J. Coll. Sci., King Saud Univ., **16** (1), pp. 41–48 (1985)

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Influence of Osmotic Stress on Free Amino Acid Pools and Protein Contents in *Aspergillus tamarii*

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Aspergillus tamarii was grown on media containing the following sucrose concentrations, 2, 30, 40, 50, 60, 70 and 80% (W/V).

The molecular weight of the detected protein bands increased with increasing sucrose concentration.

Moreover, intracellular free glutamic acid and methionine increased with increasing sucrose concentration. Histidine, methyl histidine, hydroxyproline, dihydroxy-proline, methyl-proline and 5-acetyl-ornithine were detected only in the fungus grown on media with high sucrose concentration. Alternatively, ornithine disappeared in response to osmotic stress.

Osmotolerance of several microorganisms becomes an increasing problem in industrial food production. Microbial growth at reduced water activity and high osmotic pressures attracted the attention. However, it was reported that one of the physiological adjustments in bacteria to grow at reduced water activity is to accumulate specific amino acids in their free amino acid pool. Proline, 4-amino butyric acid and glutamic acid are the only amino acids which have been shown to accumulate (Measures 1975; Koujima *et al.* 1978).

Recently, Anderson and Witter (1982) reported that proline and glutamine were found to be the predominant free amino acids in *Staphylococcus aureus* MF-31 challenged by 5.8 or 10% NaCl in the growth medium. Nevertheless, intracellular free glutamate increased rapidly in response to osmotic stress by NaCl. It accounted for 88% of the amino acid pool when the bacterium was grown in defined medium containing 500 mM NaCl a concentration approaching that of sea water (Hau *et al.* 1982).

Very little or more likely nothing is available in the literatures concerning the changes that take place in the free amino acid pool of fungi challenged by conditions of high osmotic pressure or reduced water activity. Therefore, we are trying to elucidate this problem.

Materials and Methods

The methods used in this study are the same as have been described in the previous paper by Razak *et al.* (1983).

Results

Protein analysis of A. tamarii *at different sucrose concentration*

An interesting pattern of protein analysis is shown in Fig. 1. Several protein bands of different molecular weights were detected in the fungal extract grown on medium with 2% sucrose concentration. Increasing sucrose concentration reflects the modification in protein biosynthesis under such conditions of reduced a_w and high osmotic pressure. At 30% sucrose concentration, several protein bands were detected mostly of low molecular weight types. Nevertheless, as the sucrose concentration increased in the environment the major protein fraction was found to be of high molecular weight. The molecular weight of the major fraction increased with increasing sucrose concentration.

At 60 and 70% sucrose concentration the number of detected protein bands were less than that detected at 30, 40 and 50% and of relatively high molecular weight proteins.

This may strongly support the thought that proteins of osmotolerant fungi are fundamentally different from proteins of nontolerant fungi. Similar conclusions were previously reported by Brown (1976).

Influence of changing medium osmolarity on amino acids pool of A. tamarii

The free amino acids pool of the fungus extracts grown on medium with different sucrose concentrations are manifested in Table 1.

Glycine, alanine, serine, valine, arginine, phenylalanine, proline, Leucine, tryptophane, and norvaline seem to be of no significance in osmotolerance of *A. tamarii.* These amino acids were detected in fungal extract grown on media with different sucrose concentrations. They belong to different metabolic pathways.

