Qatar Univ, Sci, J. (1997), 17(1) : 97-102

PRODUCTION OF EXTRACELLULAR ENZYMES BY SOME SOIL YEASTS

By

A. M. Falih

Department of Biology, Teachers College, Riyadh, P.O.Box 4341. Riyadh 11491, Saudi Arabia.

إنتاج عدد من الأنزيمات الخارجية بواسطة بعض خمائر التربة

عبد الله مساعد خلف الفالح

قسم الاحياء – كلية المعلمين بالرياض

ص . ب : ٤٣٤١ – الرياض ١١٤٩١ – المملكة العربية السعودية

معلوم ان الفطريات لها دور مهم في حياة الأنسان من خلال ما تنتجه هذه الكائنات الحية الدقيقة من انزيمات تعمل على تحلل المواد العضوية وأكسدة المعادن في التربة لتصبح متوفرة للنبات. كما تدخل هذه الانزيمات في كثير من الصناعات والتخلص من النفايات، ويستفاد منها ايضا في النواحي الطبية لخدمة الإنسان.

ولهذا فقد شمل هذا البحث دراسة إمكانية انتاج كل من انزيم الأميليز Amylase وانزيم السليوليز celluase وانزيم البروتييز protease خارج الخلية لعدد من خمائر التربة وهي : *Geotrichum candidum, Geotrichum capitatum, Williopsis californica, Sacchoromyces cerevisiae* وقد عزلت هذه الخمائر من عينات التربة التي جمعت من منطقة الرياض بالمملكة العربية السعودية. وتم التأكد من إنتاج انزيم الأميليز وانزيم السليوليز وانزيم البروتييز خارج الخلية من هذه الخمائر بواسطة طريقة المنطقة الشفافة (الرائقة) Cleared-zone technique .

وقد أوضحت النتائج ان جميع الخمائر تنتج نسبة بسيطة من انزيم الاميليز باستثناء خميرة *G. candidum* التي انتجت نسبة عالية من انزيم الاميليز. وقد وجد أن خميرة التربة *W. californica* تنتج السليوليز بمعدل طبيعي مقارنة بالفطريات الأخرى. بينما وجد أن الخميرة *G. capitatum* قادرة على انتاج انزيم البروتييز بمعدلات عالية جدا، وكانت اكثر الخمائر قدرة على تحلل الجيلاتين.

Key-words: soil yeasts, extracellular enzymes, amylase, cellulase, protease.

ABSTRACT

This study investigated the ability of soil yeasts, *Geotrichum candidum, Geotrichum capitatum* and *Williopsis californica* to produce extracellular enzymes (amylase, cellulase and protease) in vitro compared with that of a laboratory strain of *Saccharomyces cerevisiae.* It appears that the soil yeasts studied here were less amylolytic yeasts except the yeast *G. candidum,* which was highly effective at extracellular amylase production. The soil yeast *W. californica* was an average producer of cellulase enzyme. *G. capitatum* was an excellent proteolytic producer and it was the most active yeast in gelatin hydrolysis. The production of extracellular enzymes by soil yeasts was confirmed by the cleared-zone technique using Czapek-Dox agar medium.

INTRODUCTION

Fungi have traditionally been regarded as decomposer organisms whose primary role is the degradation of carbon and nitrogen-rich residues such as leaf litter and wood. To achieve this mineralization fungi possess a wide range of extracellular enzymes including amylase, cellulase and protease [1].

The production of various extracellular enzymes by fungi has a great influence on human life, owing to its important role in the pharmaceutical, food, paper, textile and petroleum industries [2]. The biodegradation of waste materials is another example of the beneficial effects of fungi, as the waste of garbage will become a great problem for our future generations [3].

The ability of fungi to produce extracellular enzymes is well established and hardly been studied [1, 3, 4, 5, 6]. Most of the studies to date have been limited to species of *Aspergillus, Fusarium, Trichoderma, Myrothecium* and *Penicillium* [2, 7, 8, 9, 10, 11].

