ISSN NO. 2320-5407

Journal homepage: http://www.journalijar.com
Journal DOI: 10.21474/IJAR01

INTERNATIONAL JOURNAL OF ADVANCED RESEARCH

RESEARCH ARTICLE

-ASPARAGINASE ACTIVITY OF SOME EARTH-WORM BORNE BACTERIA

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Manuscript Info

Manuscript History:

Received: 14 April 2016
Final Accepted: 19 May 2016
Published Online: June 2016

Key words:
L-L-asparaginase , Earth worm, Carbon source, Nitrogen source, *Micrococcus*

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Abstract

Asparginase activity of some earthworm-borne bacteria and actinomycetes was assayed. Though all the bacteria understudy secreted asparaginase, the degree of production of asparaginase varied with the organism. *Micrococcus roseus* and *Rhodococus sp.* secreted maximum L-asparaginase.Where as *Flavimonas orhyzihabitans,Xanthomonas maltophilia* was found to be poor in the secret of L-asaparaginase. *M. roseus, Rhodococus sp, B.mycoides* and *B.macerans* opted for D.mannose and mannitol; lactose and sorbose ; maltose and starch ; galactose respectively nitrogen source differed among the bacteria under study.

Introduction:-

In recent times L-asparaginase has gained importance in view of it's therapeutic value as an antitumor agent (Hill et. Al., 1967). Hypocholesterolemi and hepateclipidosic effect of L-asparaginase activity forced contimed search for alternative sources of this enzyme (Raj Kumari and Reddy, 1989; Elizabeth et al., 1991) and bacteria (Krishna Reddy and Reddy, 1987a, Krishna Reddy and Reddy, 1987b,) are reported to secrete L-asparaginase. Scheetz et al. (1971) and Imeda et al. (1973) have reported antineoplastic and immunological specificity of L-asparaginase studied by them. L-asparaginase from E.coli is now commercially available and is being used for clinical trials. However, no such studies were undertaken on the production of L-asparaginse by earthworm – borne microorganisms. Hence, it was considered worthwhile to study the production of L-asparaginase by some earthworm-borne bacteria. Influence of carbon and nitrogen sources in the nutrient medium was also investigated.

Materials and methods:-

Secretion of L-asparaginase by some earthworm-borne bacteria (as listed in table-1) was assessed by growing these bacteria in nutrient broth 24,48,72 and 96 hr at 30^0 C for 4 days. The biomass attained by bacteria by the end of incubation period was assessed by turbidity of culture at 660 nm. The culture suspension was taken in vial and turbidity of the culture was determined at 660 nm and the biomass is expressed in optical density. The culture broth was centrifuged at X 3000 for 30 min. the clear supernant was dialysed against distilled water over night and served as an enzyme.

The activity of L-asparaginase was assayed as suggested by Peterson and Ciegter (1969). The reaction mixture consisting of 0.2 ml enzyme, 0.9 ml, 0.1 ml sodium borate buffer (pH 8.5) and 7 ml 0.04 L-asparagine was incubated at 37^0C for 20 min. The enzyme action was stopped by the addition of 0.5 ml of trichloroacetic acid (1%). The enzyme activity is expressed in internal units. The experiment was repeated thrice as the difference among replicates was marginal, average of three was taken.

Results and Discussion:-

Table-1 reveals that all the bacteria under study secretd L-asparaginase which is increased with the progress of incubation period. However, the present bacteria differed significantly in the degree of production of L-asparaginase. Rhodococcus sp. and M. roseus secreted maximum L-asparaginase, while M.maltophila and M. kristinae were poor producers of L-asparaginase. Flavimonas orhyzihabitans failed to secrete L-paraginase upto 48 hrs of incubation. The enzyme activity was meager, but produced maximum enzyme at 72 hours of incubation. Similarly *B.brevis* produced maximum L-asparaginase by 48 hours of incubation period. Rest of the bacteria secreted intermediate amount of L-asparaginase. B.brevis, E.coli, M. lutea, P.vulgaris, P. aeruginosa and F. oryzihabitans recorded a gradual increase in the growth till the end of 4 days incubation period. On the other hand M.kristinea reached maximum cell biomass by 72 hours of incubation. PH drift in most of the media growing bacteria was mostly towards alkaline side.

Carbon and nitrogen sources present in the medium had significant influence on the production L-asparaginase by the bacteria under study (table- 2). Mannitol and maltose, lactose and L-sorbose induced maximum L-L-asparaginase in M.roseus and Rhodococcus sp. Respectively. On the other hand, B. mycoides preferred maltose and starch for production of L-asparaginase. B.macerans secreted comparatively more enzyme in medium containing D-galactose and mannitol. Rhodococcus sp. produced minimum amount of L-asparaginase in medium containing D-glucose, maltose, dextrin and succinic acid. Lactose and L-sorbose and D-mannose and succinic acid were poor sources for the production of L-asparaginase by B. mycoides and B. macerans respectively. succinie acid,citric acid and tartaric acid were responsible for inhibition of L-asparaginase production by M.roseus, and B.mycoides, while B. macerans was inhibited for growth and enzyme production

