure (1): Diameter of cellulolytic zones for all *Enterobacter cloacae* isolates

According to this figure we can conclude that *E.cloacae* E_8 produced CMCase in higher level, therfore; this isolate was chosen for purification experiment. Endoglucanase activities can be easily detected on agar plates by staining residual polysaccharides (CMC,cellulose) with various dyes because these dyes are adsorbed only by long chains of polysaccharides (7). In a study done by (28) found that there was a strong positive correlation between cellulase and phosphatase activities of the landfill soils, while there was a weak positive between cellulase and invertase.

Effect of different media on CMCase production.

E.cloacae E_8 showed CMCase (endoglucanase) activity in supernatants of all culture media examined. CMCase activity was distinct at different cellulose concentrations and composition (Figure-2).

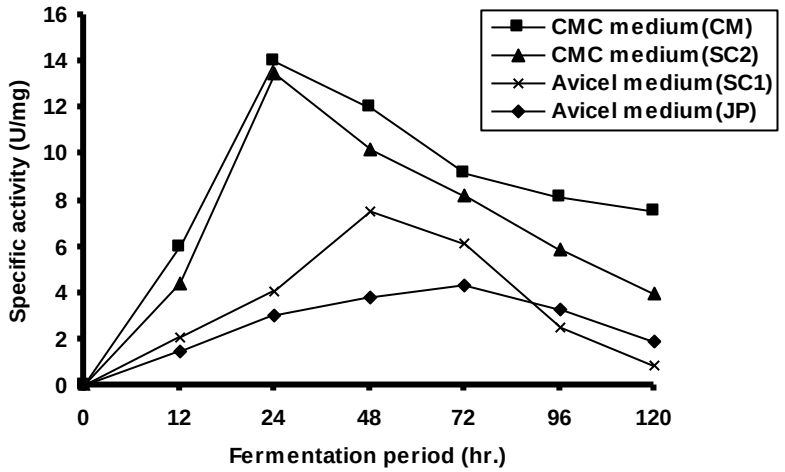

Figure (2): CMCase production in different culture media

In supernatants obtained from 3% CMC(CM medium) was 14U/mg Protein at 24h incubation. At 0.5% CMC(SC_2 medium), this enzyme showed higher specific activity 13.5 U/mg protein at 24h incubation. In SC_1 medium, CMCase showed higher specific activity 7.5 U/mg protein at 48h incubation, while in JP medium this enzyme appeared the lowest activity (maximum 4.3 U/mg protein) at 72h incubation. (30,31) found that carboxymethyl cellulose induced the highest cellulase activity in the thermophilic soil bacterium *Thermomonospora fusca* and *Aureobasidium pullulans* ,respectively. In a study done by (4) reported that *E.cloacae* produced CMCase in large a mount (higher activity) when grown at 37°C on CMC as substrate, while Avicel medium gave the lowest activity. The absence of detectable glucose in supernatants of culture media suggested an effect of substrate on the synthesis and cellulase excretion, without an end product repression, since the glucose inhibition level 0.5% ,and restored when 90% of glucose was consumed (21,23).

Purification of CMCase :

E.cloacae culture was harvested after 18-24h. The starting material for the purification was 100ml of the crude enzyme solution containing 5.2 mg of protein ml^{-1} with a specific CMCase activity of 15 U/mg.

The highest CMCase activity was found at 40-60% ammonium sulfate saturation. The dialyzed enzyme solution collected from 40-60% saturation of ammonium sulfate was loaded on to a DEAE-Sepharose Fast Flow column. From this column , three peaks of protein appeared in the eluted fractions with NaCl solution with one peak of CMCase activity located in the first protein peak (figure-3). Fractions containing the highest CMCase

