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INFLUENCE OF OSMOTIC STRESS ON CERTAIN METABOLIC ACTIVITIES IN SOME OSMOPHILIC FUNGI

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ABSTRACT

***Aspergillus quandricinctus** and a species of **Pencillium** sp., osmophilic fungi, were able to grow on a medium containing sucrose up to 80% (w/v). The morphological characteristics of the **Pencillium** sp. were not drastically affected at a higher concentrations. While, **A. quadricinctus** failed completely to form conidial heads, but gave abundant cleistothecia. Maximal growth of **A. quandricinctus** and the **Pencillium** sp. were obtained at 80 and 40% sucrose (w/v), respectively. Total soluble carbohydrates and lipids were increased with increasing sucrose concentrations in the environment. While, protein was increased in **A. quandrinctus** but decreased in the **Pencillium** sp.. Certain amino acid stimulated the fungal growth, while others inhibited their growth.*

INTRODUCTION

Extreme natural environment and the effect of osmotic stress on microorganisms have been reviewed by many investigators (Colwell and Morita, 1974 ; Holding *et. al.*, 1974 ; Gray and Postgate, 1976 ; Heinrich, 1976 ; Brown, 1976 ; Kushner, 1978 ; Razak *et. al.*, 1983 ; 1985 and 1989).

Although reducing water activity is one of the widely used methods for food presrvation, several microorganisms have the ability to tolerate and proliferate at low water activity.

Regardless of the ecological and industrial singnificance of water stress tolerant microorganisms, they warrant physiologicl studies because of their ability to thrive at an environemnt with low water activity. Several microorganisms seem able to alter their metabolic pathways, the concentration of certain intracellular metabolic products were increased with reducing water activity, e.g. : proline, 4 - aminobutyric acid, glutamic acid and methionine (Measures, 1975 ; Kaujima *et. al.,* 1978 ; Hau *et. al.,* 1982 ; Anderson and Witter, 1982 and Razak *et al.*, 1985) ; Polyls (Brown, 1974 ; 1976).

The present communication is a part of a running project aiming mainly to understand the physiological responses of highly tolerant fungi against osmotic stress in order to regulate their growth in such environment biologically.

MATERIALS AND METHODS

The most osmotolerant fungal isolate were isolated from rotted fruits. They were identified as *Aspergillus quandricinctus* and *Penicillium* sp. according to Raper and Fennell (1977) and Pitt (1979).

Determination of fungal mycelial dry weight :

0.1 ml. of fungal spores suspension was inoculated into 100 ml. conical flask capacity, containing 20 ml. Dox liquid medium, supplemented with different sucrose concentration ; 20, 30, 40, 50, 60, 70 and 80% (w/v). The flask was incubated at 28 °C, unless otherwise stated. The cultures were filtered and the produced mycelia was washed throughly with dist. Water several times and dried at 60°C for three days, then mycelial dry weights were determined.

Mean of triplicate set dry weight were taken as criterion.

Protein Determination :

Protein was determined according to the method of Lowry *et. al.*, (1951), using bovine serum albumin as a standard protein.

Carbohydrate Determination :

Carbohydrate were determined according to the method of Umbriet *et. al.* (1959), using sucrose as a standard carbohydrate.

Lipids Determination :

Lipids content were determined by the phosphovanilline method according to Barnes and Blackstock (1973), Cholesterol was used as a standard lipid.

Effect of several amino acids :

22 amino acids were individually supplied to nitrogen free medium containing different sucrose concentrations as mentioned before. Amino acids were added in amounts equivalent to nitrogen content of 0.2% $NaNO_3$. They were sterilized in a sterile weighing bottle with cotton plugs by the addition of the least volume of diethylether, just to cover the amino acid for 24 h. until dryness. They were added to the sterile basal medium under aseptic conditions. Triplicate set of flasks were used for each treatment.

