Ass. Univ. Bull. Environ. Res. Vol. 6 No. 2, October 2003

AUCES

BIOTECHNOLOGICAL APPLICATIONS OF CELLULASE AND PECTINASE ENZYMES PRODUCED BY SOME FUNGI ISOLATED FROM SOIL IN UPPER EGYPT

Afifi, M. M.

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

ABSTRACT :

This investigation was concerning with isolation and identification of fungal species from soil in upper Egypt (Assiut). Thirty fungal species belonging to eighteen genera were collected and screened for their ability to produce cellulase and pectinase enzymes. The most potent was *Mucor fuscus* (MS22) and was allowed to grow on industrial cellulose and pectin under different cultural and nutritional conditions and resulted in: the best biosynthetic abilities were induced at substrate concentrations (%) of 2 and 0.2, pH optima (7 and 8) for cellulase and pectinase respectively. Carbon source (Maltose), temperature (20°C), inoculum concentration was one disk (0.5mm) and the incubation at static condition was the best circumstances for tested enzymes. Moreover, both nitrogen source (glycine and urea) and flask's volume (ml) (250 and 2000) were the most favorable conditions for getting the best yields of cellulase and pectinase respectively. Biotechnological application of these enzymes separately and in comparison carried out with respect to clarification of ready made mango syrup resulted in: an optima enzymes' concentrations were ranged from 25–50 μl, and clarification time(minutes)150 and 5 at refrigeration condition for cellulase and pectinase respectively, corresponding to 60 minutes at open air in case of mixed enzymes. The present enzymes may be of a remarkable application in many fields, such as biodetergents, food processing technology in large scale from one hand and of silage's manufacture on the other hand.

INTRODUCTION:

Pectinase 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 & Federici, 1992) in which pecteolytic enzymes such as polygalacturonase, pectinesterase etc., as well as hemipectinases, pectinases and proteases are all present (Fogarty and Kelly 1983; Lambert 1983 and Ammar *et al.*1995b). 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 caratenoids and clarity of juice (Essa and Salama, 2002). Eight apple pomace liquefaction juices were produced to characterize soluble cell wall material released by the action of pectolytic and cellulytic enzyme preparations, very high colloid values from 9.7 to 19.6 g/l were

recovered from the juices by ethanol precipitation (Mehrlander *et al.* 2002).

Dongowski *et al.* (2002) studied the degradation of apple cell wall material by commercial enzyme preparations. Carden and Felle (2003) Studied the mode of action of cell wall-degrading enzymes and their interference with Nod factor signalling in *Medicago sativa* root hairs. The genus *Trichoderma* comprises a group of filamentous ascomycetes were widely used in industrial applications because their ability to produce extracellular hydrolases in large amounts (Mach and Zeilinger, 2003). The main object of the present work was to obtain mixture of cellulase and pectinase enzymes to get a complete hydrolysis of primary and secondary cell wall of plant cell to be applied fairly in food industry and to be ready in silage manifacture in the future as well.

MATERIALS AND METHODS:

Isolation of Fungal Species:

20 soil samples collected from different localities in upper Egypt (Assiut), using Czapeck-Dox's agar medium by serial dilution method and temperature 25°C for seven days.

Media Used:

A-Production medium:

It contains (g/l): KCl, 0.5; $MgSO_4.7H_2O$, 0.05; KH_2PO_4, 1; distilled water up to 1 liter after addition of pectin 1g or cellulose 5g.

B-Assay media:

1-Assay media for pectinase: was as in Afifi (1994,2002) and Ammar *et al.* (1995a) with some modification (Pectin 1g; agar 15g;1000 ml dist. water; pH 8.0).

2-Assay media for cellulose: was as in Ali (2000).

Analytical Methods:

Biotechnological applications of enzyme used determined by commercial methods (i.e. viscosity reduction method (V.R.M.) as in Afifi (2002) and Ammar and Afifi (2003).

Experimental:

A-Screening test: This carried out on agar media containing Czapeck-Dox's mineral salts (M.S.) with either (0.5% cellulose) or (0.1% pectin).

B-Parameters controlling enzymes production:

1-Substrate concentration: Different concentrations of substrates (%,w/v) were applied viz. cellulose (1, 2, 4, 6, 8 and 10) and pectin (0.02, 0.05, 0.2, 0.4, and 0.8).

2-pH: Various pH values covering the range of 3-10 were applied using NaOH (1N)- HCl (1N) buffer.

3-Temperature: *Mucor fuscus* (MS22) was exposed to different temperature (30-60°C) on the production medium and finally tested for enzymes activities.

4-Carbon source: Carbon sources tested (Fructose, Dextrose, Lactose, Manitol, Maltose, Sucrose and Starch) were introduced into the basal medium at the level of 1% (w/v).

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

6-Nitrogen source: Production medium was supplemented with different nitrogen sources

(urea, potassium nitrate, peptone, glycine and calcium nitrate). The nitrogen sources were added at fixed weight (0.5g/100ml) and the other conditions were performed under previously determined optimal ones.

