ASPARIGINASE ACTIVITY IN BACTERIA ISOLATED FROM THE SOIL OF KUWAIT

by

SOHAIR A. MUSTAFA* and MAYSOON SALAMA

Department of Botany, University of Kuwait, Kuwait.

INTRODUCTION

Amidases especially L-asparaginase and L-glutaminase has received considerable attention due to their practical importance in cancer treatment (CAPPIZZI et al 1970; EL-ASMAR and GREENBERG, 1966). Some neoplastic cells are unable to survive in the absence of L-asparagine yet they lack adequate L-asparagine synthetase activity. Removal of L-asparagine from these cells due to the action of L-asparaginase results in their death (COONEY and HANDSCHUMACHER, 1970). MASHBURN and WRISTON (1964) used E.coli to obtain an L-asparaginase which proved effective against experimental tumors and upon clinical evaluation revealed its special value in the treatment of acute leukaemia. Screening procedures of many bacteria from American type culture collection; International Collection of phytopathogenic bacteria; National Collection of dairy organism; National Collection of Marine Bacteria; National Collection of plant Pathogenic Bacteria revealed the widespread occurence of L-asparaginase, however, few of them produced substantial amounts of this enzyme (WADE et al. 1971).

Most, i not all, published results on screening procedures for L-asparaginase producers were carried on identified bacteria obtained from various culture collections (WADE et al. 1971). The present investigation aims at the isolation and identification of potent L-asparaginase producer by direct isolation from enriched and non-enriched soil samples .

*Present:Department of Botany, Women's College, Ein Shams Univ.,Cairo,Egypt.

Materials and Methods

Isolation, purification and identification of L-asparaginase producing bacteria.

The organisms used in the present investigation were isolated from fertile soil (collected from the agricultural experimental station, Kuwait), either directly or after enrichment. Soil enrichment with L-asparaginase producers was achieved by mixing 1gm L-asparagine with 10gm finely powdered soil and 10 ml sterile water. This was followed by incubation at 28 - 30°C for two weeks. Suitable dilutions of the enriched and non-enriched soil samples were plated on a modified Dox agar medium in which L-asparagine was the only added nitrogen source. This medium contained g/L : Sucrose, 7.5; Asparagine, 2.0; K_2HPO_4, 0.5; $MgSO_4.7H_2O$, 0.25; $FeSO_4$, 0.005; Agar 20. Incubation at 28-30°C continued for 10-12 days, after which all the bacterial colonies that developed were purified by streaking several times on agar plates of the same medium.

Purified bacterial isolates were subjected to morphological examination as well as physiological and biochemical reactions according to the methods recommended in the Manual of Microbiological methods (1957). Tentative identifications were carried out using BERGEY (1974).

Production and assay of L-asparaginase:

The procedure of WADE et al. 1971 was used after applying some modification. This modified procedure could be summarized in the following: A reaction mixture which consisted of 20 u-moles L-asparagine; 250 u-moles of tris buffer pH 7.3; 0.5 ml of a 2-6 day old shaken culture of the specified organism (as a source of enzyme) in a total volume of 2 ml, was incubated at 37°C for 30 minutes, after

which the reaction was terminated by the addition of 0.5ml of 1.5 M trichloroacetic acid (TCA). The reaction mixture was then centrifuged at 6000 r.p.m. for 10 minutes after which the amount of ammonia liberated (due to the enzyme activity) was measured spectrophotometrically as follows:0.2 ml of the supernatant was mixed with 1 ml of 1N-NaoH and after two minutes 1 ml of Nessler's reagent (BDH laboratory reagents). The colour was allowed to develop for 20 minutes at room temperature before its intensity was measured at 450 nm. Two controls were always included, a substrate free control and an enzyme control in which TCA denatured enzyme was used.

The bacterial cultures which were used as a source of enzyme were prepared by inoculating the specified isolate in 50 ml (in a 250 ml conical flask) of modified Dox liquid medium. Incubation at 28 - 30°C on a rotary shaker (120 r.p.m.) continued for 2 - 6 days.

In addition to the modified Dox medium described before, another medium similar to Dox (but with starch instead of sucrose as a carbon source and asparagine as a nitrogen source) and nutrient broth were also tested in an attempt to improve the enzyme production.

The activity of L-asparaginase in whole bacterial cultures was compared with that in the culture filterate and that in washed bacterial cells. This was carried out by centrifuging the bacterial culture at 6000 r.p.m. for 10 minutes after which the supernatant was removed and the pellet was washed three times in distilled water. The washed cells were resuspended in .04M tris buffer pH 7.5 to restore the volume to the volume of the original culture. The culture filterate

a d the bacterial suspension were used separately as a source of enzyme in the L-asparaginase reaction mixture described before.

Results

Isolation, purification and identification of L-asparaginase producers.

Twenty-four and fifteen bacterial colonies developed on agar plates inoculated with the enriched and non-enriched soil respectively. Upon purification by streaking, on modified Dox agar plates only twenty one isolates most of them obtained from the enriched soil were able to survive Gram stain, morphological and physiological studies of these isolates suggested that they represent six genera, namely Bacillus, Pseudomonas, Lactobacillus, Staphylococcus, Micrococcus, Corynebacterium.

Enzyme activity of the various bacterial isolates.

The results (of Table 1) indicate that all the isolates but one produced detectable amounts of L-asparaginase. Among these isolates the most potent enzyme producers were three strains of Bacillus sphaericus (isolates 2, 30 and 36) followed by a Corynebacterium (isolate 10).