In soil microbiology yeasts in particular have been neglected, although they are known to appear in most soils. As a result little is known about their ecology and the role that they play in mineral cycling [12]. A recent study shows that the soil yeast *Williopsis californica* is capable in the processes of nitrification, S-oxidation and P-solubilization [13]. It is suggested from that study, that soil yeasts might be used as inoculants to stimulate the beneficial processes of mineral cycling in soils [13].

The aim of the present work was to study the ability of the soil yeasts *Geotrichum candidum* Link, *Geotrichum capitatum* (Diddens & Lodder) V. Arx and *Williopsis californica* (Lodder) Krasil'nikov to produce extracellular amylase, cellulase and protease. A strain of *Saccharomyces cerevisiae* Hansen used in our laboratory was also included for purpose of comparison.

MATERIALS AND METHODS

The soil yeasts were isolated from a sandy soil (total C 0.1 %; total N 0.05 %; pH 7.5, obtained from Riyadh region, Saudi Arabia). Both of *Geotrichum candidum* and *Geotrichum capitatum* isolated were deposited at the International Mycological Institute for identification. While the yeast *Williopsis californica* was identified according to AFRC Institute for Food Research, Norwich.

Media

The basal medium used in this study was modified Czapek-Dox Agar at pH 6 for cultivation of the soil yeasts [8]. For detecting enzyme activity appropriate substrates were incorporated into the basal medium. Potato starch at 0.2 % (w/v) was included in the Czapek - Dox Agar to make a plating medium for screening the yeasts for total amylolytic activity. Similarly, gelatin at 0.4 % (w/v) was added to prepare plating medium for the detection of proteolytic enzymes. For the screening of cellulase producing yeasts, cellulase azure at 2 % (w/v) was added to Czapek Dox Agar. The inoculated plates were then incubated in 5 replicates at 25° C for 6 days.

Determination of yeast biomass

Aliquots of a yeasts cells suspension (1 ml) were used to

inoculate liquid Czapek - Dox medium (100 ml in 250 ml capacity Erlenmeyer flasks), adjusted to pH 6.0 with 2N NaOH. The medium was then amended with either starch (final suspension weight 0.2 % w/v); cellulose (final suspension weight 2.0% w/v) or gelatin (final suspension weight 0.4 % w/v). The flasks were then incubated with shaking (100 r.p.m) at 25°C for 6 days. At 2 days intervals the yeast biomass was determined in the medium after filtration through pre-dried and pre-weighed Whatman No 1 filter papers. The weight of yeast cells retained by the filter papers was then determined (after drying to constant weight at 46°C) as a measure of yeast cell biomass. The pH of the medium was determined with a glass electrode.

Detection of extracellular enzymes

After 2 day - intervals the plates were removed and the activity of extracellular enzymes were determined. Detection of amylase, cellulase and protease production by soil yeasts were carried out by the cleared-zone technique (a semiquantitative method) as described by Lim, Khew & Yeoh [8]. The percentage of cleared-zone with respect to colony size was taken as an indication of the level of enzyme activity. Percentage of cleared-zone was calculated according to the following formula:

$$\text{Percentage of cleared-zone} = \frac{\text{Diameter of cleared-zone - Diameter of colony}}{\text{Diameter of colony}} \times 100$$

RESULTS

Both *Geotrichum candidum* and *G. capitatum* hydrolysed starch by their amylolytic activity (Fig1.). *G. candidum* was particularly active in this process forming 98 % of amylase at the end of the incubation period. The soil yeast *W. californica* failed to hydrolyse starch in Czapek-Dox medium, so amylase activity was not detected in the medium. While in the case of *S. cerevisiae* amylase activity was only detected transiently in trace percentage (9 %) towards the end of the incubation period (Fig. 1). Production of extracellular amylase by soil yeasts led to a reduction in the pH of the medium, especially in the case of *G. candidum* that produced the highest percentage of amylase activity (Table. 1).