RESULTS AND DISCUSSION

The growth pattern of *A. quandricinctus* (Table 1) indicates that it is an osmophilic fungus; maximum growth was obtained at 80% (w/v) sucrose concentration. While, minimum growth was obtained at 2%. Generally, its mycelial dry weight was increased with increasing sucrose concentrations in the environment. Moreover, the carbohydrate content was also increased with increasing sucrose concentrations up to 50% (w/v), then decreased slightly. Maximum content was obtained at 30% sucrose. On the other hand, protein content was increased with increasing sucrose concentrations up to 40%. Maximum quantity was estimated at 30% sucrose concentration. The total soluble lipids was increased as well with increasing sucrose concentration in the medium up to 50%, however, lipids content at extremely high sucrose concentration ratio were nearly similar to that at 2% (w/v) sucrose concentration.

With regard to the *Penicillium* sp., the mycelial dry weight was increased with increasing sucrose concentration up to 60%. Maximal quantities of fungal mycelial dry weight was obtained at 40% / 50%. The quantities of carbohydrates and lipids content were increased with the increase of sucrose concentration up to 80%, however, maximal quantities were obtained at 40%. Contrary to that, the estimated quantities of protein decreased with increasing sucrose concentration in the environemtn. Generally, the detected quantities of protein at different sucrose concentrations were nearly similar.

Presumely, both fungi are osmophilic rather than osmotolerant.

Apparently such changes in the metabolic activities may such changes in the metabolic activities may be possibly reflected on the morphological characteristics of the fungi. The morphological characteristics of the *Penicillium* sp. seem not to be hardly affected. The fungus kept its external features undistorted at high concentrations. While *A. quadricinctus* gave abundant cleistothecia at higher concentrations. No conidial heads were detected at 50% or more (Fig. 1).

Since protein biosynthesis was badly affected in the *Penicillium* sp. when growth on high sucrose concentration containing media, the addition of certain amino acids to the growth environment were considered (Table 3). Some amino acids stimulated their growth in the presence of high sucrose concentrations. Alanine, arginine, aspartic acid, glutamine, threonine, and serine stimulated *A. quadricinctus* growth in the presence of elevated levels of sucrose in the environment, while; glycine, valine, leucine / isoleucine, cycteine, methionine, lysine, histidine, phenylalanine, ornithine, tyrosine and tryptophane inhibited it growth at high sucrose concentrations.

On the other hand, in the *Penicillium* sp., the picture is quite different. Only glycine and proline have unconsiderable effect on the fungal osomotolerance. While, mostly all the other tested amino acidshave no or an inhibitory effect. Cystine, phenylalanine, tyrosine, lysine, glutamine, isoleucine and histidine inhibited its growth when applied in very low concentrations.

From the presented results, mostly, the applied end-product amino acids ; lysine, methionine, threonine, isoleucine, as well as histidine had inhibitory effect on the fungal growth. Presumably, they exerted their feed back inhibition or feed back repression mechanisms on the fungal metabolic activities.

DISCUSSION

Fungi are known for many years as spoilage agents. Their presence is to be expected whenever processing conditions are inadeqaute. Although, reducing water activities by addition of elevated levels of sucrose or sodium chloride are wiedely used commercially in food industry, several fungi are albe to grow in environments with extremely low water activities. *A. quadricinctus* and the *Penicillium* sp. were able to thrive t extremely low water activitie ; up to 80% (w/v) sucrose concentrtion. However, from the application point of view, it is possible to use the inhibitory action of certain non toxic, unharmful and unexpensive compounds, for instance; amino acids to inhibit such fungal growth in processed foods.

A. quadricinctus, as an osmophilic fungus has been markedly inhibited by the presence of certain amino acids ; phenylalanine, cystine, lysine, tyrosine, isoleucine, valine, methionine and histidine. While, the *Penicillium* isolate was inhibited by the presence of cystine, phenylalanine, tyrosine, lysine, isoleucine, and to some extent glutamine and histidine. Since, several amino acids have no toxic effect on human health (Rosenthal, 1982), but have a toxic or inhibitory action on fungi, as indicated from the results, e.g. isoleucine and valine. Alternatively, the majority of the applied amino acids stimulated the fungal growth at low water activities. Such results are in agreement with several other reports (Christian and Waltho, 1976 ; Kaujima *et. al.*, 1978 ; Dhavises and Anagnostopouolos, 1979 ; Anderson and Witter, 1982 ; Hau *et. al.*, 1982 ; Razak *et. al.*, 1983 and 1985).

