Isolation, Screening and Morphological Identification of Fungal L-Methionase from Ripen Fruits and Vegetables

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Abstract

L-Methionase is one of the few microbial enzymes with high therapeutic value since it has been reported as a effective anticancer agent against many types of tumor cell lines like breast, lung, colon, kidney and glioblastoma. L-Methionase is present in all organisms except in mammals. It mainly catalyzes the α, ϒ-elimination of L-Methionase to α-ketobutyrate, Methanethiol and ammonia. L-Methionase producing fungi was isolated from ripen fruits and vegetables. We obtain total 24 fungal isolates from ripen fruits and vegetables. Quantitative rapid plate assay test shows the ability of 7 of these isolates to grow with yellow zone surrounding their colonial growth, while 17 of these isolates could grow and utilize L-Methionine without any yellow zone around their colonial growth. Quantitative assay test shows the rate of L-Methionine production by all isolates tested. Nesslerization assay for detection of ammonia isolate no. MFL 24 (2.95 µmol/min/ml) and isolate MFL no. 9 (3.45 µmol/min/ml) show highest L-Methionase enzyme activity. The identification of fungi was carried out by macroscopic and microscopic examinations depend on the colony color, shape, hyphae, conidia, conidiophores and arrangement of spores.

Keywords: L-Methionase, Antitumor Enzyme, Fungi, Rapid plate Assay

1. Introduction:

L-Methionase enzyme which can degrade methionine amino acid in α-ketoglutarate, ammonia and methanethiol.(Suganya et al., 2017) It can treat all type of cancers like kidney, colon, prostate, glioblastoma and neuroblastoma.(Alshehri, 2020) L-Methionase is a pyridoxal dependent enzyme.(Lin et al., 2017) L-Methionase also known as L-Methionine methanethiol lyase, L-Methionine-γ-lyase and L-Methionine-γ-demethylase.(Pokrovsky et al., 2018)

L-Methionase is isolated from bacterial species including *pseudomonas putida,*(Zolfaghar et al., 2019) *Clostridium sporogenes,*(Kulikova et al., 2017) *Aeromonas sp***,**(Tanaka et al., 1985) *Citrobacter intermedius*(Ronda et al., 2011) and *Brevibacterium lines*, (Bonnarme et al., 2000) *Trichomonas vaginalis*(Tokoro et al., 2003) and *Streptomyces variabilis*. L-Methionase are also present in fungal species including *Aspergillus flavipes*,(El-Sayed, 2009) *Candida tropicalis*,(Selim et al., 2015) *Trichoderma harzinum,*(Salim et al., 2020) *Aspergillus Niger,*(Khalaf & El-Sayed, 2009) *Penicillium spp, Geotrichum candidum*(Bonnarme et al., 2001) *and Chaetomium globosum.*(Shimaa et al., 2016) L-Methionase is an intracellular enzyme in bacterial species an extracellular enzyme in fungal species and absent in mammals.(Sharma et al., 2014) Other main important role of L-Methionase in human diet and metabolism and it can also play important role in immunological function. L-Methionase is a volatile Sulphur compound important in food industry of significant influence of the characteristic aromas of various chesse.(Baghdadi & Balobaid, 2021)

This study is aimed at identifying L-Methionase producing fungi from ripen fruits and vegetables.

1. Materials and methods

2.1 Collection of sample and fungal isolation

Different ripen fruits and vegetables were collected from market. Some ripen portion cut with sterile scalpel and plated on to Potato Dextrose Agar (PDA), containing Potato infusion (4g/l), Dextrose (20g/l) and Agar-Agar (20g/l). The pH of the medium was

adjust to 7.0 using 1N sodium hydroxide solution. The plate was incubated at 28 for 7days. The fungal isolates were sub cultured and maintained on the same media**.**

2.2 Qualitative assay

In Qualitative rapid plate assay procedure using C'zapex yeast extract agar medium. And add methionine amino acid used as carbon source and phenol red with final concentration of 0.007% just before pouring the plates, phenol red as an indicator was added to the media, as yellow color zone around the colonies for L-Methionase. The plates were incubated in inverted position at 28 for 3 to 5 days. Producing colonies were choose on the basis of formation of yellow color zone around the colonies for L-Methionase. Well isolated colonies and purified from each plate were selected for further studies.