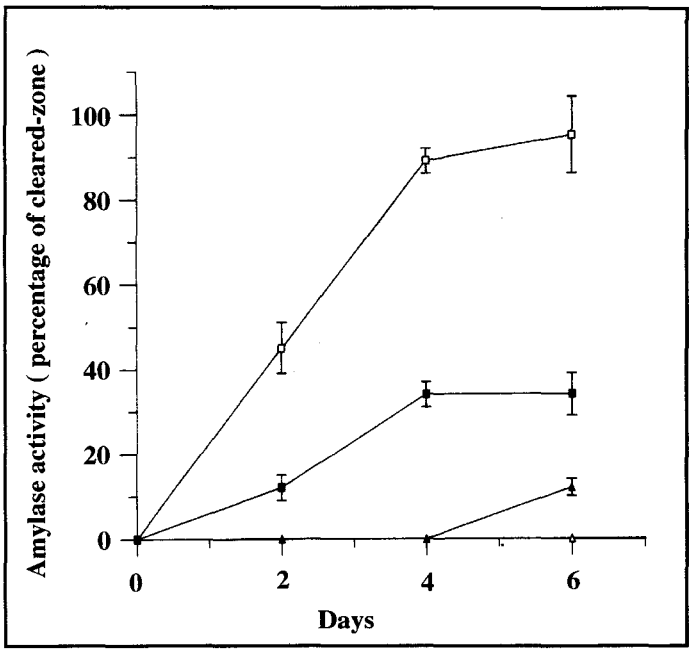

Fig. 1: Amylase activity of soil yeasts grown in Czapek-Dox agar medium (activity is expressed as a percent. of cleared-zone, all values are means of triplicates ± S.D).
(□ *Geotrichum candidum;* ■ *Geotrichum capitatum*; Δ *Williopsis californica;* ▲ *Saccharomyces cerevisiae*).

Fig (2) shows that all of the soil yeasts were able to produce cellulase in Czapek-Dox medium, a process which was also associated with a reduction in the pH of the medium (Table. 2). In this study, *W. californica* had the greatest cellulase activity with 73 % followed by the yeast of *G. capitatum* with 45 %. *G. candidum* recorded the lowest percentage of cellulase (11). In the case of *G. capitatum* yeast, cellulase activity was not detected in the medium until day 4, after which time the activity of the enzyme increased as yeast biomass increased (Table. 2).

Table 1. Biomass of soil yeasts grown on Czapek-Dox liquid medium amended with starch as a sole source of carbon and changes in the pH of medium (Values given are means of triplicates SYMBOL Symbol standard deviation).

	Days	*G. candidum*	*G. capitatum*	*S. cerevisiae*	*W. californica*
Biomass (g)	2	0.14 ± 0.07	0.08 ± 0.03	0.01 ± 0.00	0.01 ± 0.00
	4	0.22 ± 0.11	0.15 ± 0.04	0.01 ± 0.01	0.01 ± 0.00
	6	0.30 ± 0.09	0.19 ± 0.01	0.02 ± 0.00	0.01 ± 0.01
pH	2	5.3 ± 0.2	5.9 ± 0.1	5.8 ± 0.0	6.9 ± 0.03
	4	5.0 ± 0.8	5.4 ± 0.6	5.9 ± 0.5	6.7 ± 0.07
	6	4.4 ± 0.0	5.1 ± 0.4	5.6 ± 0.1	6.7 ± 0.02

Table 2. Biomass of soil yeasts grown on Czapek-Dox liquid medium amended with cellulose as a sole source of carbon and changes in the pH of medium (Values given are means of triplicates ± standard deviation).