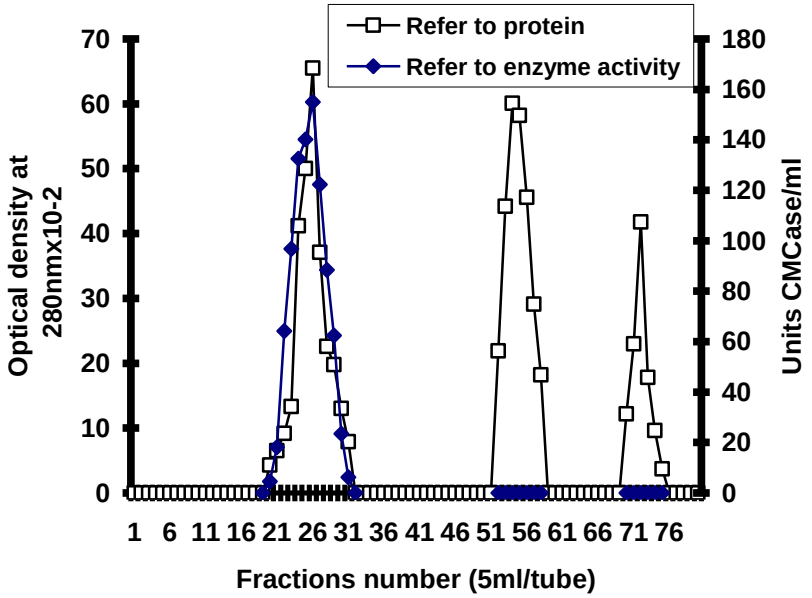

Figure (3): DEAE-Sepharose chromatography of *Enterobacter cloacae* endoglucanase (CMCase)

activity (fractions 22-29) were collected, concentrated and applied to Sephadex G-150 column. CMCase eluted as single peak of activity located in the second protein peak (figure-4) with 25mM Tris-HCl buffer.Fractions containing highest CMCase activity (fractions 61-

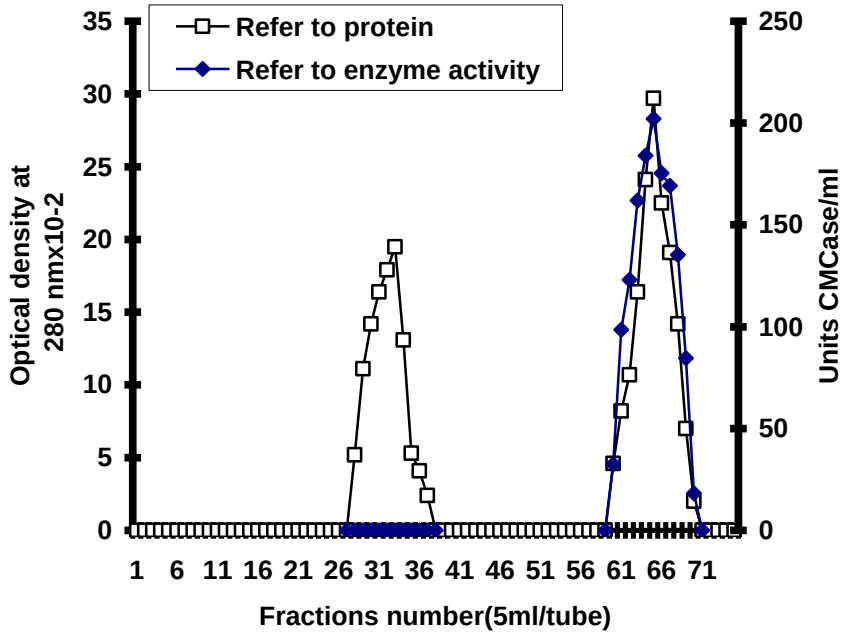

Figure (4): Sephadex G-150 chromatography of *Enterobacter cloacae* endoglucanase (CMCase)

68) were also pooled, concentrated and the elution buffer was replaced by 50 mM Tris-HCl buffer supplemented with 40% ammonium sulfate.

The last purification step was performed by hydrophobic interaction chromatography on a phenyl-Sepharose 6 column (figure-5). CMCase was eluted as single peak of activity located

