

L-Asparaginase Activity By Various Bacteria

Dr.Mohammed.A.AL- Mazini

College of Science - University of Basrah

Abstract:

Teen species of bacteria surveyed for L-asparaginase activity , Bacillus subtilis provided the highest one.

The PH indicator phenol red in medium is a fast method for screening L-asparaginase producing bacteria through 18-24 hrs. The result correlated with quantitative estimation of the enzyme activity in culture broths.

Introduction: L -Asparaginase is an effective antileukemia agent in mice and rats (4). Clinical studies indicated that this enzyme was thought as a promising agent in treating some forms of neoplastic cell disease in man (14). The principal source of L Asparaginase for clinical trials is the bacterium *Escherichia coli* (1). Although production and purification techniques have been developed they generally provide a quantity of enzyme sufficient for only limited trials. Possibly, alternative sourced L Asparaginase could overcome the problem of antigenic reactions found in some patients(14).

Asparaginase (L - asparagine amido hydro Lase, E. C.3.5.1.1) is an anti- neoplastic agent, neoplastic cells cannot synthesize L asparagine due to the absence of L asparagines synthesize (10) For this reason the commonest therapeutic practice is to inject intravenously free enzyme in order to decrease the blood concentration of L - asparagine affecting selectively the neoplastic cells (11).

The importance of microorganisms as L - asparaginase sources has been focused upon since the time it was obtained from *Escherichia coli* and its antineoplastic activity demonstrated in guinea pig serum (4). Since then a large number of bacteria and fungi have been screened for L asparaginase producing potential (18).

The present paper describe the screening types of bacteria for producing L - asparaginase .

Materials and Methods:1) Bacteria: Types of bacteria were obtained from the College of Science and Veterinary Medicine University of Basrah-Iraq. Types of bacteria were :-

Erwinia aroideae, *Proteus vulgaris*, *Serratia marcescens*

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Pseudomonas fluorescens, *Staphylococcus aureus*, *Listeria monocytogenes*, *Klebsiella pneumoniae*, *Escherichia coli*, *Enterobacter aerogenes* and *Bacillus subtilis*.

2) Primary screening :Teen bacterial species used in the study, each type was grown on modified Mq medium (8) (per 1000 ml of water: $\text{Na}_2\text{HPO}_4 \cdot \text{H}_2\text{O}$, 6.0 g; KH_2PO_4 , 3.0 g; NaCl , 0.5 g; L- asparagine, 0.5 g; 1 ml $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 2.0 ml; 0.1 M $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$, 1.0 ml; 20% glucose Stock, 10.0 ml; agar 20.0g) and phenol red (2.5% w/v in ethanol, 1 ml L^{-1}) was employed as on indicator. The media were autoclaved and plates prepared. Control plates were of Modified M-q Medium (PH7.0): (A) without dye and (B) without asparagine (instead containing NaNO_3 as nitrogen source). After standardizing the dye concentration of bacteria (0.009% w/v phenol red), all bacteria were tested by the plate assay on appropriate media at 37°C . Pink zone diameters were measured after 18-24 hr

3) L-Asparaginase assay :

ErLenmeyer flasks (250 ml) containing 50 ml of the appropriate medium were inoculated with each of the test organisms. The flasks were incubated at 37°C at 250 rev min^{-1} for 48 hr in shaker- incubator. Uninoculated media served as controls. The bacterial cultures were harvested by centrifugation at 6000 rev min^{-1} for 15 min. The enzyme activities were estimated in culture filtrates by Nesslerization A 0.1 ml sample of cell suspension or enzyme solution, 0.9 ml of 0.1 M solution of borate buffer (PH 8.5), and 1 ml of 0.04 M - asparagine solution were combined and incubated for 10 min at 37°C . The reaction was stopped by the addition of 0.5 ml of 15% (w/v) trichloroacetic acid. After centrifugation, 0.1 ml portion of the supernatant fluid was diluted to 8 ml with distilled water

and treated with 1.0 ml of Nessler's reagent and 1.0 ml of 2.0 M NaOH. The color reaction was allowed to proceed for 15 min before the OD of 500 nm was determined. The OD was then compared to a standard curve prepared from solutions of ammonium sulfate as the ammonia source. One international unit (IU) of L-asparaginase is that amount of ammonia in 1 min at 37°C (Peterson and Clegler, 1969)