7-Inoculum size: Different disks (0.5 mm diameter) of *Mucor fuscus* (MS22) was allowed to grow on the production medium. At the end of incubation period, cellulase and pectinase activities were assayed for each case.

8-Flask capacities: The production medium was inoculated by *Mucor fuscus* (MS22) using different flasks of various capacities ranged from 250-2000ml.

C-Biotechnological applications: Application studies were carried out by testing the clarification of mango syrup kindly obtained (in the form of ready made juice prepared for direct use by the consumer) from Vitrac Co. Qalbia, Egypt by using the present enzymes separately and in mixtures. These studies carried out as in Afifi (2002) and Ammar and Afifi (2003).

1-Enzyme concentration: This experiment was performed to determine the optimum concentration of enzymes tested required to get a complete clarification of mango syrup. Three types of treatments have been undertaken i.e. (cellulase, pectinase and both of them) at concentrations of (volume µl/10 ml mango syrup sample): 5, 10, 25, 50, 100 and 200 for each treatment. The activity of 100 µl is equivalent to 213.8 and 70.79 units/ml for cellulase and pectinase respectively. On the other hand the same volumes were added to each others in case of enzymes'mixture.

2-Clarification time: In this experiment, identical reaction mixtures were incubated for 5, 10, 30, 60, 120 and 240 minutes at 30°C, at the end of each incubation period, enzyme activities were determined by the viscosity reduction method (V.R.M.).

3-Storage condition: This experiment was carried out in order to determine the best conditions for enzymes action (i.e. refrigeration or open air) required for maximum clarification.

RESULTS:

A-Isolation and Identification studies :

Isolation process performed by using different soils in Assiut city followed by purification of fungal flora resulted in thirty fungal species belonging to eighteen genera viz. *Aspergillus* including 8 species (*A. oryza, A. flavus, A. fumigatus, A. niger, A. sydowii, A. ochraceous, A. terricola* and *A. terreus*); *Emericella* including four species (*E. nidulans, E. echinulata, E. dentata* and *E. stellatus*), *Fusarium* two species (*F. solani and F. oxysporum*), *Penicillium* two species (*P. duclauxii* and *P. chrysogenum*) and one species of *Syncephalastrum (S. racemosum) Absidia (A. corymbifera), Trichothecium (T.roseum), Myrothecium (M. verrucaria), Cunninghamella (C.echinulata), Circinell (C. muscae), Botryotrichum (B. piluliferum), Ulocladium sp., Drechslera* sp., *Geotrichum (G.candidum), Mucor (M.fuscus), Trichurus (T.spiralis), Curvularia* sp. and *Alternaria (A. alternata*).

B-Screening test:

As shown in table (1), almost all of fungal strains have cellulytic and pecteolytic effects and the most potent one found to be *Mucor fuscus* carrying number 22, within the three days tested.

Table (1):Screening test of cellulytic and pecteolytic productivities of identified fungal flora isolated from tested soils.

	Fungi tested	Cellulytic activity(mm)*			Pectolytic activity (mm)*		
		1st day	2nd day	3rd day	1st day	2nd day	3rd day
1	*Syncephalastrum racemosum*	14	28	39.5	12	30.5	41
2	*Aspergillus oryza*	16.7	24	28.5	15.5	21.5	24
3	*Emericella nidulans*	11.5	22	33	15	16	18
4	*Aspergillus flavus*	13	21	33.5	11	17.5	26
5	*Fusarium solani*	10.5	23	36.5	7	23	31.5
6	*Emericella echinulata*	7.5	15.5	20	7	14	17.5
7	*Absidia corymbifera*	-	-	14.5	16.5	17	22
8	*Aspergillus fumigatus*	11	15	27	6.5	17	20
9	*Trichothecium roseum*	-	-	-	-	-	-
10	*Myrothecium verrucaria*	6	10	14	7.5	10	15
11	*Cunninghamella echinulata*	19.7	33	45.5	19.5	30.5	39
12	*Aspergillus niger*	14	21	37	15	22	33.5
13	*Circinella muscae*	12.5	25	25	14	27.7	39
14	*Botryotrichum piluliferum*	-	-	14.5	7	7.7	14.5
15	*Ulocladium sp.*	-	-	-	-	-	9
16	*Penicillium chrysogenum*	7.5	19.7	37	9.5	16.5	16.5
17	*Drechslera sp.*	7	17	17	15.2	22	25
18	*Emericella dentata*	9.5	18.5	24	8	19.5	24.5
19	*Geotrichum candidum*	-	-	-	-	-	-
20	*Aspergillus sydowii*	-	-	-	7.5	14	16.5
21	*Fusarium oxysporum*	9.5	21.5	32.5	-	20	33
22	*Mucor fuscus*	24	49.5	68.5	19.5	38	57
23	*Trichurus spiralis*	8	13.5	22	10	19.5	25
24	*Curvularia sp.*	9.5	17	21	8.5	17.7	22
25	*Aspergillus ochraceous*	-	-	16.5	8.5	18	27
26	*Penicillium duclauxii*	-	-	14.5	-	-	19.5
27	*Alternaria alternate*	6.5	15	22.5	7	13	13
28	*Aspergillus terricola*	12.5	28.5	35.5	22.5	27	40
29	*Aspergillus terreus*	8.5	17	20	9	17.5	22.5
30	*Emericella stellatus*	-	-	24	-	-	-

***Mean diameters of clearing zone.**

C-Parameters controlling enzymes synthesis :

1-Carbon source: Data represented in Fig. (1) showed that the optimum carbon sources (i.e. maltose) introduced into the production medium to obtain the maximum yields (units/ ml) (79.4 and 223.85 for cellulase and pectinase respectively).