Ammonium sulphate, ammonium mylobdate and L-histidine were good sources of nitrogen for production of L-asparaginase by the bacteria under study. On the other hand, ammonium molybdate was the good source of nitrogen for M.roseus. M.roseus secreted least amount of L-asparaginase in medium containing B-alanine, L-histidine and p-aminobenzoic acid, while Rhodococcus sp. Secreted least amount of L-asparaginase in the presence of potassium nitrate, sodium nitrate and L-histidine. B.mycoides also responded poorly towards potassium nitrate, barium nitrate, L-aspartic acid and p-aminobenzoic acid. In general ammonia salts were most favoured nitrogen sources for L-asparaginase production. M.roseus preferred DL-tryptophan, L-tyrosine and barium nitrate for its growth, while Rhodococcus sp. achieved good biomass in the medium containing ammonium sulphate, L-tyrosine and ammonium molybdate. On the other hand, B.macerans preferred urea, glycine, DL-metheonine, L-tyrosine and L-histidine for its growth. No positive correlation could be observed between growth and L- asparaginase production. Krishna Reddy and Reddy (1990) have also reported the significant influence of nutrients present in the medium on the production of L- asparaginase by bacteria studied by them.

From the present investigation it can be concluded that *Micrococcus roseus* can be exploited for large scale production of this enzyme.

Thanks are due to Head, Department of Botany, Kakatiya University for kind encouragement.

Table 1: L-asparaginase production by some earthworm-borne bacteria

Name of the bacterium	Growth (O.D)				pH				L-asparaginase * (IU)			
	24 hrs	48 hrs	72 hrs	96 hrs	24 hrs	48 hrs	72 hrs	96 hrs	24 hrs	48 hrs	72 hrs	96 hrs
Bacillus anthracis	0.4	0.5	0.4	0.5	6.6	6.8	6.8	7.5	53.6	62.7	63.5	84.7
Bacillus brevis	0.3	0.4	0.5	0.6	6.5	7.1	7.4	7.5	18.6	95.7	89.8	85.6
Bacillus macerans	0.3	0.5	0.4	0.4	6.6	7.2	6.8	7.5	46.6	31.3	42.4	84.7
Bacillus mycoides	0.5	0.5	0.2	0.3	7.2	6.8	6.5	7.2	29.6	52.5	67.8	63.5
Escherichia coli	0.4	0.7	0.7	1.0	6.5	7.0	7.4	7.5	59.3	25.5	23.7	41.5
Flavimonas orhyzihabitans	0.1	0.3	0.3	0.5	6.8	7.0	7.0	7.5	_	_	16.9	10.2
Micrococcus kristinae	0.2	0.3	0.4	0.3	6.5	6.8	7.0	7.2	16.9	33.9	27.1	29.6
Micrococcus lutea	0.2	0.5	0.6	0.7	6.5	6.8	7.0	7.2	25.5	39.8	63.5	53.6
Micrococcus roseus	0.3	0.5	0.4	0.3	6.4	7.1	6.8	7.4	17	38.1	63.5	149.1
Pfroteus vulgaris	0.4	0.5	0.6	0.8	6.5	7.0	7.4	7.5	25.5	63.5	89.8	75.7
Pseudomonas aerugenosa	0.4	0.7	0.8	0.9	6.8	7.0	7.4	7.4	25.5	53.6	59.3	63.5
Rhodococcus sp.	0.5	0.5	0.3	0.4	6.6	7.0	7.2	7.5	21.2	42.4	59.3	149.1
Xanthomonas maltophilia	0.1	0.2	0.3	0.3	5.5	6.0	7.0	7.2	17	25.5	18.6	25.5

* IU = International Units - one international unit of L-asparaginase is that amount of enzyme which liberate 1 µ mole of ammonia

Table2: Effect of carbon and nitrogen sources on the production of L-asparaginase by some eartworm borne bacteria