REFERENCES

Anderson, C. B. and Witter, L. D. (1982) ; Glutamine and proline accumulation by *Staphylococcus aureus* with reduction in water activity. Appl. Environ. Microbiol. 43 (6) : 1501 - 1503.

Barnes, H. and Blackstoch, J. (1973) : Estimation of lipids in marine animals and tissue : Detailed investigation of the sulphophosphovanillines method for total lipids.

Brown, A. D. (1974) : Microbial water relations features of the interacellular composition of sugar tolerant yeasts. J. Bacteriol. 118 : 769 - 777.

Brown, A. D. (1976) : Microbial water stress. Bacteriol. Rev. 40 : 803 - 846.

Christian, J. H. B. and Hall, J. M. (1972) : Water relations of *Salmonella oranienburq* ; Acummulation of Potassium and amino acids during respiration. J. Gen. Microbiol. 70 : 497 - 506.

Christian, J. H. B. and Waltho, J. A. (1966) : Water relations of *Salmonella oranienburq* ; stimulation of respiration by amino acids. J. Gen. Microbiol. 43 : 345 - 355.

Colwell, R. R. and Morita, R. Y. (1974) : Effect of ocean environment on microbial activities. Uni. Park Press; Paltimore, U.S.A.

Dhavises, G. and Anagnostopoulos, G. (1979) : Influence of amino acids on the deplasmolysis of *E. coli.* Microbiol. Lett., 7 : 149 - 159.

Gray, T. R. G. and Postagata, J. R. (1976) : Survival of vegetative microbes. Symposium of the Society of Gen. Microbiol. V 26 : Cambridge Univ. Press. Cambridge. U. K.

Holding, A. J., Heal, Q. W. ; MaClean, J. R. S. and Flangan, P. W. (1974) : Soil organisms and decomposition in Tundra. Tundra Biome Steering Committe, Stockhorm, Swedan.

Hau, S. T., Tsai, V. Y., Lichens, G. M. and Noma, A. T. (1982) : Accumulation of amino acid in *Rhizopium* sp. strain WR 1001 in response to sodium chloride salinity. Appl. and Environ. Microbiol., 44 : (1), 135 - 140.

Koujima, I., Hayashi, H., Tomochika, K., Okabe, A. and Kanemasa, Y. (1978) : Adaptational changes in proline and water content of *Staphyllococcus aureus* after alteration of environmental salt concentration. Appl. Environ. Microbiol., 35 : 467 - 470.

Kushner, D. J. (1978) : Microbiol life in extreme environment. Academic Press, London and New York.

Lowry, O. H. Rosenbrough, N. J., Furr, A. L. and Randall, R. J. (1951) : Protein measurement with Folin - Phenol reagent. J. Biol. Chem. 193 : 256 - 275.

Measures, J. C. (1975) : Role of amino acids in osmoregulation of non-halophilic bacteria. Nature (London), 257 : 398 - 400.

Pitt, J. I. (1979) : The genus *Pencillium* and its teleomorphic states. Academic Press, London, New York, Toronto, Sydney and San Francisco.

Raper, K. B. and Fennell, D. I. (1977) : The genus *Aspergillus*. Williams and Wilkens Co., Paltimore, U.S.A.

Razak, A. A., Ramadan, S. E., Haroun, B. M. and Lashine, I. (1983) : Osmotolerance regulation in *Aspergillus tamarii.* Br. Mycol. Soc. Metting ; Mycoparasitism and mode of action of antifungal agents. Vol. 17 Suppl. 3, Manchester, 20 - 30 September, U. K.

Razak, A. A., Ramadan, S. E., Haroun, B. M. and Lashine, I. (1985) :Influence of osmotic stres on free amino acids pool and protein contents in *Aspergillus tamarii*. J. Coll. Sci. King Saud Univ. 16 (1), 41 - 48.

Razak, A. A., Ramadan, S. E., Ragab, A. M. and El-Habashy, F. (1989) : Influence of salt stress on certain biological activities in fungal isolates from an Egyptian pickles. J. Fac. Educ. *Ain Shams Univ.*, 13 : 289 - 305.