2-Nitrogen source: The results illustrated in Fig.(2) indicated that *Mucor fuscus* MS22 prefer glycine and urea as nitrogen sources to fulfill the maximum yields (units/ml) 371.5 and 223.85 for cellulase and pectinase respectively.

3-pH: The pH values in Fig. (3) showed that the maximum cellulase and pectinase activities reached at its maximum at pH 7 and 8 respe-ctively. Below or above these particular pH valuesenzymes activities decreased gradually.

4-Static and shaking conditions: It was obvious from the result detected in Fig. (4) that the best condition for cellulase and pectinase production was at static condition comparable to shaking one.

5-Substrate concentration : Data represented in Fig.(5) indicated that the maximum cellulase production was obtained in the presence of 2% g. of cellolose comparable to 0.2% g. pectin in case of pectinase production.

6-Temperature: The results in Fig. (6) showed that the temperatures optima fulfilled the maximum cellulase and pectinase production was at 20°C.The temperatures above this particular degree exerted less of enzymes activities.

7-Flask volume: Data recorded in Fig. (7) showed that 500ml flask was more favorable for cellulase production. On the other hand pectinase activity decreased gradually beyond this particular volume till 2000ml.

8-Inoculum size: An inocla optima of *Mucor fuscus* MS22 to get the best yield of enzymes tested was at one disk (0.5mm diameter) as shown in Fig.(8).

D-Biotechnological Application in food processing:

1-Enzyme concentration: Results illustrated in Fig. (9) indicated that the enzymes concentrations' optima were ranged from 25-50 µl corresponding to reduction of viscosity (R.V.%) 12.24, 55.10 and 77.50 in case of cellulase, pectinase and enzymes' mixture respectively.

2-Clarification time: Results represented in Fig.(10) showed that the optimum time required for maximum clarification, V.R.% (i.e. 36, 32 and 24) were determined at 120, 5 and 60 min. for cellulase, pectinase and enzymes' mixture respectively.

3-Storage condition: Data illustrated in Fig (11) indicated that the best conditions for storage in enzymes reaction mixtures were determined at refrigeration conditions corr-esponding to maximum clarification (i.e. 54.61 and 77.51%) in case of cellulase and pectinase respectively. On the other hand, the best condition for application of enzymes' mixture detected at open air corresponding to 47.79% clarification.

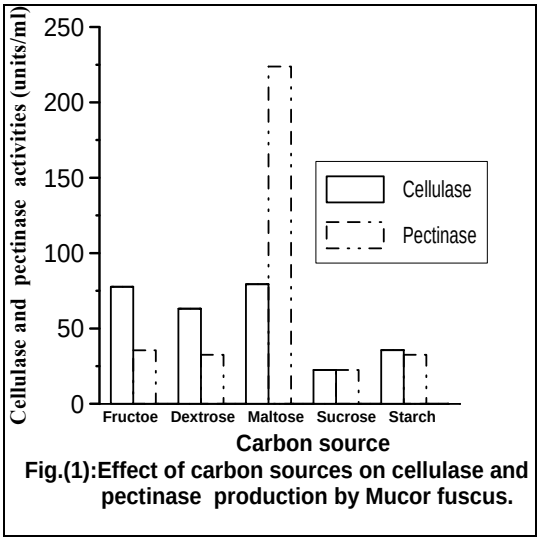

Fig.(1):Effect of carbon sources on cellulase and pectinase production by Mucor fuscus.

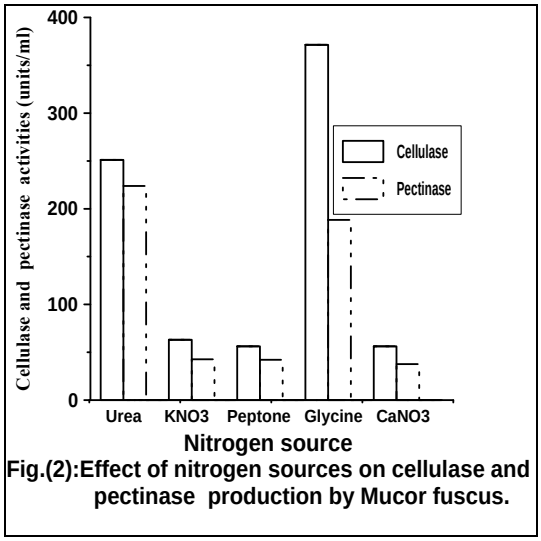

Fig.(2):Effect of nitrogen sources on cellulase and pectinase production by Mucor fuscus.

