CHARACTERIZATION OF l-ASPARAGINASE PRODUCING BACTERIA FROM WATER, FARM AND SALINE SOIL

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ABSTRACT

The habitat chosen for screening the bacteria were farm soil, saline soil and water. The activity was detected on a medium containing 1% peptone, 0.6% beef extract, 0.33% KH3PO4, 0.1% L-asparagine and phenol red. L-asparaginase activity was detected on the basis of formation of red colour around the colony. Likewise efficient L- asparaginase producing bacteria were screened. These were then studied for routine microbiological and biochemical characterization. The microorganisms we characterized belonged to the genera *E.coli, Serratia spp., Pseudomonas aeruginosa*, *Bacillus spp*., *Aeromonas species* and *Proteus spp.* L-asparaginase from halophilic bacteria is expected to be non-allergic and hence halophilic bacteria from saline soil can contribute to therapeutic value of this enzyme.

Key words: L-asparaginase, bacteria, characterization.

INTRODUCTION

L- asparaginase is enzyme acting on L-asparagine and is widely used as anticancer agent. The reason it is preferred for the purpose is it is biodegradable, non-toxic and can be administered at the local site quite easily. Other agents are found quite painful when administered to the patient and also these are quite costly. Current clinical studies indicate that this enzyme is also a promising agent in treating some forms of neoplastic cell diseases in man. The antineoplastic activity is attributed to the depletion of L-asparagine by the action of L-asparaginase from *Erwinia carotovora* is reported by Lee *et al.*, (1989). Mushburn *et al.,* (1961) purified *E. coli* L-asparaginase and demonstrated its tumour inhibitory activity. Cedar *et al.,* (1968) reported that the L- asparaginase is synthesized at constant rates by *E. coli* under anaerobic conditions. The crystal structure of the enzyme from *E. coli* was studied by Swain *et al.,* (1993).

Kidd in 1953 reported the action against tumour cells in mice and rats when Guinea pig serum was found to have this enzyme. Mushburn and Wriston detected similar activity from *E. coli* cells. But the enzyme L-asparaginase is responsible for this activity was suggested by Broome (1963). Later on some workers like Yellin and Wriston (1966) confirmed this observation that L-asparaginase is the antitumour factor. Asparaginase is preferred as it is biodegradable Stecher *et al.*, (1999), Verma *et al.*, (2007) and (Ferrare *et .al*, 2004).

Peterson *et al.,* (1969) examined L-asparaginase from *Erwinia aroideaet*. Tosa *et al.,* (1972) focused on L- asparaginase from *Proteus vulgaris* and done purification, crystallization of enzyme and has also given the enzymatic properties. Sukumaran (1978) studied to determine the production of L-asparaginase by *Serretia marcescens* grown on 14 different media. Yasser *et al.,* (2002) worked on *Pseudomonas aeruginosa* for L- asparaginase production in a solid state culture and also done an evolution of culture condition using factorial design. Younes Ghasemi *et al.,* (2008) have studied the various concentrations of modified medium ingredients and various carbon sources were tested to optimize the medium for expression and identification of L-asparaginase in *E. coli*. Other bacterium includes *Pseudomonas aeruginosa* (Manikandan *et al.,* 2010), *Citrobacter sp* (Shah *et al.,* 2010) and *Bacillus species* (Maysa *et al.,* 2010); Basha *et al.,* (2009) screened the actinomycetes for enzyme activity.

Alternatively fungi have been undertaken for L-asparaginase production (Nibha *et al.,* 2008, Sunita *et al.,* 2009, Sudha *et al.,* 2009, Siddalingeshwara and Lingappa 2011, Sarquis *et al.*). Isolates were inoculated on TGY extract broth out of the 10 isolates only 3 were found potential for L- asparaginase activity.

MATERIALS AND METHODS

Sample collection

Water samples were collected from surround area of Akola city viz 1) Dr. P.D.K.V. 2) Borgaon Manju 3) Katepurna. Saline Soil samples were collected from 4) Akot 5) Shivaji Nagar 6) Popatkhed whereas farm soil was collected from 7) Balapur 8) Dongergaon 9) Gandhigram 10) Nimba.

Soil sample were collected at specific depth upto 10 cm using 2.54 cm diameter soil borer. The borer was cleaned between samples with water followed by methanol and these samples were placed in Zip-locked plastic bags at 6° C (Gary M. Banowetz *et al.,* 2006).

Isolation of L-asparaginase producing bacteria

After colony count 10^{-7} dilution was selected. From all samples 0.1 ml was inoculated on media containing L-asparagine with phenol red as indicator. L-asparaginase producing colonies were selected on the basis of formation of pink zone around the colonies of the medium. Well isolated colonies from each plate were selected for characterization.

Identification of L-asparaginase producing organisms

These organisms were identified on the basis of morphological, cultural and biochemical studies. Bergey's manual was referred for characterization

RESULTS AND DISCUSSION

The medium employed contained asparagine with phenol red and after incubation pink zone around the colonies was observed. These colonies were taken for further studies.