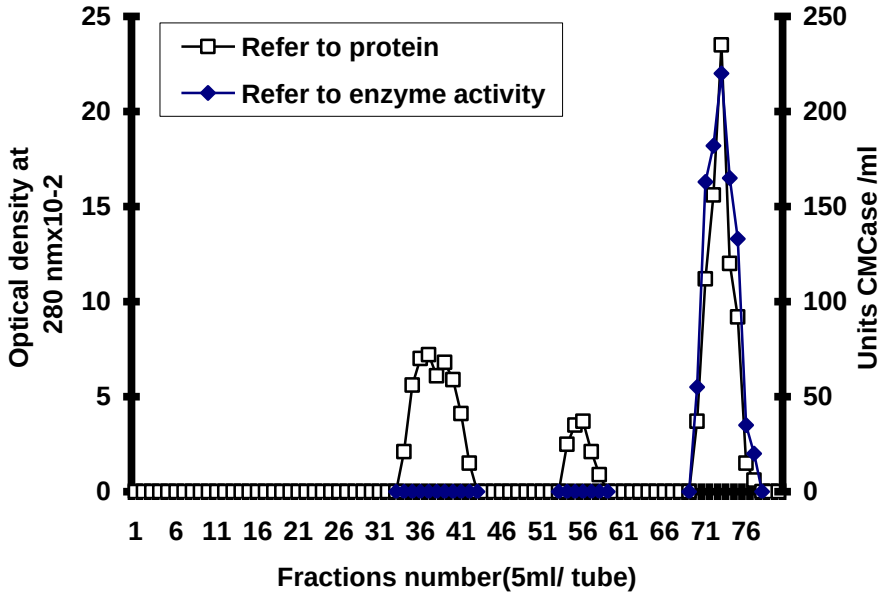

Figure (5): Phenyl-Sepharose 6 chromatography of *Enterobacter cloacae* endoglucanase (CMCase)

in the third protein peak at approximately 20% ammonium sulfate. Active fractions (fractions 71-75) were pooled and used for further studies. As summarized in (Table-1), this procedure yielded a 29.3-fold purification and 31% recovery of the enzyme with specific activity 440 U/mg.

Table (1) : Purification of *Enterobacter cloacae* CMCase

Purification step	Size (ml)	Protein Conc. (mg/ml)	Activity U/ml	Specific Activity U/mg	Total activity	Purification fold	Yield (%)
Crude extract	100	5.2	62.4	15.0	6240	1	100
(NH4)2SO4	30	3.3	164.8	49.9	4944	3.3	79
DEAE-Sepharose	15	1.3	155.4	119.5	2331	7.9	37
Sephadex G-150	10	0.9	202	224	2020	14.9	32
Phenyl–Sepharose 6	9	0.5	220	440	1980	29.3	31

CMCase was purified from *Bacillus sphaericus* 192-fold by ion exchange and gel filtration chromatography, with recovery of 23% (8). *Pseudomonas fluorescens* cellulase was purified by DEAE-Sephadex A50 and Sephadex G-100 with overall recovery of 36% (5). Also (23) found that CMCase was purified from *Sinorhizobium fredii* on a DEAE-Sepharose column and phenyl-Sepharose 6 column with the recovery was 26%. In contrast, (6) reported that endocellulase of *Clostridium cellulolyticum* was purified on Ni-NTA column with the recovery was 54%.

Characterization of CMCase:

Evaluation the molecular weight of CMCase.

The molecular weight of purified carboxymethyl cellulase (endoglucanase) was evaluated by gel filtration with Sephadex G-150. The result showed that purified CMCase of approximately 38000 daltons (figure-6). CMCase gave a molecular mass on SDS-PAGE of 85.1 and 94 kDa for *Caldibacillus cellulovorans* cellulase and *Sinorhizobium fredii* cellulase, respectively (23,32). (5)found the molecular mass for *Pseudomonas fluorescens* was 36kDa. Also(8) reported that CMCase for *Bacillus sphaericus* was a multimeric protein with a molecular mass estimated by native- PAGE of 183 kDa and 42 kDa by SDS-PAGE, and this suggested presence of four homogeneous polypeptides.

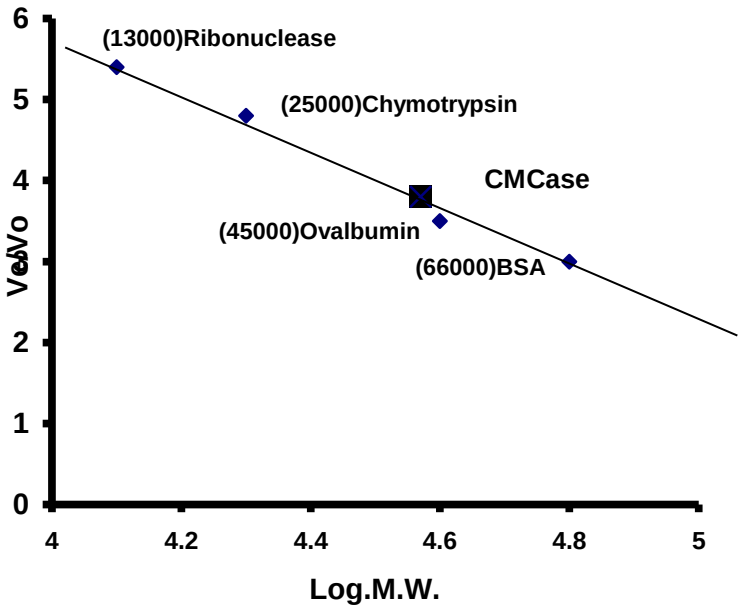

Figure (6): The standard curve of determination of molecular weight for CMCase by gel filtration on Sephadex G-150

