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Effect of prometryne and indole-3-acetic acid on the chemical composition of yeast protein

F. A. Ahmed,[a] S. A. El-Sayed,[b]* A. E. Basyony[c] & A. S. Afify[a]

[a] *Biochemistry Dept., Faculty of Agriculture, Cairo University, Cairo, Egypt*
[b] *Agricultural Microbiology Research Dept., Soils & Water Research Institute, Agricultural Research Centre, Giza, Egypt*
[c] *Crops Technology Dept., Field Crops Institute, Agricultural Research Centre, Giza, Egypt*

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The effect of different concentrations of prometryne and indole-3-acetic acid (IAA) on the protein content of *Candida utilis* 103, grown on the acid hydrolysate of sorghum stalks was investigated. Low concentrations of prometryne (5×10^{-11} M) and IAA (2×10^{-9} M) stimulated protein synthesis, while depression was observed at higher concentrations. *Candida utilis* 103, grown on the acid hydrolysate of sorghum stalks either untreated or treated with low concentrations of prometryne or IAA produced a protein containing highly soluble proteins. Electrophoresis of *Candida utilis* protein detected 14 peaks varying in their relative proportions. The treated *Candida utilis* protein contained the essential amino acids (lysine, leucine, isoleucine, phenylalanine, valine and threonine) in more than sufficient quantity as recommended by FAO. Treatment with IAA showed an increase in the amounts of methionine, valine and lysine (55.5, 29.4 and 17.2%, respectively) over that of the control.

INTRODUCTION

In Egypt, the total sorghum stalks production is about 4.2 million tonnes per year. Most sorghum stalks are used in villages as a fuel for breadmaking. Today, the use of sorghum stalks in Egypt could provide a way to ameliorate the present food crisis as sorghum stalks contain high amounts of polysaccharides (Carlson *et al.*, 1983) which could be converted by acid pretreatment to a suitable carbon source in yeast growth media for production of single cell protein.

In higher plants auxins play an important role in morphogenesis and certain physiological changes. Little attention has been devoted to the biological effects of indole-3-acetic acid, gibberellin, cytokinins and amino acids on yeast or other microorganisms. Plant growth regulators have stimulating effects on yeast growth rates (Rados & Bartfay, 1968).

Prometryne had a marked influence on the growth rate of *Candida utilis* and *Candida tropicalis* (Ghali *et al.*, 1980; Youssef, 1980) as well as protein and amino acid production (Balicka *et al.*, 1970; Strezeles, 1975).

In the present study, the effect of both prometryne and indole-3-acetic acid on the growth and protein composition of *Candida utilis* grown on the acid hydrolysate of sorghum stalks under optimised conditions has been studied.

* To whom correspondence should be addressed.

MATERIALS AND METHODS

Lignocellulosic substrate

Sorghum stalks (*Sorghum bicolor* L., Monech) from Shandaweel Research Station, Agricultural Research Centre, Egypt were used as the energy source for yeast growth.

Microorganism used

Candida utilis 103, was obtained from the Agricultural Microbiological Research Dept., Agricultural Research Centre, Egypt.

Growth medium

Acid hydrolysate of sorghum stalks was used as a fermentation growth medium for propagation of *Candida utilis* 103 using a submerged fermentation technique, as described by Abdel-Salam (1989).

Fermentation and separation of yeast biomass

Preculture shake flasks were incubated on a GFL-rotary shaker (150 rpm) at 28°C for 48 h. Growth was harvested by centrifugation at 4000 rpm under aseptic conditions and resuspended in 20 ml of sterile distilled water. This suspension was used as the inoculum. The acid hydrolysate of sorghum stalks medium (50 ml)

was dispensed into conical flasks (250 ml capacity). The flasks were sterilised at 121°C for 20 min. After sterilisation the flasks were supplemented with different concentrations of sterilised herbicide (prometryne, 2,4-bis isopropyl amino-6-methyl-thio, 1,3,5 triazine, molecular weight 241) ranging from 5×10^{-4} to 5×10^{-12} M or indole-3-acetic acid (IAA, molecular weight 175) ranging between 2×10^{-5} and 2×10^{-10} M. The flasks were then inoculated with 2 ml of inoculum suspension. The inoculated flasks were incubated on a GFL-rotary shaker (150 rpm) at 28°C for 72 h. After the incubation period, the yeast biomass was harvested by centrifugation at 4000 rpm for 20 min, then the yeast dry weight was estimated according to the method described by White (1954).

Chemical analyses

Reducing sugars in the fermented liquor were determined according to the method of Flood and Priestley (1973). Nitrogen content of the dried yeast was estimated by the Kjeldahl method and the crude protein was calculated as N × 6.25 (AOAC, 1970). Fractionation of yeast protein was carried out using sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) according to the method described by Laemmli (1970).

Qualitative and quantitative analyses of amino acids of yeast protein were carried out according to the method described by Bosi and Battaglini (1978). A Varian A.A. Analyzer Model 3700 was used.

