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EFFECT OF AMINO ACIDS AND ORGANIC ACIDS ON THE GROWTH OF STREPTOMYCES NRC-101

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While surveying the potentialities of *Streptomyces species* isolated from Egyptian soils[1] for the production of antimicrobial metabolites, an organism attracted our attention to its high antimicrobial effect on some bacteria. The active metabolite of the fermentation broth was isolated, purified and identified. The identification of the pure active principle of the fermentation broth revealed that the antibiotic substance is a new peptide antibiotic.[2] The investigations on the activities of actinomycetes have been largely with antibiotic production and the mode of action of the antibiotics. Limited considerations have also been given to certain fundamental principles of actinomycetes biochemistry.

This work deals with the biochemical changes in the fermentation medium during the growth of *Streptomyces species.*

Materials and Method

Maintenance of Streptomyces species. *Streptomyces species* was maintained on the following ingredients (g/l): glucose, 20 0; $NaNO_3$, 2 0; KH_2PO_4, 1.0; KCl, 0.5; $MgSO_4.7H_2O$, 0.5; $FeSO_4.7H_2O$, 0.005; distilled water, 1 litre. Inoculated slants were incubated at 27–30°C for 10 days to permit good growth. The slants were later kept in a refrigerator at 5°C.

Fermentation Medium. Erlenmeyer flasks (250 ml each contained 50 ml medium of the following ingredients (g/l): glucose, 20 0; $NaNO_3$, 2.0; KH_2PO_4, 1.0; $MgSO_4.7H_2O$, 0.5; KCl, 0.5; $FeSO_4.7H_2O$, 0.005; and distilled water. The medium was adjusted to pH 7.0. The flasks were plugged, sterilized and inoculated with a standard inoculum of spore suspension of *Streptomyces species.* The flasks were fixed on a rotary shaker of 200 rev/min at 28°C. Triplicate flasks were removed at 24, 48, 72 and 96 hr incubation period and final pH, sugar consumption,[3] mycelial dry weight and antibiotic production[4] were determined. Free amino acids,[5] organic acids[6] and keto compounds[7] were detected by using paper chromatographic techniques, the developing solvents were n-butanol–acetic acid–water (4:1:5) and the spraying reagent was ninhydrin.

Results and Discussions

No antibiotic activity could be detected at 24 hr incubation, but the antibiotic production began at 48 hr incubation and increased till it reached 1.65 mg/100 ml at 72 hr, while a slight decrease was recorded at 96 hr (Table 1). The final pH shifted slightly towards the alkaline side and ranged from 7.3 at 24 hr incubation to 7.7 at 72 hr. The consumed sugar was 0.6 g/100 ml at 24 hr incubation and increased till it reached 1.85 g/100 ml at 96 hr incubation. The mycelial growth, in general, was weak at 24 hr incubation and increased with the increase of the incubation period.

On paper chromatographic analysis of organic acids present in the fermentation broth at 24, 48, 72 and 96 hr incubation, lactic acid was the sole organic acid present in the fermented medium. The amount of the organic acid increased with the increase of the incubation period, this may be due to the insufficient oxygen supply.

The keto compounds (pyruvic acid, α-keto-glutaric acid and acetone) were detected at 48 hr incubation. These compounds increased with the increase of the incubation period. The presence of acetone is mainly due to the decarboxylation of acetoacetic acid. The accumulation of α-keto-glutaric acid is a result of the accumulation of the tricarboxylic acid cycle which is derived mainly from pyruvic acid. The presence of acetone (decarboxylated acetoacetic acid) is also a confirmation of the tricarboxylic acid cycle.

The absence of organic acids of the tricarboxylic acid cycle and the accumulation of lactic acid is due to the fact that the organism is highly aerobic and the aeration was insufficient.

The amino acids which could be detected by paper chromatographic technique were accumulated in very low concentration, but their accumulation increased at 72 hr incubation, while at 24 hr no amino acids or ninhydrin positive spots could be detected on the chromatograms. The *R* values of these amino acid correspond to cysteine, lysine, serine, glutamic acid and proline.

Influence of the Addition of Some Amino and Organic Acids to Fermentation Medium. Amino acids and organic acids were added in 1.0 g/litre to the fermentation medium of *Streptomyces* NRC-101 to show their effects on the production of the antibiotic NRC-101. Of these amino acids those which can be derived from the tricarboxylic acid cycle. The organic acids contained the members of the tricarboxylic acid cycle.