Effect of temperature on CMCase activity :

The effect of temperature on the activity of purified CMCase was determined at various temperatures ranging from 20 to 70°C at pH=6.5 (figure-7). The enzyme showed a good activity between 25 to 40°C with maximum activity at 35°C. The optimum temperature for cellulase activity produced by *Sinorhizobium fredii* was 35°C (23). Optimum temperature range of cellulase activity of 30-35°C has been reported for CMCase isolated from *Pseudomonas fluorescens* (5) .

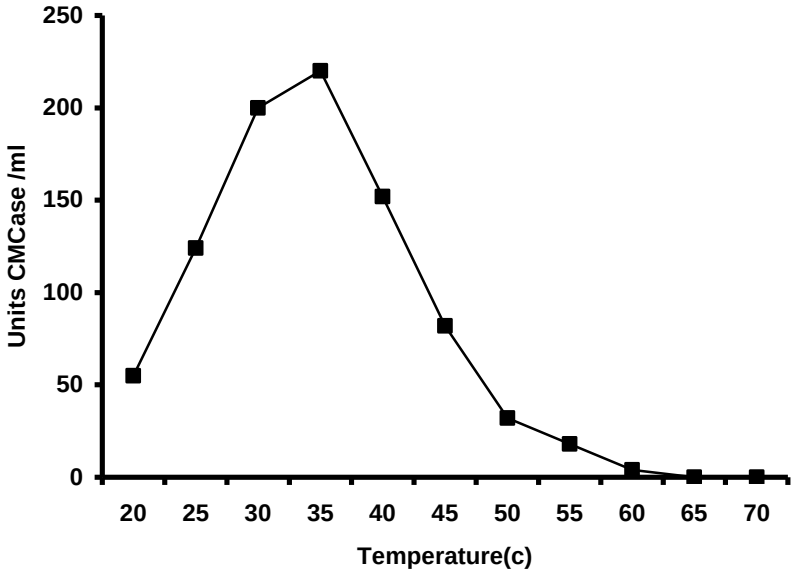

Figure (7): Effect of Temperature on CMCase activity

Effect of pH on CMCase activity :

The effect of the pH on the purified cellulase activity of *Enterobacter cloacae* was examined at various pHs ranging from 4.0 to 8.0 as shown in (figure-8). The enzyme has a broad range of pH activity (pH 5.5-7.5) with maximal activity between pH value of *Caldibacillus cellulovorans* cellulase (32). The enzyme had about 8,16 and 19% of its maximum activity at pHs 4, 4.5 and 8, respectively. Increasing or decreasing the pH changes the ionic stste of ionizing side chains in a protein, distrups ion pairs, breaks hydrogen bond, and consequently denatures the protein (33).

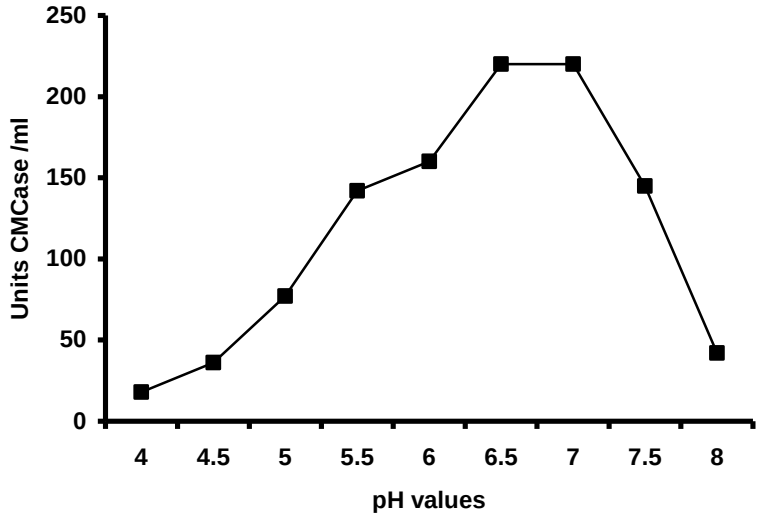

Figure (8): Effect of pH on CMCase activity

Substrate Specificty :