Rosenthal, G. A. (1982) : Plant non protein amino and imino acids. Biological, BIochemical and Toxicological properties. Academic Press, New York, London, Paris, San Diego, San Francisco, Sao Paulo, Sydney, Tokyo and Toronto.

Umbriet, W. W. ; Burris, R. H. ; Stauffer, J. F. ; Cohen, P. P. ; Johnse, W. J. ; Lee Page, G. A. ; Patter, V. R. and Schneider, W. C. (1959) : Manometric techniques, A manual describing methods applicable to the study of tissue metabolism, P. 239. Burgess Publishing Company.

Table 1: Growth pattern of *A. quadricinctus,* cultivated on Dox liquid media supplemented with different concentrations of sucrose (w/v/). Incubated at 28°C for 7 d.
Date are expressed as mgm /g dry weight, otherwise stated.

Sucrose conc. (% w/v)	Mycelial dry weight (mgm / 50 ml medium)	Carbohydrate	Protein	Lipids
2	9	81.0	43.5	48.8
30	44	99.4	76.6	64.3
40	76	95.9	52.6	55.4
50	124	82.3	40.5	49.6
60	178	73.7	43.4	47.6
70	203	71.8	34.0	43.0
80	230	69.7	27.6	43.9

Table 2: Growth pattern of the *Penicillium* isolate, cultivated on Dox medium supplemented with different concentrations of sucrose. Incubated at 28°C for 7 d.
Date are expressed as mgm /g mycelail dry weight; otherwise stated.

Sucrose conc. (% w/v)	Mycelial dry weight (mgm / 50 ml medium)	Carbohydrate	Protein	Lipids
2	290	20.1	38.9	11.2
30	309	61.4	27.9	61.0
40	330	141.4	23.3	64.7
50	326	53.7	22.6	46.3
60	315	46.3	22.9	39.9
70	280	46.8	22.4	32.3
80	263	22.2	21.5	32.0

Table 3: Effect of different amino acids on the growth of *A. quadricincts* and the *Penicillium* sp. cultivated on nitrogen free Dox media supplemented with 80 and 50% sucrose concentration respectively, for 7 d at 28°C. Amino acids were added in quantities equivalent to the nitrogen content in Dox medium constituents. Data are expressed as mycelial dry weight (mgm / 50 ml culture medium).

Amino acid	*A. quadricinctus*	*penicillium* sp.
Glycine	151	319
Alanine	228	261
Valine	43	247
L-leucine	55	259
DL-isoleucine	39	191
DL-serine	165	265
L-Cystine	16	18
L-methionine	47	247
Aspartic acid	210	275
Glutamic acid	253	303
L-lysine	18	157
Arginine	164	236
Threonine	165	265
L-histidine	79	193
L-ornithine	140	292
Phenylalanine	8	33
L-tyrosine	34	97
L-proline	296	315
L-tryptophan	72	277
L-glutamine	198	194
L-asparagine	247	271