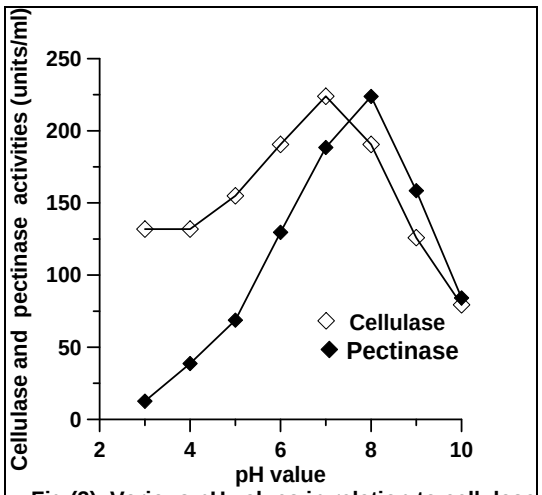

Fig.(3): Various pH values in relation to cellulase and pectinase productions by Mucor fuscus.

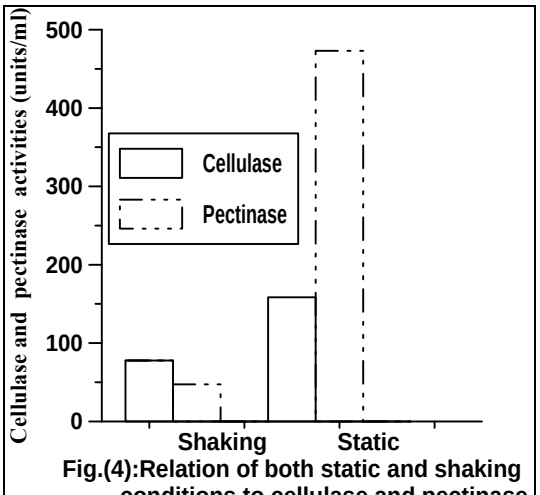

Fig.(4):Relation of both static and shaking conditions to cellulase and pectinase productions by Mucar fuscus.

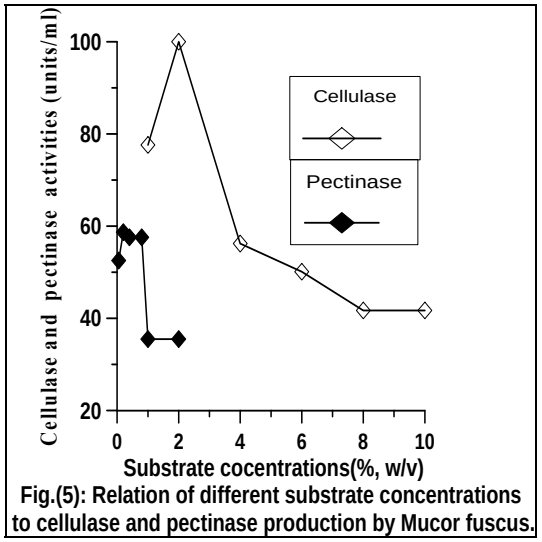

Fig.(5): Relation of different substrate concentrations to cellulase and pectinase production by Mucor fuscus.

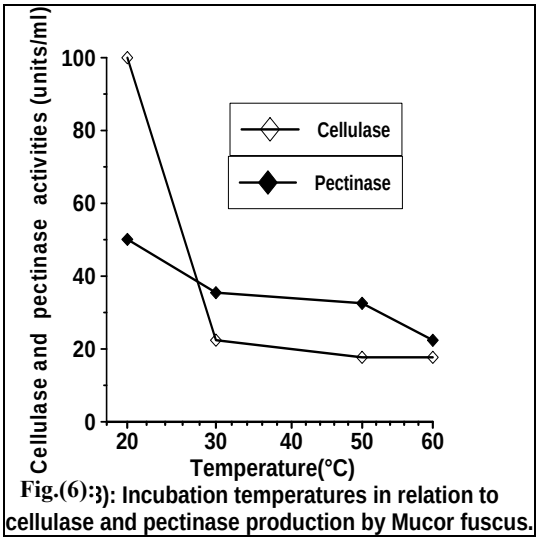

Fig.(6):3): Incubation temperatures in relation to cellulase and pectinase production by Mucor fuscus.

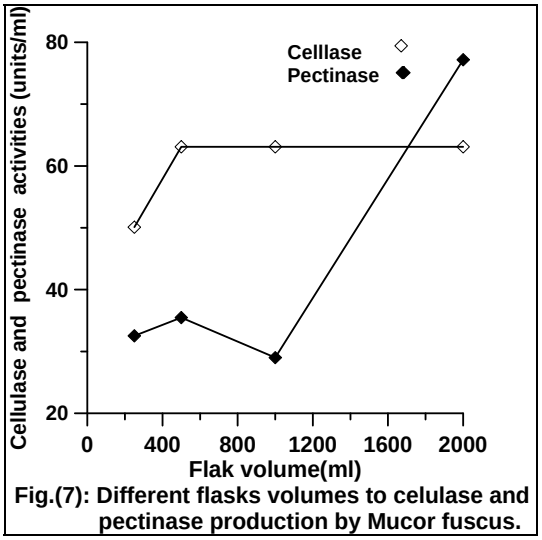

Fig.(7): Different flasks volumes to celulase and pectinase production by Mucor fuscus.