Carbon Source	Name of the Bacterium											
	M.roseus			Rhodococcus sp.			B.mycoides			B.macerans		
	Growth (O.D)	PH	Asparaginase *(I.U)	Growth (O.D)	PH	Asparaginase *(I.U)	Growth (O.D)	PH	Asparaginase *(I.U)	Growth (O.D)	PH	Asparaginase *(I.U)
D-Glucose	0.2	6.8	21.2	0.1	7.2	8.5	0.3	7.0	16.9	0.3	7.4	16.9
Sucrose	0.1	6.8	31.3	0.1	7.3	14.4	0.4	7.0	18.9	0.4	7.3	12.7
D-Fructose	0.2	6.5	29.7	0.2	7.1	10.2	0.4	7.4	27.1	0.3	7.4	14.4
Lactose	0.2	7.0	29.6	0.2	7.4	29.6	1	6.8	12.7	0.3	7.5	14.4
Maltose	0.1	6.5	38.1	0.2	7.2	8.9	0.3	7.5	31.3	0.3	7.5	12.7
D-Mannose	0.2	7.0	31.3	0.2	7.4	12.7	0.2	7.5	23.7	0.2	7.5	8.9
Xylose	0.5	7.4	27.1	0.2	7.5	18.6	0.3	7.5	25.4	0.6	7.5	29.6
D-Galactose	0.4	6.8	33.9	0.2	7.5	22.9	0.3	7.0	16.9	0.7	7.5	31.3
L-Sorbose	0.2	7.0	12.7	0.3	7.5	29.6	0.2	6.8	14.4	0.5	7.5	13.1
Starch	0.2	7.2	31.3	0.3	7.5	27.1	0.3	7.0	31.3	0.6	7.5	21.2
Dextrin	0.1	6.8	36.4	0.1	7.1	8.5	0.3	7.5	21.2	0.6	7.4	27.1
Mannitol	0.1	7.1	38.9	0.1	7.2	16.9	0.5	7.5	29.6	0.7	7.5	31.3
Succinic acid		5.0		0.1	7.1	5.9	0.1	5.5		0.6	7.0	6.8
Citric acid	0.1	5.5		0.1	7	10.2		5.0		0.1	6.5	
Tartaric acid		5.0		0.1	5.5			5.0		0.1	5.5	
Control	0.3	7.5	40.7	0.1	7.4	8.5	0.3	7.6	50.8	0.3	7.5	8.5
Nitrogen Source												
Dl-Tryptophan	1.5	8.0	21.2	1.0	7.5	21.2	0.1	7.5	25.4	0.8	7.6	18.6
Potassium nitrate	0.9	7.6	29.6	0.1	6.5	18.6	0.1	5.5	16.9	0.1	6.8	16.9
Sodium nitrate	0.8	7.5	38.1	1.2	8	16.9	0.1	5.5	52.5	0.3	6.0	14.4
Ammonium nitrate	0.8	7.6	21.2	1.0	7.4	131.3	0.3	5.5	105.9	0.3	5.0	149.1
Ammonium sulphate	0.6	7.0	127.1	1.0	7.4	211.8	1.0	7.6	144	0.9	7.2	211.8
Ammonium mylobdate	0.8	5.5	144.8	0.8	5.5	127.1	1.0	5.5	169.4	0.9	5.4	211.8
Urea	0.7	7.6	131.3	1.2	7.6	31.3	0.1	7.5	63.5	1.0	7.6	50.8
Glycine	0.6	7.5	59.3	0.1	6.5	29.6	0.5	7.5	84.7	1.0	7.0	94.9
L-Argine	0.8	7.6	33.9	0.9	7.6	35.6	0.4	7.5	127.1	1.0	7.6	127.1
Metheonine	0.8	7.5	36.4	0.6	7.6	22.9	0.3	6.0	36.4	1.0	8.0	115.2
L-Aspartic acid	0.1	5.0	21.2	0.1	5.0	40.7	0.2	6.0	18.6	0.1	5.0	21.7
B-Alanine	0.8	7.6	12.7	0.8	7.6	16.9	0.5	7.5	74.5	1.0	7.6	55.1
L-Tyrosine	1.5	7.5	67.8	1.5	7.6	74.5	1.5	7.0	80.5	1.5	7.6	97.4
L-Histidine	1.0	7.6	16.9	1.2	7.4	12.7	0.3	7.4	123.7	1.0	7.6	211.8
Barium nitrate	1.4	7.5	84.7	0.6	7.2	50.8	0.2	6.3	16.9	0.5	5.5	33.9
P-Amino benzoic acid	0.1	5.5	18.6	0.3	5.0	33.9	0.1	5.5	18.6	0.3	5.0	16.9
Control	1.2	8.0	61.8	1.4	8.4	8.5	0.5	7.5	63.5	0.1	6.5	18.6
*IU - Internatinal units ; Oneinternation unit of L-asparaginase is that amount of enzyme which liberates 1 µ mole of ammonia.												

References:-

1. Hill et.al., 1968. New procedures for purification of L-asparaginase with yield from Escherichia coli J.Bacterial. 95(6): 2117-2123
2. Raj Kumari and Reddy 1989., Asparaginase production by some species of penicillium. Nat.acad. sci. letters 12: 267-269
3. Elizabeth et.al., 1991 purification and characterization of an L-asparaginase from Fusarium solani. Indian .J. microbial Ecol. 2:61-70.
4. Krishna Reddy and Reddy; 1990. Effect of C and W sources on asparaginase production by bacteria. Indian J. microbial 30:81-83
5. Krishna Reddy and Reddy ; 1987. Synthesis of IAA by seed-borne fungi of maize (zeamays L.). Nat. Acad. Sci. letters 10: 267-269.
6. Scheetz et.al. 1971. Purification and properties of an L-asparaginase from Fusarium trinectum arch. Biochem biophys. 142 : 184-189
7. Imeda et.al 1973 Asparaginase and glutaminase activities of microorganisms Journ. Gen.microbiol. 76:85-99.