RESULTS AND DISCUSSION

Effect of prometryne and IAA treatments on the growth and protein content of *Candida utilis* 103

The results presented in Table 1, indicate that prometryne at low concentrations (from 5×10^{-10} to 5×10^{-12} M) stimulated the growth of *Candida utilis* 103, as well as protein production. Data also revealed that prometryne at a concentration of 5×10^{-11} M increased yeast dry weight up to 35.8% over the control treatment. The corresponding figure for the produced protein was 28.2%. This trend is in line with that observed by Ghali *et al.* (1980), who found that treatment of molasses medium with 1 ppm of prometryne increased the growth of *Candida tropicalis* by 68% over the control.

The results (Table 1) also reveal that addition of IAA in concentrations ranging from 2×10^{-5} to 2×10^{-7} M, decreased the growth of *Candida utilis* 103, as well as the produced protein. This action might be attributed to the fact that high concentrations of IAA have an effect on RNA polymerase leading to synthesis of abnormal ribosomal RNA, which, in turn, has a depressing effect on protein synthesis (Biswas *et al.*, 1973). A lower concentration (2×10^{-9} M) of IAA increased the growth and the produced protein by 37.1 and 33.3% over the control treatment, repectively. This might be due to the action of IAA on DNA content and cell division as reported by Polevoid *et al.* (1967).

Table 1. Effect of different concentrations of prometryne and IAA on the growth and protein content of *Candida utilis* 103, grown on the acid hydrolysate medium of sorghum stalks.

	Yeast dry wt (g/litre)	Consumed sugar (g/litre)	YER (%)[a]	Crude protein		PER (%)[b]
				%	g/litre	
Prometryne concentration (M)						
0·0[c]	6·7	28·1	23·9	58·6	3·9	14·0
5×10^{-4}	5·5	27·7	20·0	51·4	2·8	10·3
5×10^{-5}	5·2	27·6	19·0	50·0	2·6	19·5
5×10^{-6}	5·8	27·7	20·9	51·5	3·0	10·8
5×10^{-7}	6·5	27·7	23·6	51·0	3·3	12·0
5×10^{-8}	6·8	28·6	23·7	52·5	3·5	12·4
5×10^{-9}	7·5	28·7	26·2	52·0	4·5	13·6
5×10^{-10}	8·5	28·7	29·6	53·5	4·5	15·9
5×10^{-11}	9·1	28·6	31·8	54·4	5·0	17·3
5×10^{-12}	8·1	28·0	28·9	52·9	4·3	15·3
IAA concentration (M)						
0·0[c]	6·2	28·1	22·3	57·0	3·6	12·2
2×10^{-5}	4·3	27·3	15·8	50·0	2·1	7·9
2×10^{-6}	4·7	27·1	17·3	50·3	2·4	8·7
2×10^{-7}	5·0	27·2	18·4	52·0	2·6	9·6
2×10^{-8}	7·2	27·4	26·3	53·0	3·8	13·9
2×10^{-9}	8·5	28·2	30·2	57·0	4·8	17·2
2×10^{-10}	8·1	28·1	28·8	54·0	4·4	15·5

[a] Yeast efficiency ratio (YER) = $\frac{\text{yeast dry wt (g/litre)} \times 100}{\text{consumed sugar (g/litre)}}$

[b] Protein efficiency ratio (PER) = $\frac{\text{crude protein (g/litre)} \times 100}{\text{consumed sugar (g/litre)}}$

[c] Control treatment

Table 2. Relative proportions in electrophoretic pattern of protein of Candida utilis 103 grown on the acid hydrolysate medium treated with either prometryne or IAA

Peak No.	Control	Hydrolysate medium treated with	
		Prometryne	IAA
1	38·51	35·19	3·53
2	0·68	0·53	51·76
3	1·36	2·66	3·14
4	1·36	0·39	0·39
5	1·81	0·27	3·53
6	1·13	1·59	0·39
7	0·23	6·64	1·76
8	13·02	21·91	5·88
9	14·50	1·20	1·37
10	10·19	0·79	1·96
11	1·25	1·25	1·96
12	3·40	2·39	1·57
13	1·70	1·33	0·12
14	10·87	23·90	21·57

Electrophoretic pattern of *Candida utilis* protein

Table 2 shows the electrophoretic pattern of protein of *Candida utilis* 103 grown on the acid hydrolysate medium containing prometryne or IAA. The results reveal that *Candida utilis* protein has 14 peaks varying in their relative proportions. The extractable proteins of *Candida utilis*, grown on the acid hydrolysate medium containing prometryne (5×10^{-11} M) or IAA (2×10^{9} M) gave similar peaks as the control treatment but differed in their proportional amounts.