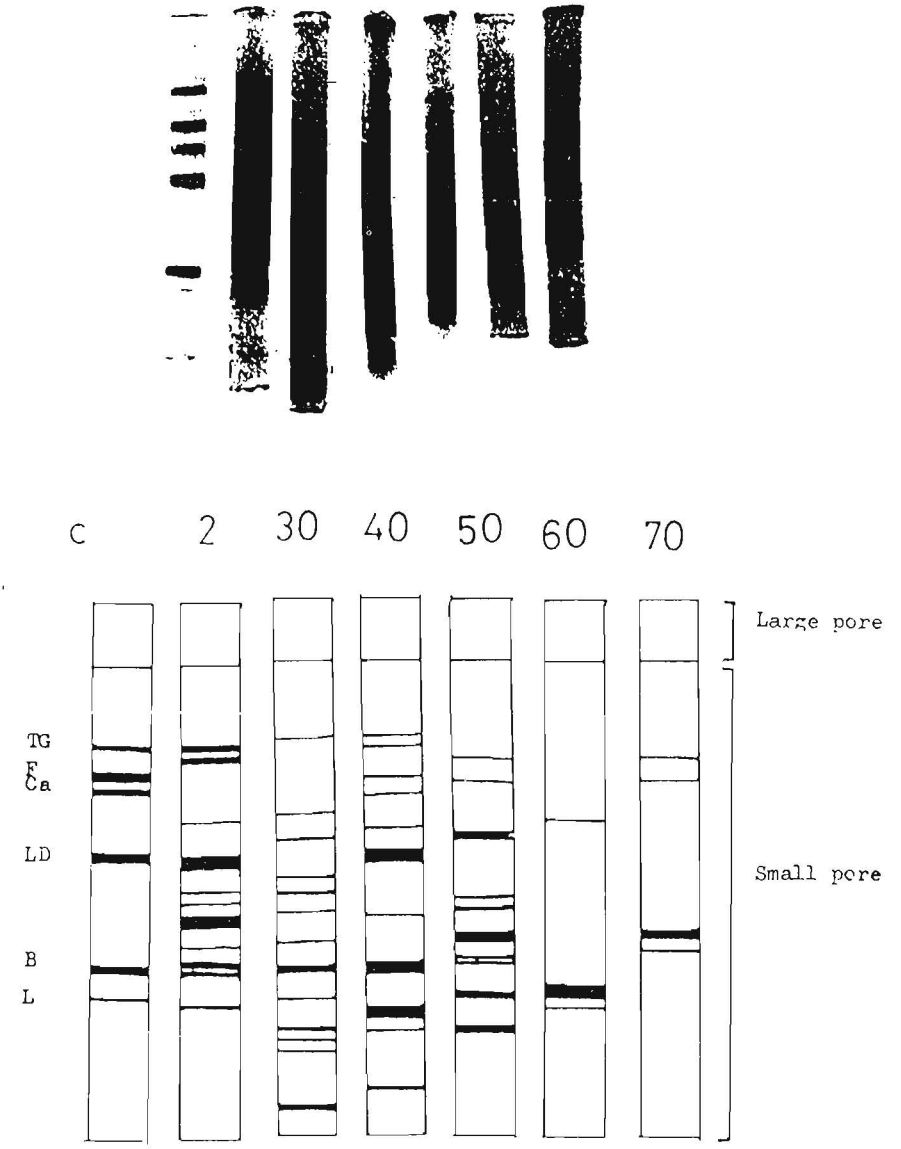

Fig. 1. Electrophoretic patterns of soluble proteins extracted from *A. tamarii* grown on Dox media, supplemented with different sucrose concentrations. C, standard proteins; TG, Thyroglobulin (669,000); F, ferritin (440,000); Ca, Catalase (232,000); LD, Lactate dehydrogenase (140,000); B, Bovine serum albumin (67,000) and L, Lipase (38,000). While 2, 30, 40, 50, 60, and 70 indicate sucrose concentrations (% W/V).

Table 1. Amino acids composition of fungal mycelia cultivated on Dox's liquid medium provided with different sucrose concentrations. Data are expressed as S, strong; M, moderate; W, weak; T, traces and O, not detected. The concentration of amino acids were judged according to the intensity of their colour with ninhydrin.

