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Research Article

A RAPID METHOD FOR SCREENING OF METHIONINASE PRODUCING *SERRATIA MARCESCENS* SPECIES FROM SOIL

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ABSTRACT

Sub-surface soil samples from cultivated land were screened for microorganisms producing L-methioninase using a rapid plate assay procedure. Screening of 147 soil samples yielded four isolates with L-methioninase activity, which was subsequently morphologically characterized and biochemically tested. Studies on enzyme production in defined medium by these isolates yielded activities in the range 0.87 IU ml^{-1} to 5.47 IU ml^{-1}, the highest being observed in isolate S_6.

Keywords: L-Methioninase, Demethiolating activity, Methionine synthase, Antitumor

INTRODUCTION

Microorganisms have shown to be potential producers of various bioactive metabolites. There are various methods to detect specifically particular bioactive metabolites. L-Methioninase (E.C 4.4.1.11) is a pyridoxal phosphate-dependent enzyme that catalyzes the direct α- and β-elimination of L-methionine to α-ketobutyrate, methanethiol, and ammonia [1]. The enzyme has the ability to catalyze the α- and β-elimination reactions of cysteine and their analogues [2]. The enzyme has received much attention, since it was reported as a potent anticancer agent against various types of tumor cell lines [3]. Physiologically, normal cells have the ability to grow on homocysteine, instead of methionine, due to their active methionine synthase [4]. Unlike normal cells, tumor cells devoid of active methionine synthase thus depend on external methionine supplementation from the diet [5]. Currently L-Asparaginase has been used widely for acute lymphoblastic leukemia, lymphosarcoma [6], Hodgkin disease [7] and a few other tumor. The problem with L-asparaginase is that it produces anaphylactic reactions. But this is not a problem with L-methioninase. Searching for new microorganisms with superior enzyme productivity is justified by the broad therapeutic applications of L-methioninase against various types of tumor. Our work is focussed on screening for L-methioninase-producing microorganisms from soil and optimizing the medium for enhanced production of the enzyme.

MATERIALS AND METHODS

Microbiological media constituents, chemicals and reagents

L-methionine and all microbiological media constituents were procured from Hi-Media Laboratories, India. All inorganic chemicals and reagents were from SD Fine Chemicals, India, and were of analytical grade.

Screening for L-methioninase producer strains

147 Soil samples were collected from several areas of cultivated land in the Madurai and Nagercoil regions of the state of Tamilnadu, India. The soils were collected from a depth of 10 to 20 cm below the surface. Soil samples were transported to the laboratory in aseptic conditions and air-dried at room temperature for 7 to 10 days. They were then packed in self-sealing polythene bags and stored until used. Appropriately diluted samples were screened for L-methioninase activity by a qualitative rapid plate assay procedure using a modified M9 medium [8] containing the following ingredients (in g l^{-1}) at the concentrations mentioned: Disodium hydrogen phosphate 6, potassium dihydrogen phosphate 3, sodium chloride 0.5, L-methionine 5, magnesium sulphate 0.24, calcium chloride 0.011 and glucose 2. The pH of the medium was 7.0, and phenol red was added to the medium as an indicator at a final concentration of 0.007% (w/v) just before pouring the plates.

Production of L-methioninase

The isolates testing positive for L-methioninase were taken up for studies on enzyme production in defined medium [9] containing the following ingredients (in $g1^{-1}$) at the concentrations mentioned: Sodium citrate 0.5, magnesium sulphate 0.1, ammonium sulphate 1.0, dipotassium hydrogen phosphate 7.0, potassium dihydrogen orthophosphate 2.0, glucose 4.0, DL-threonine 0.1, DL-leucine 0.1, and thiamine hydrochloride 0.005. The inocula (at 2% v/v) were aseptically transferred to 100 ml of defined medium in 250-ml Erlenmeyer flasks, which were then incubated at 37°C for 48 to 96 hours. Inocula were cultivated in M9 broth in stationary flasks for 48 hours. Temperature for inoculum development was also maintained at 37°C.

Extraction of L-methioninase

After incubation the crude enzyme was extracted by a simple contact method with potassium phosphate buffer at pH 7.0 [10]. The clear supernatant was used as the crude enzyme preparation.

L-Methioninase assay

The demethiolating activity of L-methioninase was expressed as the amount of methanethiol formed from L-methionine [11]. The deaminating activity was assessed by the Nesslerization method. One unit of enzyme activity was expressed as the amount of enzyme that releases one micromole of ammonia per minute under optimal assay conditions.

Morphological characterization and biochemical testing of isolates

The four isolates were subjected to morphological characterization by Gram staining, acid-fast staining and spore staining. Further, the hanging-drop method was used to detect motility of the isolates. This was followed by biochemical testing. All microscopic examinations were carried out using a bright-field microscope, using the oil-immersion (100X) objective for staining studies and the high-power (45X) objective for motility detection.

RESULTS AND DISCUSSION

From 147 soil samples screened, four isolates were identified as L-methioninase producers (L_1, M_1, C_2 and S_6), as evidenced by the pink colour of the colonies, resulting from the production of ammonia by the action of L-methioninase on L-methionine. Morphological characterization (Table 1) showed two of the isolates (L_1 and M_1) to be Gram-negative rods and the other two (C_2 and S_6) to be Gram-positive cocci. The hanging-drop test for motility revealed all the isolates to be non-motile. The spore staining and acid-fast staining also yielded negative results. The isolate S_6, which showed the maximum enzyme production was characterized and was identified as *Serratia marcescens* species.

These isolates showed activities of L-methioninase in the range 0.87 IU/ml – 5.47 IU/ml (Table 2). From the results, it may be seen that isolate S_6 (*Serratia marcescens* species) had the maximum activity of L-methioninase.

Table 1: Results of Morphological characterization and biochemical testing

S. No.	Test	*Serratia marcescens* species
1	Gram staining	+
2	Shape	Rod
3	Spore formation	-
4	Motility	+
5	Oxidase	-
6	Catalase	+
7	Indole	-
8	Citrate utilization	-
9	Methyl red	-
10	Maltose	A+, G-
11	Trihalose	A+, G-
12	Sucrose	A+, G-
13	Fructose	A+, G-
14	Lactose	A+, G-
15	Galactose	A-, G-
16	Mannose	A-, G-
17	Rhamnose	A-, G-
18	Arabinose	A-, G-
19	Raffinose	A-, G-
20	Sorbitol	A+, G-
21	Inositol	A-, G-
22	Xylose	A+, G-
23	Melibiose	A+, G-
24	Lysine	-
25	Serine	+
26	Cysteine	-
27	Asparagine	+
28	Ornithine	+
29	Threonine	+

+ indicates a positive result; - indicates a negative result

A – Acid production; G – Gas production

Table 2: Comparative yield of L-methioninase by isolates.

S. No.	Organism ID	Yield of L-Methioninase
1	L_1	2.67 IU/ml
2	M_1	1.86 IU/ml
3	C_2	0.87 IU/ml
4	S_6	5.47 IU/ml

CONCLUSION

From the above study it is clear that the isolated microorganisms have the capacity to produce L-methioninase, which was evident from their capacity to degrade methionine in the medium. Further, the organisms have diverse morphological and biochemical characteristics. Considering the fact that the crude enzyme extracts from the isolates showed a wide activity range (0.87 IU/ml to 5.47 IU/ml), they may be characterized in greater detail and their *in vivo* anticancer activity assessed using the crude enzyme extract.

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