4) Biostatistics :-

The results were statistically evaluated by calculating the correlation coefficient, r between the zone size (cm) and enzyme activities (IU ml⁻¹) in culture broths (20)

Results and Discussion:

	Bacterium	Colony diameter (cm)	Zone Diameter (cm)	Enzyme activity (IU ml ⁻¹)	pH of the culture
1	<i>Bacillus subtilis</i>	7.52	0.95	0.14	8.46
2	<i>Erwinia carotovora</i>	5.21	0.83	0.10	8.71
3	<i>Escherichia coli</i>	6.02	0.80	0.08	9.10
4	<i>Pseudomonas fluorescens</i>	5.00	0.70	0.07	7.22
5	<i>Proteus vulgaris</i>	10.44	0.60	0.04	8.30
6	<i>Serratia marcescens</i>	5.61	0.23	0.02	6.84
7	<i>Staphylococcus aureus</i>	2.8	-	-	7.53
8	<i>Listeria monocytogenes</i>	-	-	-	7.0
9	<i>Klebsiella pneumoniae</i>	-	-	-	7.0
10	<i>Enterobacter aerogenes</i>	-	-	-	7.0

Table (1): Colony and Zone diameter after 18 hrs incubation and L - asparaginase activity with changes in PH of culture filtrate after 4 hrs of various bacteria screened at 37c°

Among various bacteria tested by the plate assay and broth studies *Bacillus subtilis*, *Erwinia aroideae* , *Escherichia coli*, *Pseudomonas fluorescens* and *Proteus vulgaris* (table 1) were found to be good producers of L – asparaginase. *Bacillus subtilis* is the best bacteria that produces of L - asparaginase. A direct correlation existed between zone diameters and enzyme activities in culture broths. A high correlation coefficient (r) of 1.02 was found which bacteria.

The enzyme L - asparaginase is routinely screened in culture filtrates by using Nessler's reagent (9). The procedure is lengthy and time consuming, hence the use of rapid plate assay for screening L - asparaginase producing microorganisms. This assay is sensitive and rapid procedure that may directly give us potential asparaginase activity The incubation period required to selection of an L asparaginase procedure is significantly reduced. The incubation period for bacteria was 18 hr, while in broth studies it is 24 - 48 hr for bacteria (2). Thus, the plate assay is rapid and results correlate directly with this from broth studies.

It is generally agreed that L - asparaginase production is accompanied by an increase in PH of the culture filtrates (6).The plate assay was devised using this principle by incorporating the PH indicator phenol red in medium containing asparagine (sole nitrogen source). Phenol red at acidic PH is yellow and at alkaline PH turns pink, thus a pink zone is formed around microbial colonies producing L asparaginase.

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الإحصاء ١٩٨٤ - علي احمد عبد الرحيم وخطار ، سيف الدين منسي ، للعلوم الحياتية . وزارة التعليم

العالي والبحث العلمي / جامعة البصرة.

فعالية انزيم الاسباراجينيز بواسطة البكتريا المختلفة

د. محمد عبد الإمام المازني

كلية العلوم - جامعة البصرة

الخلاصة: درست فعالية عشرة انواع من البكتريا لانتاج انزيم الاسباراجينيز ، وكانت أكثر فعالية . وان طريقة كاشف الدالة الحامضية لصبغة الفينول الاحمر الموجودة في الوسط الزراعي هي طريقة سريعة لغريلة البكتريا لانتاج انزيم الاسباراجينيز خلال ١٨ - ٢٤ ساعة . وان النتيجة مرتبطة مع التقدير الكمي لفعالية الانزيم في الوسط الزراعي السائل *Bacillus subtilis* .