Table 3. Amino acid contents of protein of *Candida utilis* 103, grown on the acid hydrolysate medium treated with either prometryne or IAA (g/100g protein)

Amino acids	Control	Hydrolysate medium treated with		FAO protein[c] reference
		Prometryne[a]	IAA[b]	
Cysteine	0·6	0·7	0·9	—
Histidine	1·6	1·5	2·4	—
Lysine	6·4	7·0	7·5	6·3
Arginine	4·5	4·3	3·3	—
Aspartic acid	8·7	8·4	5·5	—
Serine	4·4	8·1	4·0	—
Glycine	4·3	3·7	4·1	—
Glutamic acid	13·5	13·1	12·4	—
Threonine	4·9	4·5	4·1	5·0
Alanine	4·6	4·1	4·0	—
Proline	2·9	3·0	4·6	—
Tyrosine	3·6	3·0	4·1	—
Valine	5·1	5·2	6·6	7·4
Methionine	0·9	1·0	1·4	3·1
Phenylalanine	4·1	4·1	4·2	6·3
Isoleucine	6·8	6·7	6·1	6·8
Leucine	4·3	4·2	4·1	9·0
Tryptophan	0·6	0·4	1·2	—

[a] *Candida utilis* 103, grown on acid hydrolysate medium treated with 5×10^{-11} M of prometryne.
[b] *Candida utilis* 103 grown on acid hydrolysate medium treated with 2×10^{-9} M of IAA.
[c] FAO (1957).

From the aforementioned data, it seems that *Candida utilis* 103, grown on a medium containing the acid hydrolysate of sorghum stalks (either treated or untreated with IAA or prometryne), produces soluble proteins.

Amino acid composition of *Candida utilis* 103 protein- Candida utilis 103 protein contains the essential amino acids (lysine, leucine, isoleucine, phenylalanine, valine and threonine) in more than sufficient quantity as recommended by FAO (1957). It does not have much sulphur-containing amino acids. It was interesting to note that IAA treatment caused an increase in the amount of methionine, valine and lysine (55.4, 29.4 and 17.2%, respectively) over the control treatment. The corresponding figures were 11.1, 2.0 and 9.4% for prometryne treatment, respectively.

REFERENCES

Abdel-Salam, S.M.M. (1989). Biochemical studies on stems of sorghum. PhD thesis, Faculty of Agriculture, Cairo University, Cairo, Egypt.

AOAC, (1970). *Official Methods of Analysis* (11th edn). Association of Official Agricultural Chemists, Washington, DC, USA.

Balicka, N., Kasinkiewicy, B. & Wegrzyn, T. (1970). The influence of linuron and prometryne on the production of free amino acids by microorganisms. *Mededeligen Faculteit Land Bouwete Schappen Gent*, **35**(2), 647–54.

Biswas, B.B., Mondal, H., Mondal, R.K., Gangnlly, A. & Das,A. (1973). Effect of indole acetic acid and abscisic on RNA polymerase. In *Proc. Symp. Cont. Mech. Cell Processes*, Vol.43.

Bosi, G. & Battaglini, M.(1978). Determination of amino acids. *J. Agric. Res.*, **17**(3), 152–66.

Carlson, K.D., Cunninghani, R.L. & Herman, A.I. (1983). Sweet sorghum grown on sludge-amended Stripmine soil, a preliminary look at yields, composition and ethanol production. *Trans. Ionis State Acad. Sci.*, **76**(1–2), 111–22.

FAO (1957). *Amino Acids Content of Foods and Biological Data Protein.* Food and Agriculture Organization of the United Nations, Rome, Italy.

Flood, A.E. & Priestley, C.A. (1973). Two improved method for the determination of soluble carbohydrates. *J. Sci. Food Agric.*, **24**, 953.

Ghali, Y., Nakhla, A.M., Zahra, M.K. & Eskarous, M.A. (1980). Effect of some herbicides on the growth and constituents of yeast *Candida tropicalis. Chem.Mikrobial Technol. Ceneasm*, **6**, 171–4.

Laemmli, U.K. (1970).Cleavage of structural protein during the assemby the head of bacterophage. *Nature*, **227**, 680.

Polevoid, V.V., Maksimov, G.B., Logvenkova, N.L. & Gerasimov, N.I. (1967). The effect of an auxin on the growth of Chiks excised from Coeared leaves and the nucleic acid contents. *Dotii-Akad. Nauk. SSSR*, **176**(1), 216.

Rados, G. & Bartfay, J. (1968). Stimulating effect of gibberellic acid on the fermentation of molasses. *Szeszipoc.*, **16**(4).

Strezeles, A. (1975). Effect of simazine on growth of microorganisms and decomposition of this preparation in various type of soil. *Acta microbiol. Polon. B*, **7**(1), 3–13.

White, J. (1954). *Yeast Technology.* Champan and Hall, London, UK, p.106.

Youssef, B.Y. (1980). Biochemical studies on the use of whey from dairy industry. MSc thesis, Faculty of Agriculture, Cairo University, Cairo, Egypt.