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A Preliminary Screening And Determination Of Biochemical Properties Of Bacterial Isolates Producing An Anticancerous Enzyme Asparaginase From Some Sewage Water Samples Of Bhopal, M.P. India

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Abstract:

Microorganisms have had a major impact on the development of medical science since the discovery that they not only cause infections but also produce certain organic compounds that cure infections and help treat a variety of non-infectious diseases Though, microbes are ubiquitous, but their metabolic capabilities are greatly influenced by the habitat they survive with unique conditions of pH, temperature, pressure, oxygen, light, nutrients and salinity, there is a high efficiency for those to yield metabolites to exhibit special biological activities The production of enzymes is a pursuit central to the modern biotechnology industry. Asparaginase is one such important enzyme finds their use in the pharmaceutical, biosensor and food industries and has anticarcinogenic potential for the treatment of acute lymphoblastic leukemia, lymphomas and other cancers. Asparaginase are naturally occurring enzymes expressed and produced by animal tissues, bacteria, plants, and in the serum of certain rodents, but not in mankind. *Erwinia carotovora*, *Pseudomonas stutzeri*, *Pseudomonas aeruginosa*, *E. coli* etc has been known to produce L-asparaginase, though its commercial production is restricted to use of *Erwinia chrysanthemi* and *E. coli* as per the latest information. Thus the present work was intended to screen out certain new bacterial isolates with *in vitro* asperginase producing potential from sewage water sources. The sewage water samples were collected from 5 different location of Bhopal and were subjected to brain heart infusion media containing amino acid asparagine and phenol red indicator. The bacterial isolates forming pink or red colonies or hallow around indicating asparaginase producing capability were picked and pure cultured. The three asparaginase positive isolates one from M.P. Nagar & other two from AIIMS sewage sample reported to be bacillus and coccus respectively on Gram's staining which when subjected to biochemical characterization using catalase test, IMVC test and carbohydrate fermentation tests does not matches with the biochemical characteristics of standard *E.coli* and *S. aureus*. The outcomes of this preliminary screening encourages to further investigate the sewage water samples to screen out the possibly new & efficient asparaginase producing isolates with complete biochemical & molecular characterization in view of commercial prospects.

Keywords: L-asparaginase, anticarcinogenic, enzyme production, wastewater

INTRODUCTION

Asparaginase is one such important enzyme finds its used in the pharmaceutical, biosensor and food industries and has anticarcinogenic potential against acute lymphoblastic leukemia, lymphomas and other cancers [1-3]. Asparaginase enzyme explicitly focuses the metabolism of cancer cells by manipulating deficits in metabolic pathways and catalyzing the collapse of L-asparagine into ammonia and L-aspartic acid, triggering nutrient starvation of cancer cells and bringing about their demise [4].
Microorganisms such as bacteria and fungi are promising sources for structurally diverse and potent bioactive compounds [5,6]. Enzymes are chiefly sourced from microorganism since in a short span to time they could be culture in huge masses where prospects of genetic manipulation of bacterial cells could enhances their enzyme production potential. In addition, due to the active and stable nature, microbial enzymes have been in prime choice over enzymes of plant and animal origin [7,8].
Pseudomonas aeruginosa, *Pseudomonas stutzeri*, *Escherichia coli*, *Erwinia carotovora*, and several other bacteria have been known to produce L-asparaginase [9-12]. But *E. chrysanthemi* and *E. coli* are being used for commercial production of two types of asparaginase enzymes for clinical used as per the latest information [13].
Under harsh and toxic environments, the enzyme producing capacity and growth of microorganisms itself gets retarded mostly. However, many microorganisms undergo likely physiological or genetic adaptations their by enabling them to survive and release enzyme under harsh conditions [7, 14]. Sometimes, the sewage water becomes a tough environment for microbes because of toxicity generated due pharmaceutical pollutants, disinfectants, chemical, dyes etc., and leads to survival of microbial population different metabolic potentials. The present preliminary investigation is intended to screen out such microbial isolates from local sewage sources with biopharmaceutically significant metabolic potential with reference to asparaginase enzyme.

MATERIALS & METHODS

Sample Collection:
For the screening and isolation of suspected bacteria, sewage water samples were used to collect in sterile containers and taken to the laboratory. The sewage water samples were collected from various locations of Bhopal namely M.P Nagar zone-II, Anna Nagar, Meera Nagar, Saket Nagar and Barkheda Pathani.

Media Preparation:
For screening of asparaginase producing bacteria a modified brain heart infusion broth (Himedia M210) media containing 10 gm/l amino acid asparagine, 6 gm/l K2HPO4, 4 ml/l MgSO4 2 ml/l CaCl2 gm/l 2.5 ml of 2.5% phenol red indicator and 15 gm/l agar was prepared [16]. All the contents were mixed followed by adjusting the pH at 7. After autoclaving, the media was poured aseptically into sterile petri plates.

Screening of Asparaginase Producing Bacteria:
The water samples collected from different sources were subjected to serial dilution in sterile distilled water in aseptic conditions. 10^{-5}, 10^{-7} and 10^{-8} dilutions from series were used to raise the microbial cultures by spreading 0.1 ml of samples in separate culture plates. After incubation at 37°C for 24 hours in bacteriological incubator the bacterial colonies forming pink or red or hallow around indicating asparaginase producing capability were picked and pure cultured.

Biochemical Characterization:
The suspected asparaginase producing bacterial isolates were subjected to partial characterization through Gram's staining, and some biochemical tests like catalase test, indole test, methyl red reduction test, voges proskauer test, and citrate utilization test. The carbohydrate fermentation test with Sucrose, Fructose, Lactose, Dextrose and Mannitol was also performed.