	Days	*G. candidum*	*G. capitatum*	*S. cerevisiae*	*W. californica*
Biomass (g)	2	0.12 ± 0.05	0.04 ± 0.00	0.11 ± 0.01	0.13 ± 0.06
	4	0.19 ± 0.01	0.57 ± 0.01	0.21 ± 0.07	0.54 ± 0.03
	6	0.22 ± 0.04	0.66 ± 0.02	0.25 ± 0.00	0.75 ± 0.04
pH	2	5.4 ± 0.5	4.9 ± 0.4	5.9 ± 0.1	5.5 ± 0.02
	4	5.5 ± 0.2	4.6 ± 0.1	5.7 ± 0.6	4.8 ± 0.04
	6	5.2 ± 0.3	4.7 ± 0.0	5.0 ± 0.3	4.3 ± 0.01

Table 3. Biomass of soil yeasts grown on Czapek-Dox liquid medium amended with gelatin as a sole source of carbon and changes in the pH of medium (Values given are means of triplicates ± standard deviation).

	Days	*G. candidum*	*G. capitatum*	*S. cerevisiae*	*W. californica*
Biomass (g)	2	0.01 ± 0.00	0.16 ± 0.03	0.01 ± 0.00	0.11 ± 0.02
	4	0.04 ± 0.01	0.27 ± 0.04	0.05 ± 0.01	0.16 ± 0.07
	6	0.11 ± 0.04	0.32 ± 0.11	0.09 ± 0.02	0.24 ± 0.05
pH	2	5.6 ± 0.1	4.3 ± 0.3	5.4 ± 0.1	5.2 ± 0.02
	4	5.5 ± 0.2	3.5 ± 0.01	5.5 ± 0.5	4.6 ± 0.04
	6	5.4 ± 0.5	2.1 ± 0.6	5.0 ± 0.7	4.1 ± 0.03

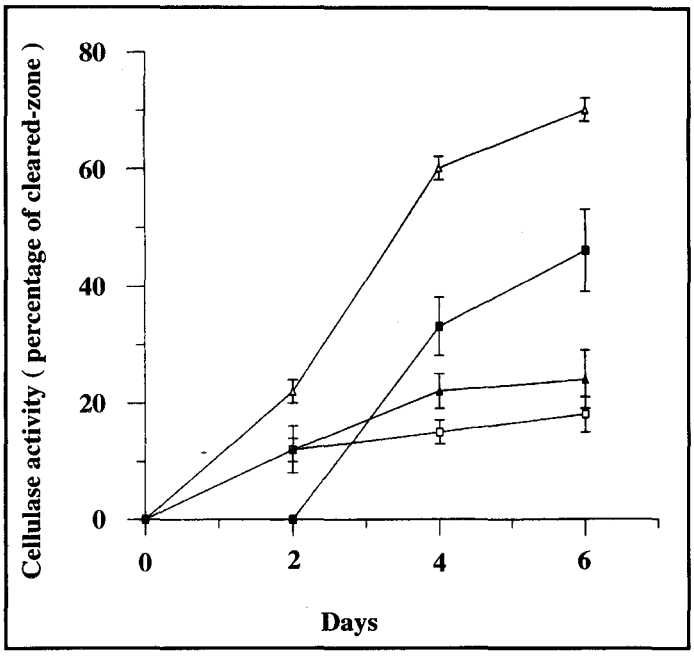

Fig. 2: Cellulase activity of soil yeasts grown in Czapek-Dox agar medium (activity is expressed as a percent of cleared-zone, all values are means of triplicates ± S.D).
(□*Geotrichum candidum;* ■ *Geotrichum capitatum;* Δ *Williopsis californica;* ▲ *Saccharomyces cerevisiae*).