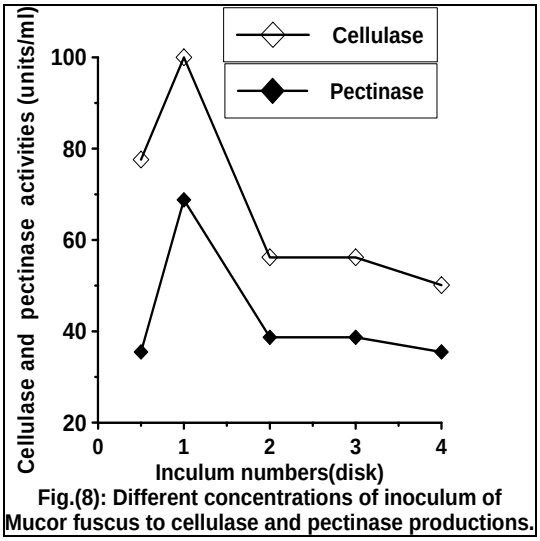

Fig.(8): Different concentrations of inoculum of Mucor fuscus to cellulase and pectinase productions.

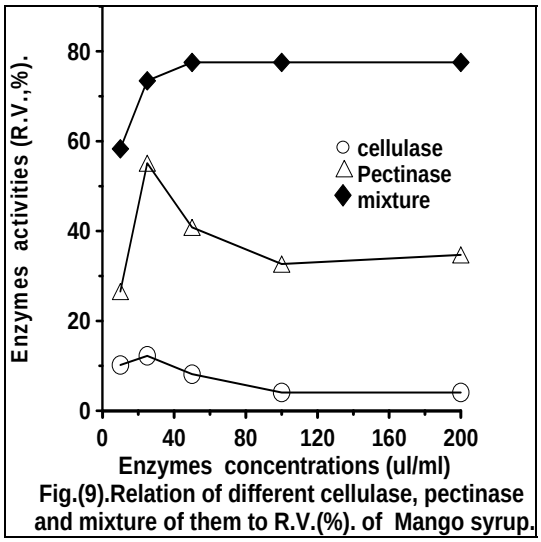

Fig.(9).Relation of different cellulase, pectinase and mixture of them to R.V.(%). of Mango syrup.

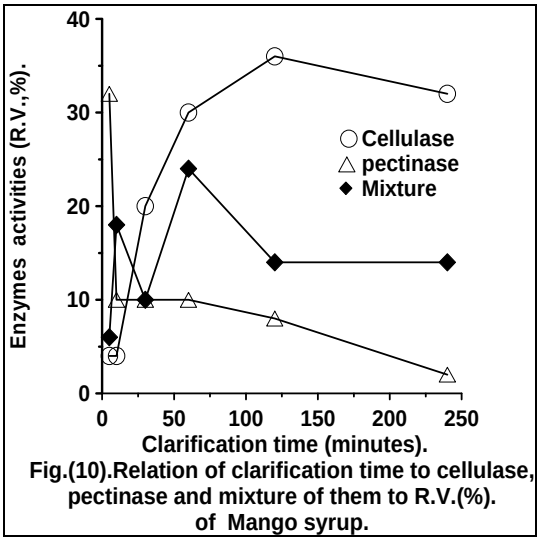

Fig.(10).Relation of clarification time to cellulase, pectinase and mixture of them to R.V.(%). of Mango syrup.

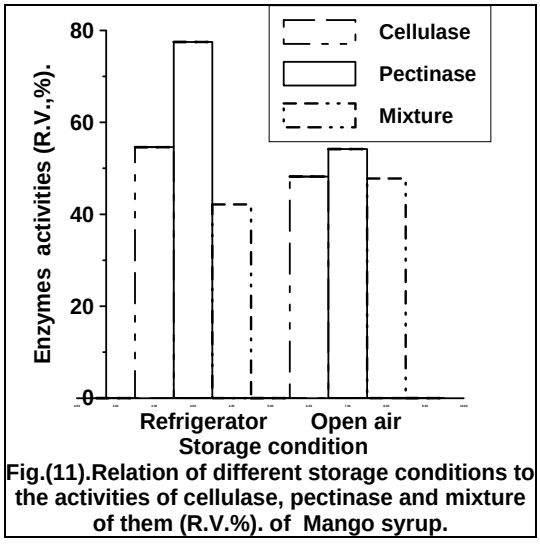

Fig.(11).Relation of different storage conditions to the activities of cellulase, pectinase and mixture of them (R.V.%). of Mango syrup.

DISCUSSION:

The main object of the present work was to obtain mixture of enzymes (cellulase and pectinase) to get a complete hydrolysis of primary and secondary cell wall of plant cell to be applied fairly in food industry from one hand and to be ready in silage manifacture in the future as well. So we have investigated isolation, identification of fungal genera and species from soils in upper Egypt (Assiut). Thirty cellulytic and pecteolytic species belonging to eighteen genera (viz. *Aspergillus, Emericella, Fusarium*, *Penicillium, Syncephalastrum, Absidia, Trichothecium, Myrothecium, Cunninghamella, Circenella, Botryotrichum, Ulocladium, Drechslera, Geotrichum, Mucor, Trichurus, Curvularia and Alternaria*) were collected. Screening test was performed to evaluate the most potent cellulytic and pecteolytic activities(i.e. *Mucor fuscus* MS22).

