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SHORT COMMUNICATION

Screening of Streptomyces for L-asparaginase Production

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INTRODUCTION

Asparaginase is an anticancer agent used in the chemotherapy. Several group of microorganism have potential of asparaginase production and enzymes derived from microorganisms are the major sources of the enzymes for practical clinical use [1]. *Streptomyces* are gram positive sporulating soil bacteria. These organisms synthesize and produce several compounds that posses antimicrobial and antitumour activity [2]. Very rare reports are available on the asparaginases producing microbes from mangrove origin [3-6]. Hence, a wide scale screening was made of the *Streptomyces* isolates obtained from this mangrove area with objective to achieve some good microbial strains. Marine organisms have been reported as potential sources for anti cancer compounds [7]. Our studies may be one of preliminary tool for the development of therapeutic agents active against cancer.

The present study was carried out on *Streptomyces* isolated previously from Bhitarkanika mangrove forests of Orissa coast. These isolates of *Streptomyces* were screened for the L asparaginase activity in Biomass and extracellular condition by culturing them in Glucose aspargine medium (25ml in 150ml Ehrlenmeyer flasks) of 4.5 pH, 30°C for 10 days in static incubation. The cultures were harvested after the due incubation period and the mycelial mats were washed with ice cold dist. water. One gram freshly weighed fungal biomass was thoroughly homogenized in 10 ml of cold normal saline with sterilized sand and mycelial extract obtained was centrifuged at 3000 rpm for 20 min and supernatant was directly uses as the source of enzyme. To evaluate the extracellular enzyme activity, culture filtrate was centrifuged at 3000 rpm and supernatant was used as enzyme. The enzyme L asparaginase was assayed by estimating the amount of ammonia released in the reaction [8]. The amount of Ammonia released by the test sample was calculated with reference to the standard graph. The enzyme activity was expressed in terms of enzyme units (i. u./ml).

In present study, almost 56.75 % *Streptomyces* have shown the L asparaginase activity (Table-1). All isolates were significantly different ($P<0.001$) from each other with respect to the L asparaginase enzyme production. Among 74 isolates, 27 Streptomyces produced enzyme in biomass indicated the production of intracellular and cell bound enzymes. However maximum intracellular enzyme produced by ST 58 (10.62 i.u./ml/g) followed by *Streptomyces ST 20* (9.901 i. u./ml/g). The amount of intracellular asparaginase produced by ST20 and ST58 were significantly higher than others ($P<0.001$).

Only 15 *Streptomyces* (20.27%) exhibited the extracellular production of L asparaginase into the culture medium after 7 days of growth. The highest enzyme activity recorded was 5.88 and 4.87 i.u. /ml produced by *Streptomyces ST 46* and *Streptomyces ST 14*, respectively. Both isolates produced significantly higher extracellular asparaginase over others. It is very interesting to note that all Streptomyces, which are producer of biomass bound enzyme in this present study, were not necessarily found to be producer of extracellular L asparaginase. Total eight *Streptomyces* were found to be producer of L asparaginase in intracellular as well extracellular condition. Among them three isolates namely ST1, ST 46 and ST 51 exhibited higher extracellular enzyme activity. *Streptomyces* (ST20 and ST58) exhibited more quantity of intracellular enzymes as compared to extracellular (Table-2).

Asparaginases is an antineoplastic agent, used in the lymphoblastic leukaemia chemotherapy. Neoplastic cells cannot synthesize L asparagines due to the absence of L asparagines synthetase [9]. L asparaginases from bacterial origin can cause hypersensitivity in the long term used, leading to allergic reactions and anphylaxis [10]. Antibiotic production and L asparaginase activity from

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Table 1: L- asapraginase activity at intracellular level (biomass) produced by Streptomyces (IU/ml/g)

Streptomyces strains	
Streptomyces strain ST 1	0.96±0.45
Streptomyces strain ST 3	0.78±0.02
Streptomyces strain ST 11	0.89±0.27
Streptomyces strain ST 18	2.068±1.13
Streptomyces strain ST 20	9.899±0.63
Streptomyces strain ST 56	1.373±0.41
Streptomyces strain ST 58	10.61±0.05
Streptomyces strain ST 59	1.940±0.52
Streptomyces strain ST 27	2.524±1.02
Streptomyces strain ST 36	2.213±0.86
Streptomyces strain ST 37	4.18±2.88
Streptomyces strain ST 38	1.15±0.49
Streptomyces strain ST 39	2.99±2.10
Streptomyces strain ST 42	5.20±0.42
Streptomyces strain ST 43	2.99±2.10
Streptomyces strain ST 46	3.73±1.51
Streptomyces strain ST 47	0.73±0.06
Streptomyces strain ST 48	1.56±0.76
Streptomyces strain ST 49	1.08±0.30
Streptomyces strain ST 50	2.65±1.12
Streptomyces strain ST 51	2.07±0.20
Streptomyces strain ST 52	0.528±0.02
Streptomyces strain ST 61	2.72±1.19
Streptomyces strain ST 62	1.14±0.76
Streptomyces strain ST 69	3.348±0.77
Streptomyces strain ST 70	3.66±0.56
Streptomyces strain ST 72	0.65±0.08

Table 2: L- asapraginase activity at extracellular level (culutre filtrate) produced by Streptomyces (IU/ml)

Streptomyces strains	Average
Streptomyces strain ST 1	2.25±1.41
Streptomyces strain ST 7	2.54±1.92
Streptomyces strain ST 14	4.86±1.13
Streptomyces strain ST 17	2.15±1.22
Streptomyces strain ST 20	2.548±0.30
Streptomyces strain ST 58	2.35±0.0
Streptomyces strain ST 30	0.58±0.0
Streptomyces strain ST 37	1.47±0.72
Streptomyces strain ST 42	0.58±0.0
Streptomyces strain ST 46	5.88±0.0
Streptomyces strain ST 50	0.78±0.48
Streptomyces strain ST 51	3.53±0.36
Streptomyces strain ST 54	0.58±0.0
Streptomyces strain ST 63	1.56±0.61
Streptomyces strain ST 64	3.52±0.0

±, Standard deviation of 6 replications

Streptomyces of coastal environment was reported by few workers [4, 11]. Our findings of achieving L asparaginases from mangrove origin is also an important record. The search for the L asparaginases from this source may provide better non immunogenic therapeutic agents. This preliminary observations certainly leads to extensive and elaborative study on *Streptomyces* isolate ST20, *ST58, ST46 and ST 14* in order to achieve more potent microbial strains as anticancer agent.

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