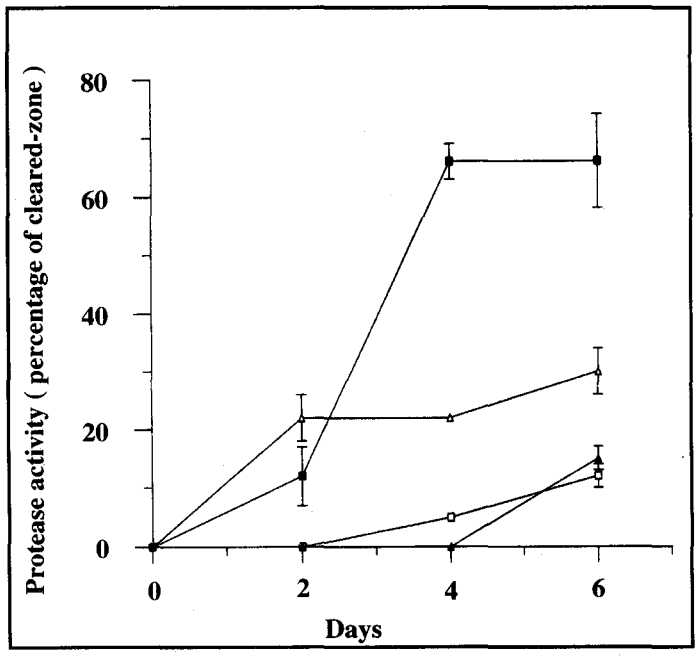

Fig. 3: Protease activity of soil yeasts grown in Czapek-Dox agar medium (activity is expressed as a percent. of cleared-zone, all values are means of triplicates ± S.D).
(□*Geotrichum candidum;* ■ *Geotrichum capitatum;* Δ *Williopsis californica;* ▲ *Saccharomyces cerevisiae*).

The three genera of the soil yeasts exhibited extracellular proteolytic activity (Fig. 3). Again differences in the levels of enzyme activities of the soil yeasts were observed. For example, *G. capitatum* has shown to be the best proteolytic enzyme producer with 65 %. Proteolytic activities of soil yeasts led to a marked reduction in the pH of the medium, particularly in the case of *W. californica,* probably due to the formation of amino acids (Table.3). Another interesting observation here was that the proteolytic and amylolytic activity of *G. capitatum* was stable after certain time, so the enzyme activities of this yeast was reaching the maximal level after 4 days of incubation.

The largest amount of biomass was produced by W. californica (0.75 g), when the medium was amended with cellulose (Table. 2). However when the Czapek-Dox medium supplemented with starch or gelatin, the highest amount of biomass observed, was 0.30 g (produced by G. candidum) and 0.32 g (produced by *G. capitatum*) respectively (Tables. 1 and 2).

DISCUSSION

The production of extracellular enzymes by a particular soil yeast varied with the source of carbon e.g. *G. candidum* exhibited 98 % cleared-zone when the medium was amended with starch, while this yeast exhibited only 10 % cleared-zone when the medium was amended with gelatin. On the other hand, the yeast of W. californica which grew poorly on starch medium, showed no cleared-zone, indicating it was not a producer of amylase. Result of this study shows that the *S. cerevisiae* was the poorest in the production of amylase (9 %), while *G. candidum* was the best (98 %). Bokhary & Parvez [9], in a survey of soil mycoflora capable of producing extracellular amylase, reported that *Penicillium chrysogenum* was the best producer of amylase (forming 82 % cleared-zone) among 84 species of soil mycoflora. The soil yeast *G. candidum* appears unusual, however, in that it forms large amounts of amylase.

A wide variety of filamentous fungi have been previously reported as amylolytic fungi [2, 6, 8, 9]. However the potential role of yeasts in biodegradation of waste materials in soils has been ignored, largely because yeasts are thought to make up an insignificant proportion of the

soil microbial population [13]. This study clearly shows however, that at least two soil yeasts can achieve substantial rates of amylase production.

A previous study of 61 funji species recorded that *Absidia corymbiferae* produced the largest cleared-zone with 86.8 % [10] and *Aspergillus awamori* found to be the best amylolytic enzyme producer with 53 % [8]. The present results show that the soil yeast *W. californica* produced a large cleared-zone of cellulase activity (73 %), indicating that it was an average producer of cellulase enzyme. In general, all soil yeasts tested were recorded as cellulose degraders.