Screening of L- asparaginase production

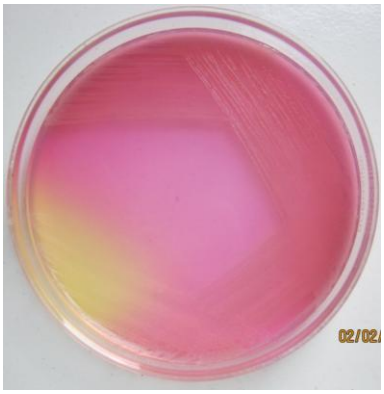

Fig:1 Screening of L-asparaginase production by *P. aeruginosa*

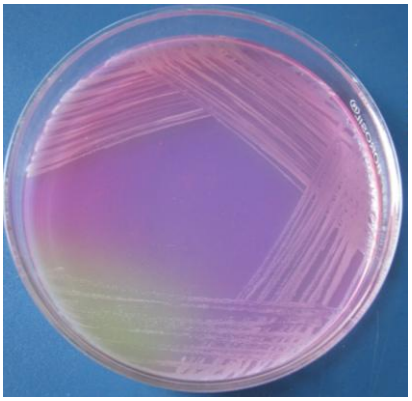

Fig:2 Screening L-asparaginase production by *Bacillus spp.*

Table 1: Biochemical analysis of bacteria from different soil samples (IMViC Test)

Soil Samples	Gram's Nature	I	MR	VP	Citrate	Organisms
Dr. P.D.K.V.	Gm –ve Short rods	+ve	+ve	-ve	-ve	*E. coli*
Borgaon	Gm –ve Short rods	-ve	+ve	+ve	-ve	*Aeromonas species*
Katepurna	Gm +ve Short rods	+ve	+ve	-ve	-ve	*Proteus spp.*
Akot	Gm –ve Short rods	-ve	-ve	-ve	+ve	*P. aerugionsa*
Shivaji Nagar	Gm –ve Short rods	-ve	-ve	-ve	+ve	*P. aerugionsa*
Popatkhed	Gm +ve rods	-ve	+ve	+ve	-ve	*Bacillus spp.*
Balapur	Gm -ve Short rods	-ve	-ve	-ve	+ve	*P. aerugionsa*
Dongergaon	Gm +ve rods	-ve	+ve	+ve	-ve	*Bacillus spp.*
Gandhigram	Gm -ve Short rods	+ve	+ve	-ve	-ve	*Serratia spp.*
Nimba	Gm -ve Short rods	+ve	+ve	-ve	-ve	*Serratia spp.*

Table 2: Analysis of sugar fermentation for bacteria characterized from different soil samples

Sites of Sample Collection	Glucose		Lactose		Mannitol		Organisms
	Acid	Gas	Acid	Gas	Acid	Gas	
Dr. P.D.K.V.	+ve	+ve	+ve	+ve	+ve	+ve	*E. coli*
Borgaon Manju	+ve	+ve	-ve	-ve	+ve	+ve	*Aeromonas spp.*
Katepurna	+ve	**+**ve	**-** ve	+ve	**+** ve	**+** ve	*Proteus spp.*
Akot	+ve	-ve	-ve	-ve	-ve	-ve	*Pseudomonas spp.*
Shivaji Nagar	+ve	-ve	-ve	-ve	-ve	-ve	*Pseudomonas spp.*
Popatkhed	+ve	-ve	+ve	-ve	+ve	+ve	*Bacillus spp.*
Balapur	+ve	-ve	-ve	-ve	-ve	-ve	*Pseudomonas spp.*
Dongergaon	+ve	-ve	+ve	-ve	+ve	+ve	*Bacillus spp.*
Gandhigram	+ve	+ve	+ve	+ve	+ve	+ve	*Serratia spp.*
Nimba	+ve	+ve	+ve	+ve	+ve	+ve	*Serratia spp.*

The L-asparaginase positive colonies were identified by formation of pink zone around the colonies of the medium. It indicates deamination with release of ammonia. For the confirmation the L-asparaginase activity was detected by spot inoculation on L-asparaginase producing medium. After incubation at 37°C for 24 hrs zone around inoculated spots were observed. These isolated organisms were *E.coli, Aeromonas species*, *Proteus spp, Serratia spp., Pseudomonas aeruginosa*, *Bacillus spp*. which was found positive for L-asparaginase production.

CONCLUSION

The characterized bacteria are isolated from farm soil; saline soil and water sample are potential source for high yield of enzyme and high substrate specificity. The bacteria produces enzyme optimally at 37°C and at pH 7. The strong L-asparaginase producing bacterium includes *E. coli* from Dr. P.D.K.V., *Aeromonas species* from Borgaon Manju and *Proteus spp* from Katepurna. Saline Soil samples contained *Pseudomonas species* in Akot and Shivaji Nagar whereas *Bacillus species* was isolated from Popatkhed. The farm soil contained *Pseudomonas* in Balapur and *Bacillus* in Dongargaon. *Serratia sp* was found in Gandhigram and Nimba.

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