Emtiazi *et al.* (2001) reported that the biodegradation of lignocellulosic waste by *Aspergillus terreus* was reported and the isolate produced 250 CM Case(carboxymethyl cellulase or endogluconae) U.ml(-1) and biodegraded hey and straw during 3 days and the biomass production on straw was 5g.L^{-1} dry weight from 0.25 cm^2 inoculated mycelium. Ammar, *et al.* (1995g) studied the pecteolytic activities and identities of the fungal flora of Tut Ankhamen Tomb (TAT) which allowed to grow under solid state fermentation (S.S.F.). Some environmental and nutritional parameters controlling the productivity of cellulase(s) by *Trichoderma hamatum* (Bon.) Bain, S-6 and *T.ongibrachiatum Refai*, S-7 under bench scale fermentation conditions has been undertaken (Ammar, *et al* (1995c). Production, Purification and Properties of cellulase(s) biosynthesis under bench scale

fermentation by *Trichoderma hamatum* (Bon.) Bain was studies (Ammar *et al.*1995e).

In a trial to determine some environmental and nutritional parameters controlling cellulase and pectinase production by *Mucor fuscus MS22*, resulted in: the best substrate concentration 2 and 0.2(%g.) respectively. In addition the best incubation temperatures was (20˚C) for both enzymes.

The pH 7 and 8 were the optima for growing *Mucor fuscus MS22* to produce the highest yield of cellulase and pectinase respectively. Effect of commercial pectinase and cellulase addition on in vitro digestibility of two common bean (*Phaseolus vulgaris*) varieties, black and red, was measured constant conditions of pH (6.3-6.4), time (60min), and temperature (40˚C) were held, and three enzyme concentrations were tested (Viquez and Bonilla, 2002). On another side, an optimum carbon source was (Maltose), and the incubation in static conditions were the best circumstances for tested enzymes. Some authors reported that as the applied agitation rate increases, the apparent activity of the endoglucanases from *Trichoderma reesei* towards cotton pectin increases more markedly than does the apparent activity of the cellobiohydrolases (Cortez *et al.* 2001).

Biotechnological application of these enzymes separately and in comparison carried out with respect to clarification of ready made mango syrup resulted in: an optima enzymes' concentrations were ranged from 25-50 µl and clarification time (minutes) were 150 and 5 at refrigeration condition for cellulase and pectinase respectively, corresponding to 60 minutes at open air in case of enzymes' mixture.

In accordance to our results, Mclellan *et al.*(1985) investigated the effect of pectinase levels (0.00-0.006%) on apple juice clarification, they found that 0.006% was the recommended level for apple juice clarification using a pectinase commercially available. Partially depectinated apple cell walls were digested by pectin lyase or endoglucanase or a combination. Henderson *et al.* (1982) reported that adding 4% pectinase (wet bases) to grasses and legumes resulted in marked pectin hydrolysis.

Similarly, Mchan (1986) improved ensiling and fiber digestion of coastel bermudagrass with an alkalizing agent and a pectinase enzyme complex. Pathak and Sanwal (1998) studied the multiple forms of PG from banana fruits and indicated that three multiple forms of PG in ripe and two in unripe banana(*Musa acuminata*) fruits, were separated by DEAE-pectin and further purification was performed using sephadex G-150 chromatography. Afifi(2002) studied the application process of pectinases enzymes by using four fruit juice samples included Carob,Tamarind,Guava and Mango. Ammar and Afifi (2003) studied on the treatment of separation, turbidity, and malclarification problems occurring in mango syrup by three kinds pectinase, their study emphasized that the optimum enzyme concentrations (units/ml) were 8721.44, 4498.72 and 6748.08, incubation period optima (30, 90 and 120 min.) for crude ST, purified ST and KLL-pectinases respectively. De-Vries (2003) Described that the *Aspergillus* enzymes involved in the degradation of cellulose, xyloglucan, xylan, galacto (gluco) mannan and pectin, and the regulatory systems involved in the expression of the genes encoding these proteins. The latter was of major importance in the large-scale production of these enzymes for industrial applications.

It was concluded that there is a great hope in the use of the present cellulase and pectinase in many purposes: To solve the problem of clarification and increasing the clarification

percentage; the validity of these enzymes to obtain a permanent stable liquid form emphasized the validity of this technique in concentrated fruit juice; May be applied in increasing fruit juice yield on large scale and availability to use these enzymes in silage manufactures in the future as well. This may encourage to apply the present enzymes in many fields of large/industrial scale.

Acknowledgement :

Author's thanks and gratitude to Prof. Dr. A. El-shanawany and Mr. A. Mostafa, Bot. and Micro. Dept., Faculty of science, Al-Azhar University, Assiut, Egypt, for their efforts in the isolation and identifications of fungal species under investigations.