2.3 Rapid Plate Method

In a Rapid plate method fungal mycelium was inoculated into C'zapex broth. After inoculation broth were incubated in a rotary shaker at 120 rpm for 3 to 5 days. After incubation fungal mycelium grow in a broth and These fungal mass harvested by centrifugation at 6000 rpm for 30 min then filtrate by filter paper. Agar plates were prepared by pouring 20 ml of C'zapex dox media in to sterile petri dish. Phenol red and methionine are added before pouring the plates. Plates was solidified 7mm wells were punching, using sterile cork borer, 100µl of fungal culture was loaded in the wells, the plates kept in upright position at 28 for 24 to 48 hrs.

2.4 Quantitative assay by Nesslerization Method

The L-Methionase production was detected by determining the amount of ammonia released from L-methionine. The optimal reaction system includes 1 ml of 1% methionine in 0.5 M potassium phosphate buffer (PH 7.0), 0.1 ml pyridoxal phosphate, and 1ml of raw enzyme. The reaction system was incubated at 30 for 1 h. The enzymatic activity was blocked by adding 0.5 ml of 1,5 mol/L trichloroacetic acid or by boiling for 5 min. The system was centrifuge at 5,000rpm for 5 min to eliminate the precipitated proteins. 0.1 ml prior mixture was added to 3.7 ml of distilled water and the liberated ammonia was detected utilizing 0.2 ml Nessler reagent, and the developed color

compound was measured at 480 nm using spectrophotometer. Enzyme and substrate blanks were utilized as control. One unit of L-Methionase was measured as the amount of enzyme that releases ammonia at 1µmol/min under standard examination conditions.

2.5 Morphological identification

In Morphological identification using Lactophenol cotton blue. Take small part of fungal mycelium with help of sterile pointed needle and placed on to sterile slide. Put one drop of lactophenol cotton blue on slide. Then a slide covered with a coverslip and observed under 10X.

3. Result and Discussion

Isolation of fungal strain

Based on fungal isolation we obtain 24 fungal isolates from different soil sample. Isolates MFL 24 was obtain from ripen pineapple (Figure.1) and MFL 9 was isolate from ripen onion. (Figure.2) Various fungal strain which were isolated from ripen fruit and vegetables were preserved on potato dextrose agar (PDA) for further studies

Qualitative Assay of L-Methionase

L-Methionase activity was tested on C'zapex dox agar supplemented with L-methionine, as the nitrogen sources and add phenol red as the pH indicator. **Yellow color** zone around fungal colonies for L-Methionase. (Figure.3) (Figure.4)

Rapid Plate Method

The cell free filtrate of two selected fungi was evaluated L-Methionase production by agar well diffusion assay on solid C'zapex dox plates with phenol red, after 24 to 48 hr of incubation, the measurement of yellow zone in mm were recorded based on diffused of L-Methionase. (Table 1) (Table 2)

Quantitative Assay by Nesslerization Method

In Nessler's method isolates MFL 9 (3.45 µmol/min/ml) and MFL 24 (2.95 µmol/min/ml) give a high enzyme activity. (Table 3)

Morphological Characterization

Based on morphological identification isolates MFL 9 and MFL 24 are observe under microscope and we have been shown, forming filamented hyphae that make them appear like small plant. (Figure 5)

Conclusion

In this study Isolation of Fungal L-Methionase from ripen fruits and vegetables. A total 7 fungal isolates as L-methionine decomposer. In which 2 fungal isolates MFL 9 and MFL 24 has been shown higher L-Methionase activity. In rapid plate method isolates MFL 9 and MFL 24 is give maximum zone of diameter.