The purified CMCase from *Enterobacter cloacae* degarded CMC, avicel, cellohexaose, cellopentaose, cellotetraose, cellobiose, cellotriose and lichenan (figure-9). The rate of CMC

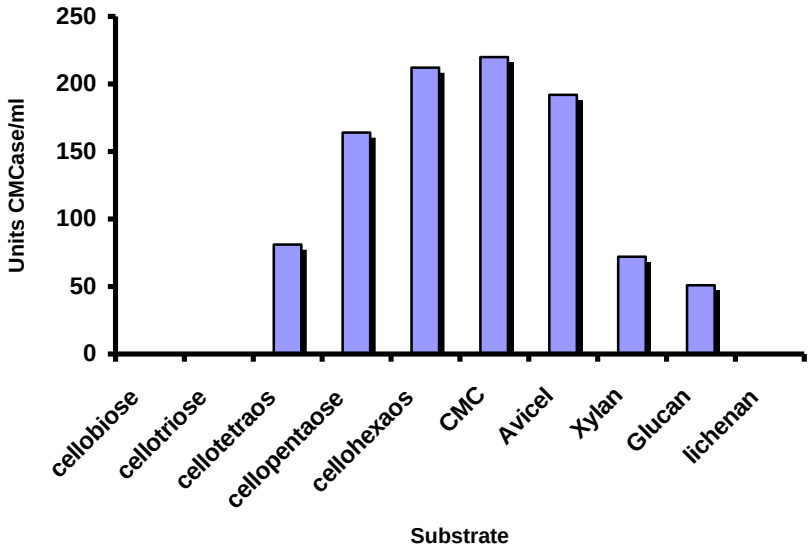

Figure (9): Activity of CMCase on various substrates

degradation was higher than any other substrates used in this study. The enzyme exhibited significant activity toward cellohexaose, avicel and cellopentaose with much lower activity on glucan; a low level of activity was also found against xylan and cellotetraose. No activity could be detected or cellobiose,

cellotriose, or lichenan. We can conclude that cellotriose and cellobiose were not degraded, and at least four contiguous glucosyl residues were necessary for degradation by the enzyme. Endoglucanase was most active on CM-cellulose and had the ability to produce a high proportion of soluble reducing ends and this indicated that it was a processive enzyme (6). Some activity was noted on glucan containing 1,3-1,4-linkages showing reasonable sensitivity of the 1,3-linkage to the enzyme (11). (32,34) reported that *Caldibacillus cellulovorans* and *Xylella fastidiosa* CMCase showed activity on CMC, acid swollen cellulose, avicel, xylans and the oligosaccharides cellotetraose and cellopentaose, but cellotriose and cellobiose were not degraded.

REFERENCES

1-Nishijima,K.A.; Alvarez,A.M.; Heppeny,P.R.; Shintaku,M.H.; Keith,L.M.; Sato,D.M.; Bushe,B.C.;Armstrong,J.W.and Zee,F.T. Association of *Enterobacter cloacae* with rhizome rot of edible ginger in Hawaii . Plant Dis.88(12):1317-1321.(2004).

2-Cruickshnk,R.;Duguid,J.P.;Marmion,B.P.;and Swain,R.H.A. "Medical Microbiology" Vol.2,12th ed.p:279-280 .Churchill livingstone pub.,London(1975).

3-Neto,J.R.; Yano,T.; Beriam,L.O.; Destefano,S.A.; Oliveira,V.M.; and Rosato,Y.B. Comparative rflp-its analysis between *Enterobacter cloacae* strains isolated from plants and clinical origin . Arq.Inst.Biol.70(3):367-372 (2003).

4- Sami,A.J.;Awais,M. And Shatoori,A.R. Preliminary studies on the production of endo-1,4-B-D-glucanases activity produced by *Enterobacter cloacae*. African J.Biotech.7(9):1318-1322 (2008).

5-Bakare,M.K.;Adewale,I.O., and Shonukan,O.O. Purification and characterization of cellulase from the wild type and two improved mutants of *Psudomonas flourescence* .African J.Biotech.4(9):898-904 (2005).