REFERENCES:

1-Acebal, C.M.P. Castillon, P. Estrada, I. Meta, E. Casta, j. Aguado, D. Romero, and F. Jimenez (1986). Enhanced pectinase production from *Trichoderma ressei* QM 9414 on physically treated wheat straw. Appl. Microbiol. Biotechnol. 24:218.

2-Afifi, M.M.(1994). Studies on Microbial Pectinases. M. Sc. Thesis, Bot. Dept., Fac. Sci., Al-Azhar Univ. Cairo, Egypt.

3-Afifi, M.M.(2002). Application of *Aspergillus niger*, S-48 TAT enzyme in food processing as compared with those of natural microflora of fermented potato wastes. Ph.D. Botany and Microbiology Dept., Faculty of Science, Al-Azhar Univ., Cairo, Egypt.

4-Ali, I.O.(2000). Microbial Utilization of *Eichhotnia crassipes* under SSF. M. Sc. Thesis, Bot. & Microbiol. Dept., Fac. of Sci., AL-Azhar Univ., Cairo, Egypt.

5-Ammar M.S. and Afifi, M. M. (2003). Solution of the problems of malclarification, turbidity and separation in mango syrups by fungal pectinases. Socity of general microbiology, 152nd meeting, University of Edienburgh, UK.7-11 April 2003., pp.41.

6-Ammar, M.S.; Loubody, S.S.; Azab, M.S. and Afifi, M. M. (1995a). A new method for the estimation of fungal pectinase(s) using the pectin clearing zone (P.C.Z.) and its application in food industries. Al-Azhar Bull.Sci. 6 (1): pp.325-339.

7-Ammar, M.S.; Desouki, E., El-Houseiny, M. M. and Sedki, Nagwa, M. (1995b). Cellulolytic fungi from phylloplane and phyllosphere of some Egyptian plants.Al-Azhar Bull. Sci. 6(2): 1823-1841.

8-Ammar, M.S.;Desouki, E., El-Houseiny, M.M. and Sedki, N.M. (1995c). Some environmental and nutritional parameters controlling the productivity of cellulase(s) by *Trichoderma hamatum* (Bon.) Bain, S-6 and *T. longibrachiatum* Refai, S-7 under bench scale fermentation conditions. Al-Azhar Bull. Sci. 6(9): 423-437.

9-Ammar, M.S.; Desouki, E., El-Houseiny, M. M. and Sedki, N.M. (1995e). Production, purification and properties of cellulase(s) biosynthesis under bench scale fermentation the phylloplanic fungus *Trichoderma hamatum* (Bon.) Bain, S-6, Al-Azhar Bull.Sci.6 (1): 375-391.

10-Ammar, M.S.; Loubody, S.S.; Azab, M.S.; A.El-Deeb and Afifi, M.M.(1995g).Pecteolytic activities and identities of the fungal flora of Tut Ankhamen Tomb (TAT) allowed to grow under solid state fermentation (S.S.F.) conditions. Al-Azhar Bull. Sci., 6(1): 361-374.

11-Carden DE, and Felle HH. (2003).The mode of action of cell wall-degrading enzymes and their interference with Nod factor signaling in *Medicago sativa* root hairs). Planta Apr; 216(6): 993-1002

12-Cortez JM; Ellis J; and Bishop, DP. (2001). Pectinase finishing of woven, cotton fabrics in

jet and winch machines.J. Biotechnol Aug 23;89(2-3):239-45.

13-De-Vries, RP. (2003). Regulation of *Aspergillus* genes encoding plant cell wall polysaccharide-degrading enzymes; relevance for industrial production. Appl. Microbiol Biotechnol, Mar; 61(1):10-20

14-Dongowski G., Sembries S., Bauckhage K., Will F., and Dietrich H. (2002). Degradation of apple cell wall material by commercial enzyme preparations. Nahrung Apr; 46(2): 105-11.

15-Emtiazi, G.; Naghav, N; and Bordbar, A.(2001). Biodegradaton of lignocellulosic waste by *Aspergillus terreus.* Biodegradation. 12(4): 259-263.

16-Essa, HA and Salama, MF. (2002). Effect of macerate enzymes on the yield, quality, volatile compounds and rheological property of prickly pear juice. Nahrung, Aug; 46(4): 245-50.

17-Fogarty, WM. and Kelly, TC. (1983). Pectic enzymes. Microbial Enzymes and Biotechnology, ed Fogarty WM. Applied Science puplishers, London, pp 131-182.

18-Henderson, A. R; McDonald, P. and Anderson, D. (1982). The effect of pectinase preparation derived from *Trichoderma veride* on the chemical changes during the ensilage of grass, lucerne and clover. J. Sci. Food Agric. 33:16.

19-Lambert, PW(1983). Industrial enzyme production and recovery from filamentous fungi. The filamentous fungi. Vol. 4, eds Smith JE, Berry OR and Kristiansen B.Edward Arnold Publishers, London, pp 210-237.

