Summary

The present study was carried out to achieve two main objectives :

The first object was to evaluate the possibility of utilization of sugar - beet industry by - products (beet pulp, and beet molasses) for production of some microbial enzymes (pectinases).

The second goal of the present work deals with the evaluation of fungal biomass of Myrothecium verrucaria (involved in pectinases production) as a possible source of protein.

The whole frame of the work and the findings obtained can be summarized in the following:

1. Screaning studies were carried out using nine strains of M. verrucaria, as to select the suitable strain for production of pectinases. These cultures were obtained from different microbial cultures collections in Netherland, United Kingdom, Canada, United State of America and Egypt, for production of pectinases. Modified Czapek— Dox media were used in the screening studies with the replacement of the original carbon source (sucrose) with,

glucose, apple pectin or beet pulp at 1% final concentration.

2. The results obtained revealed that M. verrucaria CBS 28846, CBS 17627 and Ls-NRC could produce high levels of pectolytic activities on Czapek - Dox medium - containing beet pulp as a carbon source whereas the rest of strains tested exhibited feeble or no pectolytic activity at all. On the other hand all tested strains yielded very low or no activity in media containing glucose or apple pectin as carbon sources.

3. The highly active pectolytic strains of M. verrucaria were subjected to a comparative study as to find out the type of pectolytic enzymes produced. The results demonstrated that the observed pectolytic activities are due to the presence of endo polygalacturonase enzymes; no pectin lyase activity could be detected.

4. Further characterization polygalacturonase (PG) produced revealed that the PG activities of the tested strains namely CBS 28846, CBS 17627 and Ls-NRC of M. verrucaria belong to the endo type

as judged by their effect on viscosity and release of reducing groups.

5. Physiological studies on formation of endo-PG enzymes by the active pectolytic strains of M. verrucaria grown in beet pulp containing medium have demonstrated that the optimization of growth conditions of the fungal cultures for production of high levels of pectolytic activities could be as follows:

a) With respect to the incubation period it was found that 3-4 days of incubation is optimal for endo-PG production from M. verrucaria CBS 28846 and CBS 17627 whereas 8 days were required for strain Ls-NRC to obtain high levels of enzyme under study.

b) Using M. verrucaria strains CBS 28846 and CBS 17627 under all medium: air ratios tested in the experimental flasks the static cultures gave better enzyme yields as compared to shake cultures. This finding was more accentuated at the highest tested Air: mediam ratio namely 54:6. However high levels of PG activities were also produced under the shaking conditions although the enzyme yield was less than in case of static cultures.

observed non homogeneity of the cultures grown under the shaking conditions, may be another factor affects the level of produced PG activities side by side with the aeration level.

c) The optimum initial pH values of the medium for production of PG activity was in the range 3-5.2 for strain CBS 28846 and 2.6-4.6 for strain CBS 17627 of M. verrucaria. However the tested strains produced appreciable amounts of PG activities over a wide range of the initial pH values ranged from 2.6-7. In spite of the variation of initial pH value of the medium the pH at harvest was always in the acidic side.

d) Various carbon sources were tested with respect to their effect on PG formation. These included arabinose xylose, fructose, glucose galactose, Galacturonic acid, lactose, malatose. Sucrose, raffinose, cellulose, pectin, starch beet molasses, and beet pulp. The beet pulp proved to be best carbon source for both test strains CBS, 28846 and CBS 17627 of M. verrucaria.

Galacturonic acid induced much lower endo-PG activities (14.3 and 15.2% RV respectively) compared with those obtained with beet pulp (65.8 and 53%

RV) using M. verrucaria CBS 28846 and CBS 17627 respectively. On the other hand the apple pectin exhibited no inducing effect for endo-PG formation by M. verrucaria. The other carbon sources gave no or feeble endo-PG activities.

e) No marked differences could be detected within the enzyme levels produced at 1-4% (W/V) of beet pulp in the growth medium. However the lowest concentration (0.5% W/V) gave the lowest activities.

f) The levels of PG activities produced was slighthly affected by the size of inoculum. Thus inoculum size of 4% (V/V) could be employed favourably for attaining highest activities with respect to the test strains.

g) Various nitrogen sources were tested, namely sodium nitrate, ammonium sulphate, ammonium chloride, urea, peptone and yest extract.