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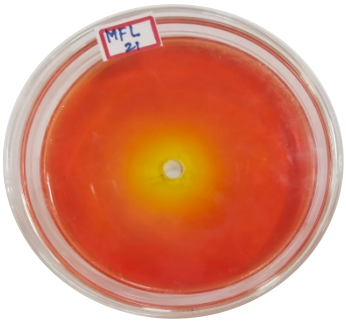
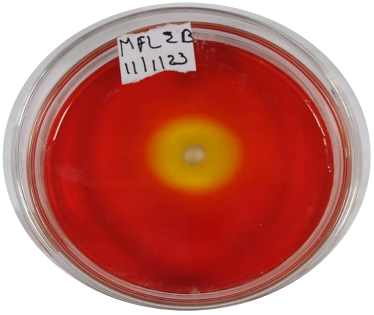
Fungal Isolates	24 hours	48 hours
MFL 9		
MFL 24		

Table: 1 Quantitative assay of L-Methionase in fungal cell free filtrate by agar well diffusion method

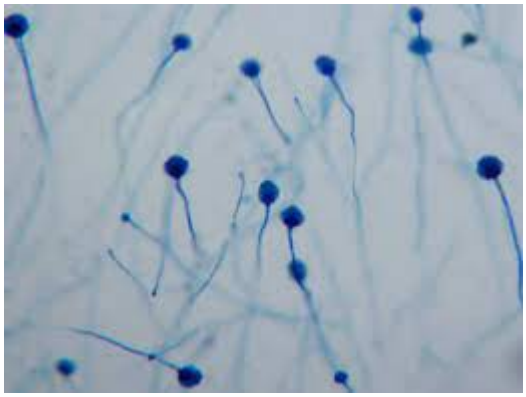
Fungal Isolates	Zone of diameter in mm after 24 hr.	Zone of diameter in mm after 48 hr.
MFL 9	25	35

MFL 24	15	20

Table: 2 Zone of diameter of MFL 9 and MFL 24

S. No	Organism ID	co Activity of L-Methionase
1	MFL 9	3.45 U/ml
2	MFL 24	2.95 U/ml

Table: 3 L-Methionase Enzyme Activity of fungal isolates



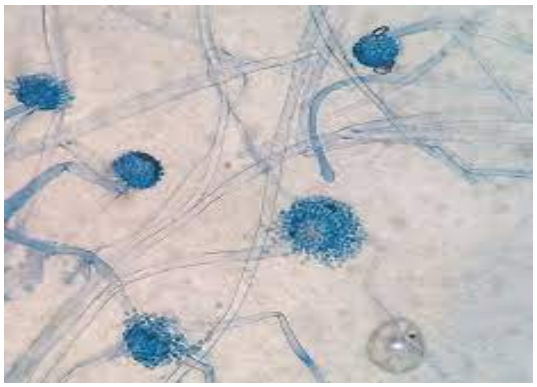


Figure: 5 Micro Morphological identification of fungal isolates

Figure: 1 Isolation Of fungus MFL 24 from Ripen Pineapple

Figure: 2 Isolation of fungus MFL 9 from Ripen Onion

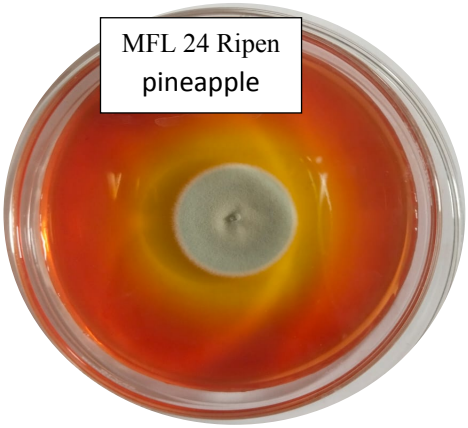


Figure: 3 Qualitative assay of L-Methionase with yellow color zone isolate no. (MFL 24)

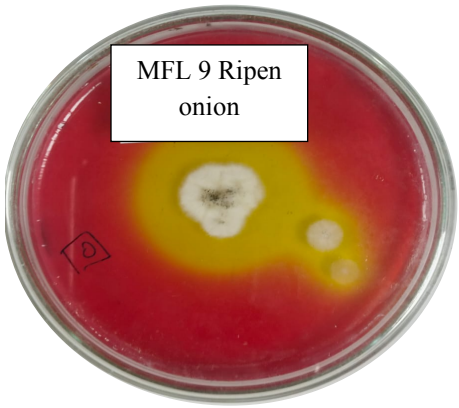


Figure: 4 Qualitative assay of L-Methionase with yellow color zone isolate no (MFL 9)