The role of soil yeasts in the production of exteracellular protease has been neglected. For example, in survey by Hudson [3], Casida [6] and Hankin & Anagnostakis [14], of fungi capable of participating in the process, yeasts are not mentioned. Although, the present investigation clearly shows that all yeasts studied are almost capable of extracellular protease production. *Aspergillus niger* was shown, by Lim, Khew & Yeoh [8], to be the best proteolytic enzyme producer with 55.1%. While Hankin & Anagnostakis [14] reported that the highest percentage of protease activity was found to be 43 % produced by *Penicillium expansum.* The present results show that *G. capitatum* was the most active yeast in gelatin hydrolysis (65 %), so it was an excellant proteolytic producer.

Since most environments contain only small amounts of carbon, it is unlikely that the rates of production of extracellular enzymes observed by some soil yeasts using Czapek - Dox medium would be relevant to biodegradation in soils. However, in vitro studies such as this do at least indicate the potential ability of yeasts to hydrolysis; a fungus which is incapable of hydrolysis in vitro is unlikely to do so in the environment. This observation could be useful for future researches on the role of yeasts in biodegradation of waste materials.

In conclusion, it appears that the soil yeasts studied here were less amylolytic except *G. candidum*, which was highly effective at extracellular amylase production [9]. *W. californica* was an average producer of cellulase enzyme [8, 10]. *G. capitatum* was an excellant proteolytic producer and it was the most active yeast in gelatin hydrolysis [8, 14].

REFERENCES

[1] **Wainwright, M. 1992**. The impact of fungi on environmental biogeochemistry. In: The fungal community (Eds, Carroll, G. C. and Wicklow, D. T.). Marcel Dekker, Inc., New York.

[2] **Pestana, F. and F.J. Castillo, 1985.** Glucoamylase production by *Aspergillus awamori* on rice flour medium and partial characterization of the enzyme. MIRCEN J. 1, 225-237.

[3] **Hudson, H. J. 1980.** Fungal Saprophytism, Edward Arnold, U. K. p. 75.

[4] **Enari, T.M and P. Markhanen, 1977**. Production of cellulolytic enzymes by fungi. Adv. Biochem. Eng. 5: 8-12.

[5] **Sawao, M and K.J. Motoo, 1979**. Isolation and identification of cellulolytic enzyme-producing microorganisms. J. Ferment, Technol. 57 (3): 151-156.

[6] **Casida, L. E. Jr. 1991**. Industrial Microbiology, Wiley Eastern Limited, New Delhi. p. 460.

[7] **Kassim, E. A. 1983**. Cellulolytic activity of some soil fungi. J. Coll. Agric, King Saud Univ. 5: 169 - 177.

[8] **Lim, G., E. Khew and H.H. Yeoh, 1985**. Extracellular enzymes of some black aspergilli in Singapore. MIRCEN J. 1: 55-61.

[9] **Bokhary, H. A. and S. Parvez, 1992** . Production of extracellular amylase by soil mycoflora. Arab Gulf J. Scient. Res. 10 (3): 117-127.

[10] **Bokhary, H. A. and S. Parvez, 1994**. Extracellular cellulase enzyme production by soil mycoflora in Saudi Arabia. J. King Saud Univ. Science. 6 (2): 137-148.

[11] **Falih, A.M. 1997**. Cellulolytic activity of some fungi isolated from desert soils in Saudi Arabia. Mycopathologia (submitted).

[12] **Falih, A.M. and M. Wainwright, 1996**. Microbial and enzyme activity in soils amended with a natural source of easily available carbon. Biol Fertil Soils. 21 (3): 177-183.

[13] **Falih, A.M. and M. Wainwright, 1995**. Nitrification, S-oxidation and P-solubilization by the soil yeast *Williopsis californica* and by *Saccharomyces cerevisiae*, Mycol. Res. 99(2): 200-204.

[14] **Hankin, L. and S.L. Anagnostakis, 1975**. The use of solid media for detection of enzyme production by fungi. Mycologia. 67: 587-607.