In general no drastic effect for the type of nitrogen source could be detected on PG activities proudced. However, ammonium sulphate supported the formation of slighthly higher levels of PG activities in both tested strains.

6. Kinetics studies of crude endo-PG of M. verrucaria

CBS 28846 and CBS 17627 revealed the following properties:

a) The optimum temprature for PG activity was 60°C for both CBS 28846 and CBS 17627 strains of M. verrucaria. Moreover the two enzymes exhibited different heat stability reaching 60 and 50°C for 15 minutes respectively.

b) With respect to enzyme concentration linear activity response was obtained with enzyme concentation up to 0.048 mg protein/ml for both CBS 28846 and CBS 17627. The activity increased at higher protein concentrations but not in linear manner.

c) For both enzymes of M. verrucaria CBS 28846 and CBS 17627, a linear response of reaction rate with reaction time was obtained up to 10 minutes. Then the reaction progressed with time but not in a strictly linear manner for about 15 minutes. The reaction reached steady state and leveling off for reaction rate took place after 35-40 minutes of the incubation time.

d) A linear increase in enzyme activity was observed by increasing substrate concentration (apple pectin) up to 0.8% W/V pectin concentration, for crude enzymes of M. verrucaria CBS 28846 and CBS 17627.

The reaction rates leveled off at higher substrate concentrations 2% and 1.2% W/V of pectin for both strains respectively).

e) The optimum pH values for enzyme activity were the range between 3-5 and 4-5 for the enzymes obtained from both fungal strains tested.

7. Partial purification of PG enzyme of M. verrucaria CBS 28846 was carried out using fractional precipitation with acetone, and gel filtration on sephadex G100 coulmn. Electrophoresis technique was used to show the degree of purity.

The acetone fractionation yielded enzyme fractions of different purity, some fractions gave about 6 and 9 fold of purification. The highest purification fold (about 38 times) was obtained in a two-stage acetone fraction through which the appropriate fraction from the first stage was refractionated for obtaining a higher level of enzyme purity. Yet the enzyme was unstable. Thus when applied on sephadex G100 column, only feeble activity was obtained in the resultant sephadex fractions. However the gel filtration gave partially purified enzyme of about 26 fold purification using

some other acetone fractions.

The gel electrophoresis on polyacylamide gel showed that the enzyme obtained from the sephadex column is only partially purified.

8. Kinetics studies of partially purified enzyme of M. verrucaria CBS 28846 showed that:

a) The optimum temprature for PG activity was as that of crude enzyme i.e 60°C. The enzyme exhibted thermal stability up to 60°C. Then a sharp decrease of enzyme activity was observed at higher tempratures.

b) With respect to the enzyme concentration the enzyme exihibted a linear response in reaction rate to protein concentration at least up to 0.03 mg/ml. The activity increased at higher protein concentration but not in linear manner.

c) The reaction rate progressed with time in an approximately linear manner at least up to 10 minutes (viscometerically measured). whilst the reducing group determination showed a reaction linearity at least for 15 min. Upon extending the reaction time the rate of reduction of viscosity and release of reducing groups were relatively slow during the following five minutes bebore leveling off

at longer reaction time.

d) With respect to the substrate concentration showed a linear increase of the reaction rate up to 0.8% substrat (Apple pectin) concentration.

e) The optimum pH value was 4.5, then a sharp drop of reaction rate was noted by increasing the pH values of the reaction mixture. At pH 7 no detectable activity was obtained.

9. The chemical and biological evaluation of the fungal biomass of M. verrucaria showed that the amino acid methionine was revealed the first limiting amino acid. Histidine and threonine were the second and the third limiting amino acids respectively. The other essential amino acids are present in adquate amounts and exceeded their conterpart amounts in the FAO pattern, (1973). On the other hand the animal feeding experiment indicated that the fungal biomass of M. verrucaria was palatable, digistable and enhanced the growth and gain in weight of the test animal (rats). The nutritional evaluation parameters namely PER, AD and TD, BV, NPU and up proved that this kind of protein could be considered of high quality.