The potent isolates were used to examine enzyme production after different incubation periods on nutrient broth and Dox liquid media.

The results which are shown in table 2 suggest that enzyme production was generally higher on Dox liquid medium than on nutrient broth. Moreover on the former medium all the isolates consistently produced more enzyme at a culture age of one week. It was also found that sucrose as a carbon

source in Dox liquid medium stimulated more enzyme production than starch did.

The results of Table 3 indicate that the majority of the enzyme activity is intracellular or associated with the cells, a negligible part was present in the cutlure filterate.

Discussion

The results of the present investigation support the general belief of the wide spread occurrence of L-asparaginase in microorganisms. The enrichment technique however, increased the number of L-asparaginase producing isolates by sixty per cent. Isolates which failed to utilize asparagine as a nitrogen source were unable to survive as pure cultures on the medium which contained asparagine as the only added nitrogen source.

The enzyme activity is fully expressed in the intact organisms. This agrees with the findings of CEDAR and SCHWARTZ, 1967 who were able to conclude that the antitumor L-asparaginase obtained from E. coli is located in the periplasm. It should be pointed out that by using whole cells in the enzyme assay, any cytoplasmic asparaginases that are present will be overlooked. The cytoplasmic asparaginase are unlikely to have a high affinity for asparagine (in competition with enzymes of protein synthesis) and unlikely therefore to be of much practical importance (BROOME 1965).

One of the first bacterial species that received special attention with view to the therapeutic use of their amidases was Pseudomonas aeruginose (GREENBERG et al 1964). In the present investigation however, Pseudomonas aeruginosa was not among the most potent enzyme producer. This points out to strain variation among members of the same species and also the environmental and nutritional condition should not be ignored.

SUMMARY

Twenty-one L-asparaginase producing bacteria were isolated and identified from the soil. Soil enrichment increased the number of enzyme producers by 60%. The isolates represented six different genera and the most potent enzyme producers were 3 strains of Bacillus sphaericus and one Corynebacterium sp. All the potent isolates had their enzyme associated with the cells and negligible amounts were found in the culture filterate. More enzyme was produced on a synthetic liquid medium in which asparagine was the only added nitrogen source than on nutrient broth.

Table 3

Isolate number and identity.	Relative enzyme activity $O.D_{450}$		
	Whole culture	culture filterate	washed cells
2 Bacillus sphaericus	.27	.017	.25
10 Corynebacterium sp.	.54	.03	.48
30 Bacillus sphaericus	.18	0	.15

L-asparaginase activity in whole cultures, culture filterate and washed cells of two isolates of B.Sphaericus and one isolate of Corynebacterium,.

Table 1

Isolate number	Identity	Relative enzyme activity $O.D_{450}$
1	Bacillus sphaericus	0.27
2	Bacillus sphaericus	0.54
3	Pseudomonas aeruginosa	0.08
4	Lactobacillus coryneformis	0.02
5	Staphylococcus aureus	0.15
6	Lactobacillus coryneformis	0.17
10	Corynebacterium Sp.	0.34
11	Micrococcus varians	0.01
12	Pseudomonas aeruginosa	0.08
13	Bacillus brevis	0.13
14	Lactobacillus coryneformis	0.01
15	Staphylococcus aureus	0.0
30	Bacillus sphaericus	0.42
31	Bacillus sphaericus	0.28
32	Bacillus firmus	0.17
33	Bacillus macerans	0.31
34	Bacillus sphaericus	0.06
35	Micrococcus luteus	0.25
36	Bacillus sphaericus	0.52
37	Bacillus firmus	0.23
38	Bacillus alvei	0.14

Table 3

Isolate	Culture age (hrs)	Relative enzyme activity $O.D_{450}$	
		Dox	Nutrient Broth
2 (*Bacillus sphaericus*)	48	.185	.26
	168	.270	.1
	216	.08	-
10 (*Coryne-bacterium*)	48	0	.13
	168	.54	.03
	216	-	-

References

BERGEY'S Manual of determinative bacteriology. (1974).
8th ed. Eds., BUCHANAN and GIBBONS, N.E.
The WILLIAMS and WILKINS Comp., Baltimore.

CAPIZZI, R.L.; BERTINO, J.R. and HANDSCHUMACHER,R.E. (1970)
L-asparaginase.
Annual Review of Medicine, 21, 433.

CEDAR, H. and SCHWARTZ, J.H. (1967).
Localization of the two L-asparaginases in anaerobically grown Escherichia Coli.
Journal of Biological Chemistry, 242 , 3753.

COONEY, D.A. and HANDSCHUMACHER, R.E. (1970).
L-asparaginase and L-asparagine metabolism.
Annual Review of Pharmacology, 10, 421.

EL-ASMAR, F.A. and GREENBERG, D.M. (1966).
Studies on the mechansim of inhibition of tumor growth by the enzyme glutaminase.
Cancer Research 26, 116.

GREENBERG, D.M.: BLUMENTHAL, G.H. and RAMADAN, M.A. (1964).
Effect of administration of the enzyme glutaminase on the growth of cancer cells.
Cancer Research, 24, 957.

Manual of the microbiological methods. (1957).
The Society of American Bacteriologists.
McGraw Hill.

MASHBURN, L.T. and WRISTON, J.C. (1964).
Tumor inhibitory effect of L-asparaginase from
Escherichia Coli.
Archives of Biochemistry and Biophysics, 105, 450.

WADE, H.E., ROBINSON, H.K. and PHILLIPS, B.W. (1971).
Asparaginase and glutaminase activities of bacteria.
J. Gen. Microbiol. 69, 299.