6-Levoy,C.;Pages,S.;Belaich,A.;Belaich,J. And Tardif,C. The processive endocellulase Cel F, a major component of the *Clostridium cellulolyticum* cellulosome: purification and characterization of the recombinant form. J.Biotech.179(1):46-52 (1997).

7-Zhang,Y.H.;Himmel,M.E.and Mielenz,J.R.Outlook for cellulase improvement: screening and selection strategies. Biotech.Advances 24:452-481 (2006).

8-Jagtar,S; Navneet,B. And Sobti,R. Purification and characterization of alkaline cellulase produced by a novel isolate , *Bacillus sphaericus* JS1 . J. Indust. Microbiol. Biotech. 31(2):51-56 (2004).

9-Sindhu,S.S. and Dadarwal,K.R. Chitinolytic and cellulolytic *Psudomonas* sp. Antagonistic to fungal pathogens enhances

nodulation by *Mesorhizobium* sp. Cicer in chickpea.Microbiol.Res.156(4):353-358(2001).

10-Hinton,D.M. and Bacon,C.W. *Enterobacter cloacae* is an endophytic symbiont of corn.Mycopathol.129(2):117-125 (1995).

11-Ibrahim,A.S.S. and El-diwany,A.I.Isolation and identification of new cellulases producing thermophilic bacteria from a Egyptian hot spring and some properties of the crude enzyme . Australian J.Basic &Appli.Sci. 1(4):473-478 (2007).

12-Anone.Cellulase.Advan.enz.technolo. 1-3 (2006).

13-Heaton,K. Industrial uses of thermophilic cellulase .Biochem .: 1-9 (2004).

14-Kar,S.;Mandal,A.;Mohapatra,P.K.;Mondal,K.C. and Pati,B.R. Production of cellulase - free xylanase by *Trichoderma reesi* saf 3. Brazilian J.Microbio.37:462-464 (2006).

15-Agot,N.;Nybroe,D.F.;Nielsen,P. And Johnes,K. An altered *Psudomonas* diversity is recovered from soil by using nutrient poor *Psudomonas*–selective soil extract media. Appli.Environ.Microbio.67(11): 5233-5239 (2001) .

16-Johnson,M.T. Microbiology Laboratory Notebook. 7th ed. Indian university school of medicine. Blackwell publishing. (2007) .

17-Patrick, R.M; Ellen,J.; Baron,J.J. Manual of clinical microbiology. Vol.1 Blackwell publishing .(2007).

18-Baron,E.J. and Finegold,S.M. Diagnostic microbiology. 8th (ed.) .Bailey &Scotts .Mosby,com.Missuri. (1990).

19-Apun,K. Cellulase production. Pract.Biotech. National center for Biotechnology Education. (1995).

20-Chattejee,A.;Cui,Y;Liu,Y.;Domenyo,C.K. and Chatterjee,A.K. Inactivation of rsm A leads to overproduction of extracellular pectinases,cellulases and proteases in *Erwinia carotovora* subsp.*carotovora* in the absence of the starvation/cell density–sensing signal , N-(3-oxohexanoyl)-L-homoserine lactone. Appli. Environ. Microbiol. 61(5):1959-1967 (1995).

21-Junior,J.A.;Correia,M.J. and Oliveira,N.T. Cellulase activity of a *Lentinula edodes* (Berk)pegl.strain grown in media containing carboximetlicellulose or microcrystalline cellulose. Brazillian Archiv.Biol.Technol.46(3):333-337 (2003) .

22-Lowry,O.H.;Rosebrongh,N.J.;Farr,A.L. and Randall,R.J. protein measurement with the folin phenol reagent.J.Biolo.Chem.193:265-275 (1951).

23-Chen,P.;Wei,T.;Chang,Y.and Lin,L. Purification and characterization of Carboxymethyl cellulase from *Sinorhizobium fredii*.Bot.Bull.Acad.Sin.45:111-118 (2004).

24-Andrews,P. Estimation of the molecular weights of proteins by sephadex gel – filtration .Biochem.J. 91:222-232 (1964).

25-Georgieva,O. *Enterobacter cloacae* bacterium as a growth regular in greenhouse cucumbers. Cucurb.Genetics 26: 4-6 (2003).

