ISOLATION AND SCREENING OF BACTERIAL L-METHIONASE FROM SOIL AND DAIRY PRODUCTS.

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Abstract

Due to expression of oncogenes in normal cells, they get converted into tumor cells. Chemotherapy and radiotherapy used nowadays have many side effects and it kills normal cells. It has been established that a number of enzymes have anticancer property such as L-Asparaginase, L-Glutaminase, L-Arginase and L-Methionase. In this study, we focused on isolating and screening of bacterial L- Methionase enzyme from different soil samples and dairy products. During isolation from different soil samples and dairy products 14 isolates were purified and 6 of them showed positive results giving pink colored colonies. Out of 6 isolates, MGL 2, MGL 3, MGL 10 and MGL 13 showed maximum enzyme activities. MGL 3 showed highest enzyme activity of 8.38U/ml. In Morphological characterization MGL2, MGL 3, MGL 10 and MGL 13 showed purple color and are rod shaped Gram positive bacteria. In biochemical characterization four isolates MGL 2, MGL 3, MGL 10 and MGL 13 gave catalase test and methyl red test positive.

Keywords: L-Methionase, Anticancer enzyme, Nesselerization method, Enzyme Activity.

Introduction

Cancer is uncontrolled mass of rapidly growing cells.(Liu et al., 2021)Tumor cells uptake required nutrients from normal cells and divide at very high rate. Chemotherapy and Radiotherapy used to treat cancer have many side effects and it also destroys normal cells(Mh, 2020).Enzyme therapy is good alternative to treat cancer cells(Sharma et al., 2014).Many enzymes such as L-Asparaginase(Zolfaghar et al., 2019), L-Glutaminase(Binod et al., 2017), L-Arginase(Fernandes et al., 2017) and L-Methionase(Alshehri, 2020) can be used in enzyme therapy to treat tumor cells. These enzymes degrade different amino acids and starvation condition occurs in cancer cells due to which the tumor cells are killed. These enzymes are found in organisms such as bacteria, fungi, archaea, plants and some eukaryotes, but they are completely absent in mammals(Kotramada Bopaiah et al., 2020).

L-Methionase is also known by different names such as L-Methioninase, L- Methionine Methanethiol-lyase, L-Methionine–γ-lyase, L-Methionine-γ-demethiolase. It is pyridoxal phosphate (PLP) dependent enzyme(Kulikova et al., 2017). It has molecular weight of 149kDa to 173kDa. L-Methionase works efficiently as anticancer enzyme against tumor cell lines of kidney, breast, colon, lung and glioblastoma(Kavya & Nadumane, 2020). L-Methionase degrades L-Methionine into α-ketobutyrate, Methanethiol and ammonia(El-Sayed, 2010). Due to degradation of L-Methionine the tumor cells get arrested in S or G2 phase of cell cycle and undergo apoptosis(Shrivastava et al., 2016).

This study was aimed for isolating and screening of L-Methionase enzyme producers. Morphological and biochemical characterization was performed for isolates that showed positive results through isolation and screening.

Materials and Methods

Sample Collection

Dairy products such as curd, raw milk, cheese and other soil sources from sugarcane field soil, cotton field soil, corn field soil, groundnut soil, pigeon pea field soil, garden soil and marine soil were collected from different places in sterile polythene bags and kept at 4°C.

Chemicals

Chemicals were used in study like Disodium phosphate (Na_2HPO_4), Monopotassium phosphate(KH_2PO_4), Sodium chloride (NaCl), Magnesium sulfate heptahydrate ($MgSO_4 \cdot 7H_2O$), Calcium chloride ($CaCl_2$), D-glucose, Tri- sodium citrate, Ammonium sulfate $(NH_4)_2SO_4$, Dipotassium hydrogen phosphate (K_2HPO_4), Monopotassium phosphate (KH_2PO_4), D- Leucine, L-methionine, Thiamine hydrochloride, Nessler's reagent, Potassium phosphate buffer, Trichloroacetic acid, Ammonium chloride (NH_4Cl) and Deionized water