In vitro Asparaginase Production & Assay:
50 ml of nutrient broth (Himedia MM244) containing 1% asparagine maintained at pH 7 was prepared in 125 ml conical flasks. After autoclaving, the media was inoculated with 1 ml of the culture of suspected

bacterial isolates and incubated at 37ºC for 48 hours with intermittent shaking. After incubation, 2 ml of fermentum was centrifuged at 10000 rpm then supernatant was retained in new sterile microfuge tube. The activity of asparaginase was assayed by well diffusion method again on modified BHI medium containing 1% asparagin and 0.01% phenol red indicator. Wells were prepared on mediums of 6 mm diameter and 10 µl of fermentation extract from each isolates separately were poured aseptically in wells. After 24 hours of incubation at ambient temperature pink coloured zones were observed around the wells for any asparaginase activity due to release of ammonia [15-16].

RESULTS & DISCUSSION

Screening of Asparaginase-Producing Bacteria:

In terms of preliminary investigation for search of asparaginase enzyme producing bacteria sewage water samples were collected from 5 different locations of Bhopal which are M.P Nagar zone-II, Anna Nagar, Meera Nagar, Saket Nagar and Barkheda Pathani. The sample from M.P Nagar zone-II and Saket Nagar were reported to have bacterial colonies with red or pink colour or hallow around them due to the breakdown of asparagine amino acid present in the culture media. Sewage water both from M.P Nagar zone-II and Saket Nagar nears AIIMS were observed to contain heavy microbial load even at 10^{-7} and 10^{-8} dilutions indicates heavy load of untreated organic pollutants in sump. Though the isolates with positive asparaginase from these samples were taken forward for further confirmatory studies. Recently, Tomar *et al*., (2019) [17] isolated asperaginase producing bacteria soil samples using M9 media plate; where, Bhat *et al*., (2015) [18] reported *Salinicoccus* sp. as asparaginase production microbe from soil. Though, Tarulli *et al*., (2013) [19] reported 21 bacterial isolates with L-asparaginase activity on plate method assay by pink colour zones.

Biochemical Characterization:

There were three different types of bacterial isolates were reported with asparaginase producing ability & were coded as Sp-1, Sp-2 and Sp-3. The three asparaginase positive isolates one from M.P. Nagar zone-II & other two from AIIMS sewage Saket Nagar sample reported to be bacillus, short bacillus & coccobacillus type respectively on Gram's staining which when subjected to biochemical characterization using catalase test, IMVC test and carbohydrate fermentation tests does not matches with the biochemical characteristics of E. coli (MTCC-1687) and S. aureus (MTCC-737). The results of the biochemical tests are mentioned in table 1.

Wastewater can carry many opportunistic pathogens like Enterobacter cloacae, Enterococcus faecalis, Escherichia coli, Klebsiella pneumoniae, Proteus vulgaris or Pseudomonas aeruginosa [20]. Clostridium perfringens, Legionella pneumophila and Mycobacterium tuberculosis like species were found to be common [21]. Using a combination of FISH probing and 16S rDNA clone inserts, Plumb, et al., (2001) [22] revealed the methanogenic population of Methanobacterium and Methanospirillum species from wastewater containing food dyes degraded under sulfidogenic and methanogenic conditions. Liu et al.,

(2015)[23], identified 18 species of genera Longilinea, Georgenia, Desulforhabdus, Thauera, Desulfuromonas and Arcobacter in the sewerage system which are responsible for methane fermentation of sewage sludge, facilitating decomposition of macromolecular organic matter into simpler compounds (Cyprowski, *et al*., 2018) [20]. The present investigation has the provision for precise molecular identification of the isolates Sp-1, Sp-2 and Sp-3 in later studies.

Table 1: The observation of microscopy & biochemical tests for 3 asparaginase producing bacteria

S.N.	Tests for Characterization	Bacterial Isolates		
		S-1	**S-2**	**S-3**
1.	**Gram's Staining**	Gram +ve Rods	Gram –ve Short Rods	Gram –ve Coccobacillus
2.	**Catalase test**	+ve	+ve	+ve
3.	**Indole test**	–ve	–ve	–ve
4.	**Methyl red reduction test**	+ve	+ve	+ve

5.	**Voges Proskauer test**		+ve	+ve	+ve
6.	**Citrate utilization test**		–ve	–ve	–ve
7.	**Carbohydrate Fermentation Tests**	**Mannitol**	+ve	–ve	–ve
		Lactose	–ve	–ve	–ve
		Sucrose	+ve	–ve	–ve
		Fructose	–ve	–ve	–ve
		Dextrose	+ve	–ve	–ve

***In vitro* Asparaginase Production & Assay:**

The three bacterial isolates earlier screened for asparaginase activity when subjected to fermentation in broth containing amino acid asparagine, yields the asparaginase enzyme in medium during fermentation. The cell free fermentation extract when poured into well bored into same media with asparagine and phenol red indicator gives a clear pink zone. This indicates that all the three bacterial isolated have the ability to release asparaginase enzyme when allowed to grow in fermentation medium which encourages their possibilities explore microbes and their sources in wider perspective.

CONCLUSIONS

The treated or untreated wastewater gathers organic matter, variety of chemicals, and microorganisms including pathogens and multi-resistant bacteria from several sources which may be released into the environment [24]. The outcomes of this preliminary short study indicates that the wastewater samples from local sewage discharge can provide a rich source of L-asparaginase producing bacteria. The outcomes of this preliminary screening encourages to further investigate the sewage water samples to screen out the possibly new & efficient asparaginase producing isolates with complete biochemical & molecular characterization in view of commercial prospects. However, the bacterial isolates culture in this study will be optimized for enzyme production as well as purification.

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