26-Roberts,D.P.;Dery,P.D.;Yucel,I. And Buyer,J.S. Importance of pfkA for rapid growth of *Enterobacter cloacae* during colonization of crop seeds.Appli.Environ.Microbiol. 66(1):87-91 (2000).

27-Hassen,A.A.;Xu,J. And Yang,J. Growth conditions of associative nitrogen-fixing bacteria *Enterobacter cloacae* in rice plants.Agricultu.J.2(6):672-675 (2007) .

28-Halsz,J.L.;Chonka,I.;Dobron,M.;Boyko,N. And Balszy,S. Microorganism and enzyme activity in the soil of landfill sites of Bereg county. Arch.Agrono.Soil Sci. 54(5):465-476 (2008).

29-Khan,H.R.;Mohiuddin,M. And Rahman,M. Enumeration , isolation and identification of nitrogen-fixing bacterial strains at seedling stage in rhizosphere of rice grown in non-calcareous grey flood plain soil of Bangladesh. J.Facult.Environ.Sci.Tech. 13(1):97-101 (2008).

30-Spiridonov,N.A.; and Wilson,D.B. Regulation of biosynthesis of individual cellulases in *Thermomonospora fusca* .J.Bacteriol.180(14):3529-3532 (1998).

31-Kudanga,T and Mwenje,E. Extracellular cellulase production by tropical isolates of *Aurobasidium pullulans* .Con.J.Microbiol.51(9):773-776 (2005).

32-Huang,X.P. and Monk,C. Purification and characterization of a cellulase (CMCase) isolated from thermophilic aerobic bacterium *Caldibacillus cellulovorans*. World J.Microbiol.Biotech.20(1):85-92 (2004).

33-Rawn,J.D. Biochemistry .Neil.Palterson pub.U.S,A.(1989) .

34-Wulf,N.A.;Carrer,H. And Pascholati,S.F. Expression and purification of cellulase Xf 818 from *Xylella fastidiosa* in *Escherichia coli*.Curr.Microbiol.53(3):190-203(2006).

تنقية ودراسة الصفات البايوكيميائية لانزيم كاربوكسي مثيل سليليز-(endo-1,4-ß-D-glucanase) المنتج من العزلة المحلية *Enterobacter cloacae*

الخلاصة

تم الحصول على 10 عزلات تعود لبكتريا *Enterobacter cloacae* من مجموع 35 عينة تربة زراعية مأخوذة من مواقع مختلفة من مزارع في منطقة قناة الجيش شرق مدينة بغداد.

درست الظروف المؤثرة في الانتاج ولوحظ ان وسط لانتاج انزيم الكاربوكسي مثيل سيليليز (اندوكلوكانيز) هو وسط الكاربوكسي مثيل (CM) ، وتلقيحه بـ5% من اللقاح البكتيري وحضنه بدرجة حرارة 35مْ لمدة 18–24 ساعة في الحاضنة الهزازة وبرقم هيدروجيني 705.

تم اختيار العزلة التي اعطت اعلى فعالية تحلل للسيليلوز لاستخلاص وتنقية انزيم اندوكلوكانيز باستخدام اربعة مراحل تضمنت(الترسيب بكبريتات الامونيوم، التبادل الايوني باستخدام DEAE-Sepharose ، الترشيح الهلامي باستخدام Sephadex G-150 والكروماتوغرافيا الكارهة للماء باستخدام phenyl-Sepharose 6) بفعالية نوعية 440 وحدة/ملغم ، عدد مرات تنقية 29.3 وبحصيلة نهائية 31%.

تم توصيف انزيم اندوكلوكانيز المنقى ووجد بأن وزنه الجزيئي يقارب 38 كيلودالتن باستخدام كروماتوغرافيا الترشيح الهلامي. لقد وجد بأن درجة الحرارة المثلى لاقصى فعالية للانزيم هي 35 °م ، بينما الرقم الهيدروجيني الامثل لاقصى فعالية للانزيم يتراوح بين 6.5–7.0 . لقد اظهر الانزيم اعلى فعالية تجاه الكاربوكسي مثيل سيليلوز (CMC) كمادة اساس بينما اظهر اقل فعالية تجاه مادة الكلوكان. اما السيلوترايوز والسيلودايوز فهي لم تتحلل بواسطة الانزيم، وبذلك وجد بأنه على الاقل اربع وحدات كلوكوسيل متجاورة ضرورية للتحلل او التحطم بواسطة الانزيم.

Diala, Jour, Volume, 37, 2009