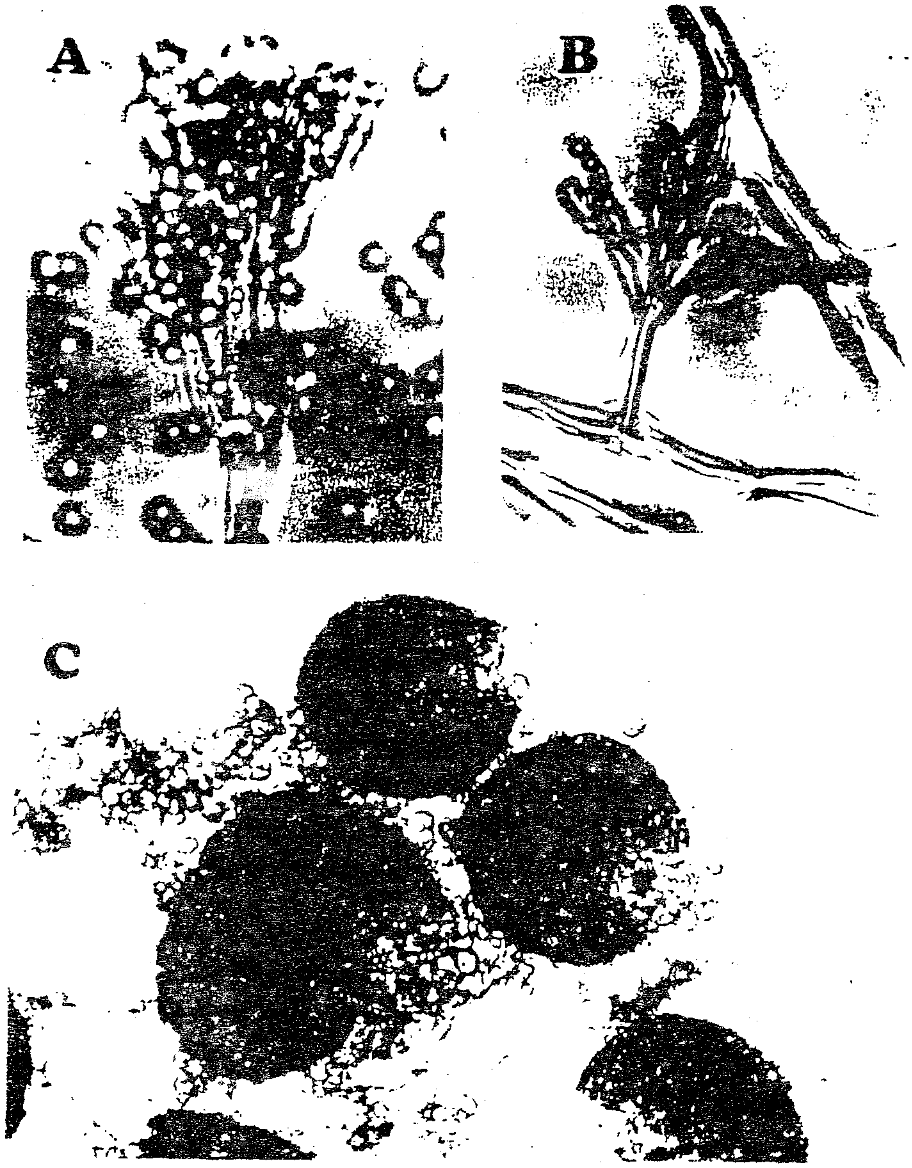

Fig.(1) . Microphotographs of The Penicillium isolate; A , growth at normal sucrose concentrations containing medium (2 %, w/v) ; B , growth at 80 % (w/v) sucrose comcentration and C, A. quadricinctus at 50 % (w/v) sucrose concentration .

الملخص العربي

تأثير الضغط الاسموزى على انشطة غذائية معينة على بعض الفطريات المتحمله للضغط

فى دراسة اجريت على فطر الأسبرجلس كوادريسنكتس وفطرة البنسليوم ، وجد أن هذة الفطريات قادرة على النمو فى وسط غذائى يحتوى على تركيز من السكروز حتى تركيز ٨٠٪ (وزن/حجم) .

وقد وجد أن الشكل الخارجى لفطرة البنسليوم لم يتأثر تأثرا شديدا بالتركيزات المرتفعة من السكروز ، بينما فطرة الأسبرجلس كوادريسنكتس فشلت تماما فى تكوين الأبواغ الجرثومية ،ولكن تكونت الأجسام الثمرية بوفرة .

واتضح من الدراسة أن فطرة البنسيليوم أقل قدرة على تحمل الضغط الأسموزى من قطرة الأسبرجلس وتعطى أعلى معدل نمو لها عند ٤٠٪بينما قد يصل التركيز إلى ٨٠٪ لكى تعطى فطرة الآسبرجلس أعلى معدل لها.

وجد أن الليبيدات والكربوهيدرات الذائبة قد إزدادت معدلاتها بزيادة تركيز السكروز فى كلا الفطرين ، بينما إزداد معدل البروتينات فى قطرة الاسبرجلس كوادريسنكتس وقل فى فطرة البنسيليوم .

يتباين اثر الأحماض الأمينية على نمو الفطرتين فبعضهم يحث على النمو مثل البرولين والجلوتامين والألانين بينما يتثبط النمو بالسيستين بالسيستين .