Isolation of L-Methionase producing Bacteria

Dairy and Soil samples were serially diluted from 10^{-1}up to 10^{-5} and spreaded on M9 media containing [Na_2HPO_4 (6g/L) KH_2PO_4 (3g/L), NaCl (0.5g/L), $MgSO_4.7H_2O$ (0.24g/L), $CaCl_2$ (0.011g/L), Glucose (2g/L). L-Methionine and phenol red (0.007%) as indicator dye were added before pouring the plates. The plates were incubated at 37°C for 24 to 48 hours. After incubation pink colored colonies were enriched on same media using streak plate technique and incubated at 37°C for 24 to 48 hours. Formation of pink colored colonies indicate the L-Methionase producers.

Enzyme Production

The isolates showing positive results were used for further studies. Production media contains Sodium citrate(0.5g/L), Magnesium sulfate(0.1g/L), Ammonium Sulfate(1.0g/L), Dipotassium hydrogen phosphate(7.0g/L), Potassium dihydrogen orthophosphate(2.0g/L), Glucose(4.0g/L), D,L-threonine(0.1g/L), D,L-leucine(0.1g/L) and Thiamin hydrochloride(0.005g/L). The inoculum were aseptically transferred to 100 ml of defined media in 250 ml Erlenmeyer flasks, which were then incubated at 37°C for 48 to 96 hours in shaker.

Morphological Identification

Morphological identification was performed through Gram's staining.

Biochemical Test

Catalase test, Gelatin hydrolysis test, Methyl red test, Voges-Proskauer test, Indole production test, starch hydrolysis test, Simon citrate test and Casein hydrolysis test were performed for all positive isolates.

Enzyme Assay

In Nesslerization method 1ml of 50mM Potassium phosphate buffer, 0.1 ml of 20mM L-methionine solution and 0.9 ml deionized water were mixed in a clean test tube and equilibrated at 37°C for 5 minutes. To this mixture, 0.1 ml of enzyme solution was added and mixed immediately by inversion, after which the test tube was incubated at 37°C for 60 minutes. After incubation, 0.1 ml of 1.5M TCA was added to the test tube. The mixture was then centrifuged for 2 minutes to clarify the solution, after which 0.2 ml of the supernatant, 4.3 ml of deionized water and 0.5 ml Nessler's reagent were mixed in test tube and absorbance of the mixture was measured at 480 nm. A blank was also prepared without the enzyme. One unit of enzyme activity was expressed as the amount of enzyme that released micromoles of ammonia per minute under optimal assay conditions.

Result and discussion

Isolation of L-Methionase producing bacteria

Based on this study 14 isolates were obtained from different samples. Out of them 6 isolates showed positive results as they form pink colonies (table 1).

Sr.No.	Isolate Number	Source
1	MGL 2	Garden soil
2	MGL 3	Sugarcane field soil
3	MGL 4	Sugarcane field soil
4	MGL 8	Cheese
5	MGL 10	Corn field soil
6	MGL 13	Curd

Table 1: Isolation of L-Methionase producing bacteria from soil and dairy products

Enzyme Assay

Out of 6 positive isolates, MGL 2, MGL 3, MGL 10 and MGL 13 showed higher enzyme activities. Enzyme activities are shown in (table:2)

Isolate number	Source	Nesseler's assay OD at 480nm	Enzyme activity (U/ml)
MGL 2	Garden soil	0.219	6.90
MGL 3	Sugarcane field soil	0.266	8.38
MGL 10	Corn field soil	0.241	7.59
MGL 13	Curd	0.131	4.13

Table 2: Enzyme activities obtained from Nesslerization reaction.

Morphological identification

Morphological identification of bacterial isolates MGL 2, MGL 3, MGL 10 and MGL 13 identified by gram staining. We observed positive isolates as purple colored and rod shaped bacteria.(figure 1a, 1b, 1c, 1d).