Amino acids	Sucrose concentrations (% W/V)					
	2	30	40	50	60	70
Glycine	M	M	M	M	M	M
Alanine	M	M	M	M	M	M
Serine	M	O	M	O	M	M
Threonine	M	M	M	M	O	O
Methionine and its sulphoxide	M	M	S	S	S	S
Cystine	S	S	M	O	O	M
Valine	S	S	M	O	M	S
Aspartic acid	O	O	O	M	O	O
Asparagine	O	M	W	M	S	S
Glutamic acid	M	S	S	M	S	S
Glutamine	M	M	M	O	M	M
Arginine	M	O	M	W	M	M
Phenylalanine	M	S	M	M	M	S
Tyrosine	M	M	M	M	W	O
Histidine	O	M	O	M	M	W
Proline	S	S	S	S	S	S
Hydroxyproline	O	M	O	M	M	M
Ornithine	W	O	O	O	O	O
Leucine	S	M	W	M	M	M
Dihydroxy proline	O	S	O	O	O	O
Tryptophane	M	M	O	M	M	M
Lysine	W	W	M	M	M	M
Citrulline	W	M	W	M	M	O
Methylhistidine	O	O	O	W	W	O
Y-aminobutyric acid	M	O	W	W	W	O
2,3-Diaminopimelic acid	O	O	O	T	T	T
Norvaline	W	M	O	M	M	M
hydroxy norvaline	O	O	W	O	O	O
5-acetyl ornithine	O	M	O	M	O	M
Methyl proline	O	O	O	O	S	O
Y-glutamyl phenyl alanine	O	O	W	O	O	O
Y-glutamyl S-methyl	O	O	W	O	O	O
Y-glutamyl tyrosine	O	O	M	O	O	O
unknown I	M	M	M	M	W	O
unknown II	O	O	M	O	W	O
unknown III	O	O	W	O	O	O
unknown IV	O	O	M	O	O	O

It was not possible to detect the blocked pathway as a result of high sucrose concentration (low a_a). However, the distribution pattern of certain other amino acids may spot the light on certain metabolic disorders in the fungus.

Nevertheless, the amino acids analysis shows very interesting and promising erra in regulating microbial osmotolerance.

Asparagine, aspartic acid, hydroxy, dihydroxy- and methylproline, as well as histidine, methyl histidine, 5-acetyl-ornithine and diaminopimelic acid were detected only in the fungus grown on media with high sucrose concentrations moreover glutamic acid and methionine levels were increased. On the other hand, ornithine was detected only in the fungus grown on medium with normal sucrose concentration (2%).

Most of the detected amino acids only at high sucrose concentrations could be synthesized directly or *via* short biosynthetic pathway from other amino acids detected at both low and high sucrose concentrations. Aspartic acid, asparagine and diaminopimelic acid could be biosynthesized from other members of asparatate family of amino acids which were detected in different concentrations, e.g., serine, leucine, norvaline, valine and lysine or from tryptophane or alanine *via* well operated pathways. Similarly, hydroxy, dihydroxy and methyl proline could be biosynthesized from proline or members of the glutamate family of amino acids which were detected in the fungus grown over different sucrose concentrations.

Surprisingly, 5-acetyl-ornithine as unusual, restricted amino acid was detected at high sucrose concentrations only while ornithine was not detected at such high concentrations. Other members of the ornithine cycle; arginine and citrulline were detected at different concentrations. This may indicate the formation of 5-acetyl-ornithine which was performed *via* the acetylation mechanism of glutamate and not *via* the interconversion of other members of the ornithine cycle of amino acids.

Asparagine, histidine, methyl-histidine, hydroxy- and dihydroxy-proline, methyl-proline and diaminopimelic acid perhaps may have a role in stimulating the growth of the fungus at high sucrose concentrations. However, the detection of methyl-proline, methyl-histidine, 5-acetyl-ornithine, hydroxy norvaline, hydroxy-proline and dihydroxy-proline may lead to the suggestion that, methylation, hydroxylation and acetylation mechanisms are well operated at such low water activities. However, mostly these mechanisms are involved in both aspartate and glutamate families of amino acids.