20-Mach RL. and Zeilinger S.(2003).Regulation of gene expression in industrial fungi: *Trichoderma*. Appl. Microbiol Biotechnol Jan; 60(5): 515-522

21-Mchan, F. (1986). Pectinase-treated coastel bermudagrass silage and production of soluble charbohydrates, silage acids, and digestibility. J. Dairy Sci. 69:431.

22-Mclellan, M.R., Kime, R.W. and Lind. R. (1985). Apple juice clarification with the use of honey and pectinase. J. Food. Sci. 50:206-208.

23-Mehrlander, K; Dietrich, H.; Sembries, S ; Dongowski, G.and Will, F.(2002). Structural charachterization of oligosaccharides and polysaccharides from apple juice produced by enzymatic pomace liquefaction. J. Agric Food Chem. feb.27; 50(5) : 1230-1236.

24-Pathak, N. and Sanwal, G.G. (1998).Multiple forms of polygalacturonase from banana fruits. Phytochemistry 48:2, p. 249-255.

25-Petruccioli M., and Federici F(1992). Utilization of a yeast pectinase in olive oil extraction and red wine making processes. J. Food Sci. Agric. 58, 253-260.

26-Viquez, R.F. and Bonilla, L., A. (2002). Effect of pectinase and cellulase addition on in vitro digestibility of commen bean (*Phaseolus vulgaris*). Archlatinoam Nutr. jun; 52(2):151-154.

Ass. Univ. Bull. Environ. Res. Vol. 6 No. 2, October 2003

التطبيقات التقنية الحيوية لإنزيمات السيلوليز والبكتيناز المنتجة من بعض فطريات التربة بصعيد مصر

مجدى محمد عفيفى بدران

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

يهدف هذا البحث للحصول على أفضل العزلات الفطرية والمعزولة من التربة بصعيد مصر بمحافظة أسيوط لتحفيز إنتاج إنزيمات السيلوليز والبكتيناز للاستفادة منها فى تكنولوجيا صناعة الغذاء واستغلالها بصورة فردية أو في مخاليط للحصول على التحلل الكامل للجدر الأولى والثانوية للخلية النباتية ولاستخدامها فى تكنولوجيا صناعة الأعلاف.

وقد تم عزل وتنقية وتعريف ثلاثين نوعاً فطريا تتبع ثمانية عشر جنسا، كما تم عمل مسح شامل لاختيار أقوى العزلات الفطرية قدرة على إنتاج الإنزيمات وكانت هى فطرة ميوكر فوسكس –مس–٢٢، كما تم دراسة الظروف المثلى لإنتاج إنزيمات السيلوليز والبكتيناز ووجد أن : أنسب درجة حموضة (pH) كانت عند ٧ و ٨ في حالة إنزيمات السيلوليز والبكتيناز على التوالى، وأنسب مصدر كربوني كان (المالتوز)، وأنسب تركيز للحقنة كان (واحد قرص ٠,٥ مليمتر قطر) والتحضين عند ظروف ثابتة ودرجة الحرارة ٢٠°م، وكان أنسب تركيز لمادة التفاعل (٢، ٠,٢%)، أفضل مصدر نيتروجيني هو (الجليسين واليوريا)، حجم المخمر (٢٥٠، ٢٠٠٠ مل) مثلت أفضل الظروف لإنتاج إنزيمات السيلوليز والبكتيناز على التوالي.

وقد تمت العمليات التطبيقية بواسطة استعمال (شربات المانجو) المستخدمة للمستهلك والمنتجة من شركه فيتراك للأغذية بمحافظة القليوبية بواسطة استعمال الإنزيمات الحالية بصوره فردية أو مختلطة على النتائج التالية : أثبتت الدراسة أن نشاط الإنزيمات المنتجة يزداد تدريجياً بزيادة تركيزها حتى ٢٥ (ميكروليتر) يقابله ٥٠ (ميكروليتر) في حالة مخلوط الإنزيمات. كما أوضحت الدراسة أن درجة نشاط كل من الإنزيمات تزداد تدريجياً مع وقت الترويق حتى ١٥٠ و ٥ دقيقة بالنسبة لإنزيمات السيلوليز والبكتيناز على التوالي يقابلها ٦٠ دقيقه في حالة مخلوط الإنزيمات.

وجد أن أنسب ظروف لتطبيق الإنزيمات كانت عند درجة حرارة الثلاجة يقابلها ظروف التطبيق عند درجة حرارة الغرفة في حالة مخلوط الإنزيمات. وبناءً على هذا فيمكن تطبيق هذه الإنزيمات على المستوى المعملي والتجريبي أو على المستوى الصناعي والتجاري نظراً لكفاءتها الملحوظة في العمل والنشاط عند درجة حرارة الغرفة وخصوصا في مجال العصائر المركزة وأشباهها، كما يمكن التركيز على إمكانية استخدامها في مجالات الصناعة والغذاء نظراً للنتائج التي تم التوصل إليها فى مجال التطبيق بالإضافة إلى قدرة الإنزيمات الحالية بالعمل بصفة فردية أو في مخاليط للتأكد التام على تطبيقها مستقبلا في العديد من المجالات مثل صناعة الأعلاف.