Colony characterization

Characteristics	MGL 2	MGL 3	MGL 10	MGL 13
Size	1 mm	3mm	1mm	1mm
Shape	Circular	Circular	Circular	Circular
Surface	Glistening	Viscid	Glistening	Dull
Color	Pinkish	Pinkish	Pinkish	Pinkish
Opacity	Opaque	Transluscent	Transluscent	Opaque
Elevation	Flat	Raised	Flat	Flat
Margin	Even	Even	Even	Even

Table 3: Colony characterization

Biochemical Test

MGL 2, MGL 3, MGL 10 and MGL 13 showed catalase and methyl red tests positive (table 4).

Biochemical tests	MGL 2	MGL 3	MGL 10	MGL 13
Catalase test	+	+	+	+
Gelatin hydrolysis test	-	-	-	-
Methyl red test	+	+	+	+
Voges Proskauer test	-	-	-	-
Indole production test	-	-	-	-
Starch hydrolysis test	+	+	+	-
Simmon citrate test	+	+	+	-
Casein hydrolysis test	-	-	-	-

Table 4 : Biochemical characterization

Conclusion

From this study we can conclude that, MGL 2, MGL 3, MGL 10 and MGL 13 are potent for producing L-Methionase anticancer enzyme. L-Methionase enzyme can be purified from these isolates and can be efficiently used as therapeutic drug through enzyme therapy.

Acknowledgement

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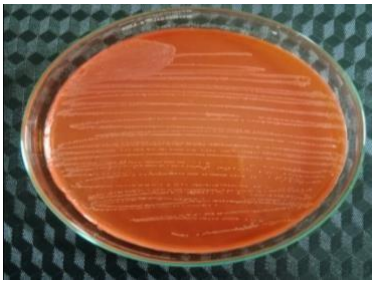
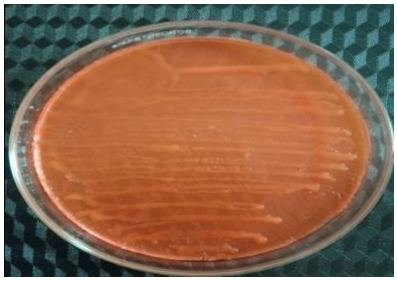
Figures

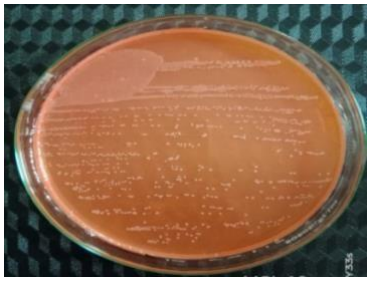
Isolation



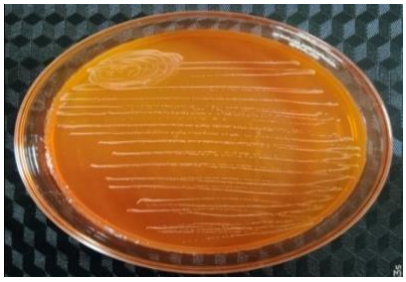
MGL 2



MGL 3



MGL 10



MGL 13

Morphological Identification

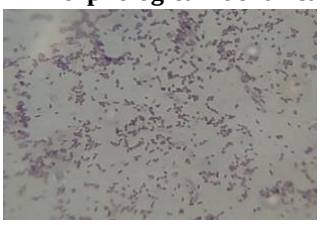


Figure 1a : MGL 2

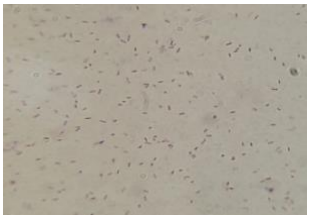


Figure 1b : MGL 3

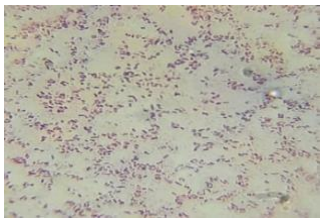


Figure 1c : MGL 10

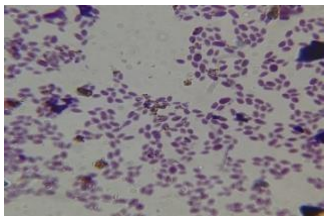


Figure 1d : MGL 13