The failure of synthesizing ornithine seems to have a marked role in fungus osmotolerancy; ornithine was not detected at high sucrose concentrations while its acetylated derivative was detected only at these conditions. This will be considered in some detail.

Conclusively, the results obviate the significant increase of intracellular methionine, glutamic acid in response to osmotic stress by sucrose. Nevertheless, the accumulation of asparagine, histidine, hydroxy-proline, dihydroxy-proline, and glutamyl derivatives of certain amino acids were found to be the predominant patterns at various challenging conditions with sucrose concentrations. Alternatively, the non-detection of ornithine and the reduction in cysteine/cystine and γ-amino-butyric acid levels within the organism seems to be of high significance in osmotolerance regulation.

Discussion

The ability of osmophilic fungi to grow successfully at reduced a_w is related in part to the accumulation of certain amino acids and to the biosynthesis of others. Glutamic acid was accumulated in the fungus challenged with high osmotic pressure whereas, asparagine, aspartic acid, hydroxy-proline, dihydroxy-proline, methyl-proline, histidine, methyl-histidine and 5-acetylornithine were synthesized only at high osmotic pressures. Certainly these amino acids stimulate the respiration or/and the growth of the fungus. Glutamine has been shown to stimulate the growth of *Salmonella typhimurium* challenged with NaCl (Csonka 1981). On the other hand proline has been shown to stimulate both growth and respiration in some bacteria at low a_w (Christian 1955; Christian and Waltho 1966). Proline, γ-aminobutyric acid and glutamic acids were shown as the amino acids accumulated in some bacteria grown at reduced a_w (Measures 1975; Koujima *et al.* 1978; Anderson and Witter 1982; Hua *et al.* 1982). Nevertheless, the results may suggest that adjustments in fungi is rather different than in bacteria. Proline level was the same and not changed with changing osmolarity of the media while, γ-amino-butyric acid showed variable and non-significant levels. However, glutamic acid was accumulated in the fungus at high osmotic pressures.

Alternatively, the ability of *A. tamarii* to tolerate the high osmotic pressure media is more likely due to its ability to synthesize several other amino acids. Consequently, the predominant feature was the biosynthesis of several derivatives of certain amino acids. We may conclude that, formation of such derivatives is probably a mechanism to accumulate amino acids in the most active form for rapid consumption, to stimulate respiration and growth.

The detection of ornithine only at normal medium while its acetylated derivative, 5-acetylornithine with increasing osmolarity of the medium attracted our attention to study its metabolism. This amino acid increased fungal osmotolerance as well as, conidiation markedly (data under publication).

It could be concluded that fungal osmotolerance is dependent on the biosynthesis of members and amino acids of both aspartate and glutamate families. Their exogenous feeding to the fungus were shown to increase the fungal osmotolerance significantly (data under publication).

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تأثير شدة الأسموزية على الأحماض الأمينية الطليقة والمحتويات البروتينية في فطرة أسبيرجيللوس تامارى

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تمت تنمية فطرة أسبيرجيللوس تامارى على بيئة غذائية تحتوى على التركيزات التالية من السكروز : ٢ ، ٣٠ ، ٤٠ ، ٥٠ ، ٦٠ ، ٧٠ ، ٨٠ وزن/حجم .
وقد وجد ان الوزن الجزيئي لأشرطة البروتين التي تم فصلها زاد بزيادة تركيز السكر .
كما وجد أيضا ان كل من الحمض الأميني جلوتاميك والميثيونين الطليقة داخل الخلية قد زادت بزيادة تركيز السكر ، كما لوحظ وجود الهستيدين ، ميثيل هستيدين ، هيدروكي برولين ، ثنائي هيدروكسي برولين ، ميثيل برولين ، ٥ ـ أسيتيل أورنيثين فقط في الفطر النامي على بيئة محتوية على تركيز مرتفع من السكروز ، هذا وقد لوحظ اختفاء الحامض الأميني أورنيثين نتيجة لتأثير شدة الأسموزية .