The addition of amino acids to the fermentation medium in concentrations of 1.0 g/litre were unfavourable (Table 2). The medium without amino acids produced the maximum yield of the antibiotic. Glycine gave no increase or decrease in the antibiotic yield, while addition of L-glutamic acid, DL-lysine, DL-threonine and β-alanine gave a slight decrease in the antibiotic production. L-Proline, L-arginine L-tyrosine, DL-tryptophan and DL-β-phenylalanine depressed the antibiotic yield to about one-half the yield of the flasks without amino acids. L-Leucine and DL-valine inhibited the antibiotic production completely. The amounts of the consumed sugar were low, in all cases, than the flasks without treatment. In case of L-tyrosine and L-arginine, the consumed sugar was 7.5 g/litre, while it was 13.0 g/litre in case of L-glutamic acid and 16.5 g/litre in the flasks without any supplement of the amino acids.

TABLE 1. BIOCHEMICAL CHANGES IN THE FERMENTATION MEDIUM DURING THE GROWTH OF *Streptomyces species.*

	Incubation period (hr)			
	24	48	72	96
Final pH	7·3	7·6	7·7	7·76
Consumed sugar (g/100 ml)	0·60	1·25	1·70	1·85
Mycelial dry weight (g/100 ml)	0·0425	0·0425	0·1320	0·1540
Antibiotic NRC-101 (mg/100 ml)	—	1·20	1·65	1·60
Organic acid				
Lactic	+	++	++	+++
Keto compounds				
Pyruvic acid	—	+	++	+++
α-Keto-glutaric acid	—	+	++	+++
Acetone	—	+	++	+++
Amino acids				
L-Cysteine	—	—	++	++
DL-Lysine	—	—	++	+
L-Serine	—	+++	++	++
L-Glutamic	—	—	++	++
L-Proline	—	—	+++	++

+, ++, +++ refer qualitatively to the colour density (concentration) of the spot on the chromatograms.

The final pH was shifted towards the alkaline side in all cases ranged from 7.4 in case of L-arginine to 9.0 in case of L-glutamic acid. The mycelial growth were more or less unaffected with the addition of the different amino acids. The aromatic amino acids, DL-tryptophan and DL-β-phenylalanine gave the least mycelial growth, while DL-valine was the most favourable one for the building of the mycelial growth.

The addition of organic acids (maleic, fumaric and succinic) increased the yield of the antibiotic, while gluconic, oxalic and citric acids were unfavourable for the antibiotic production. The final pH ranged from 8.0 in the case of citric and gluconic acids to 8.8 in case of maleic acid. The consumed sugar was low, in general, this may be due to the utilization of the added organic acids as simple carbon compounds. The mycelial growth was somewhat increased to 3.6 g/litre in case of succinic acid. This also may be due to the suitability of the organic acids as simple carbon compounds for cell building.

TABLE 2. ADDITION OF SOME AMINO ACIDS AND ORGANIC ACIDS TO THE WELL-DEFINED FERMENTATION MEDIUM OF *Streptomyces* NRC-101.

Amino acids and organic acids added	Final pH	Consumed sugar (g/100 ml)	Mycelial dry wt	Antibiotic NRC-101 (mg/100 ml)
Control	8·9	1·65	0·2646	1·70
Amino acids				
Glycine	8·0	1·15	0·2486	1·70
L-Glutamic	9·0	1·30	0·2604	1·60
DL-Lysine	8·4	1·20	0·2386	1·60
DL-Threonine	8·6	1·10	0·2396	1·50
L-Alanine	8·6	1·20	0·2386	1·35
L-Proline	8·6	0·80	0·2565	0·87
L-Arginine	7·4	0·75	0·2640	0·62
L-Tyrosine	8·6	0·75	0·2264	0·62
DL-Tryptophan	8·7	0·95	0·1934	0·62
DL-β-Phenylalanine	8·7	0·90	0·1890	0·62
DL-Isoleucine	8·4	0·85	0·2584	0·06
DL-Valine	8·4	0·85	0·2986	—
L-Leucine	8·8	0·80	0·2124	—
Organic acids				
Maleic	8·8	1·13	0·3080	2·25
Fumaric	8·4	1·32	0·3350	1·80
Succinic	8·4	1·23	0·3610	1·80
Acetic	8·5	1·05	0·2647	1·65
Gluconic	8·0	1·15	0·3008	1·35
Oxalic	8·4	1·10	0·3190	1·35
Citric	8·0	1·15	0·2586	1·20

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