September 2010 ISSN : 0974 - 7435 Volume 4 Issue 3

Trade Science Inc.

BioTechnology

An Indian Journal

SHORT COMMUNICATION

BTAIJ, 4(3), 2010 [153-155]

L-asparaginase activity of different thermophilic fungi

R.Ranjith Kumar*, B.Sreelatha, S.Girisham, S.M.Reddy

Department of Microbiology, Kakatiya University, Warangal - 506 009, (INDIA)

Received: 2nd April, 2010 ; Accepted: 12th April, 2010

INTRODUCTION

L-asparaginase (E.C.3.5.1.1 asparagine amino hydrolase) catalyses the deamination of L-Aspargine resulting in L-aspartate and ammonia. This enzyme is reported to be useful for treatment of selected types of haematopoitic leukemia and non Hodgkin lymphomas[14].

The beneficial role of L-asparaginase administration is usually attributed to the fact that the tumor cells have a compromised ability to generate L-asparaginase endogenously, either due to low expression levels of asparagine synthetase[18] or insufficient amount of its substrate, aspartate or glutamine[3]. Though L-asparaginase are available from number of microbial sources but tumor inhibitory activity has been demonstrated only with the asparginases obtained from *E.coli*[13] marine actinomycete[6] and fungi. It has been observed that eukaryotic microorganisms like yeast and filamentous fungi such as *Aspergillus, Penicillium and Fusarium* have a potential for L-asparaginase production. Serquis and Olivera[17] worked on its distribution, biochemical and immunological properties. A large number of bacteria[9] and fungi[8] have been reported to secrete L-asparaginase. Gulati and Saxena, Gupta[10,16] have developed a simple rapid plate assay which is most sensitive by using phenol red. Present investigation was aimed to screen variety of thermophilic fungi for secretion of L-asparaginase.

TABLE 1 : Asparaginase production by different thermophilic fungi

Name of the fungi	Concentration of dye(in ml/100ml media)			
	0.03	0.06	0.09	0.1
	Zone of coloration(in mm)			
Aspergillus fumigatus (TT)	1.0	2.0	3.0	5.0
A. nidulans (TT)	2.0	4.0	6.0	0.8
A. terreus (TT)	2.0	3.0	4.0	0.6
A. flavus (TT)	2.0	4.0	8.0	0.9
A. niger (TT)	1.0	2.0	3.0	0.4
Chaetomium thermophile (TP)	1.0	2.0	3.0	4.0
C. thermophilium (TP)	1.0	1.0	4.0	6.0
Humicola insolens (TP)	2.0	4.0	5.0	8.0
H. grisea (TP)	3.0	4.0	6.0	8.0
H. lanuginosa (TP)	4.0	--	7.0	9.0
H.stelleta (TP)	3.0	5.0	8.0	8.0
Malbranchea pulchella (TP)	3.0	7.0	9.0	12.0
Mucor miehei (TP)	5.0	8.0	9.0	12.0
M. pusillus (TP)	4.0	9.0	12.0	13.0
Torula thermophila (TP)	6.0	9.0	12.0	15.0

TT- Thermotolerent fungi TP-Thermophilic fungi

EXPERIMENTAL

Materials and methods

Total of 46 thermophilic fungal strains representing 8 genera 17 species 46 strains isolated from different natural substrates like coalmine soil, municipal waste and vermin compost. Decomposing materials and Zoo waste material etc were screened for thermophilic fungi were grown in Yeast Extract Starch Agar (YESA) at 45°C ±2° C for 7 days. Modified Czepek dox's medium containing glucose 2.0g, L-aspargine 10.0g, KH_2PO_4 1.52g, KCl 0.52g, $MgSO_4.7H_2O$ 0.52g,

SHORT COMMUNICATION

TABLE 2 : Asparginase production (in mg/ml) by some thermophilic fungi

Name of the fungus	Incubation period(in days)	pH	Dry weight(mg/ml)	Asperginase activity(in IU/ml)
Aspergillus fumigatus	4	6	134	0.03
	8	6.7	239	0.04
	12	6.9	202	0.02
A.nidulans	4	6.4	145	0.01
	8	6.9	254	0.02
	12	7.3	196	0.01
A.terreus	4	6.2	155	0.01
	8	6.8	265	0.03
	12	7.4	201	0.02
A.flavus	4	6.1	143	0.02
	8	7.1	235	0.04
	12	6.9	182	0.03
A.niger	4	6.2	137	0
	8	7	243	0.02
	12	6.8	179	0.01
Chaetomium thermophile	4	6.4	187	0.04
	8	6.9	258	0.06
	12	7.2	210	0.03
C.thermophilium	4	6.2	192	0.03
	8	7.3	246	0.04
	12	7.1	183	0.02
Humicola insolens	4	6.0	180.0	0.04
	8	7.0	252.0	0.12
	12	6.4	200.0	0.09
H.gresia	4	6.0	175.0	0.03
	8	7.2	248.0	0.09
	12	6.9	202.0	0.07
H.lanuginosa	4	6.0	162.0	0.04
	8	7.2	265.0	0.10
	12	7.0	201.0	0.08
H.stelleta	4	6.8	192	0.03
	8	7.4	254	0.09
	12	7.2	220	0.07
Malbranchea pulchella	4	7.0	135.0	0.03
	8	7.5	210.0	0.06
	12	7.4	185.0	0.05
Mucor meihei	4	6.0	150.1	0.09
	8	7.0	275.0	0.12
	12	6.5	240.0	0.08
M.pusillus	4	6.0	172.0	0.08
	8	7.1	285.0	0.10
	12	6.7	240.0	0.09
Torula thermophila	4	7.0	150.0	0.06
	8	7.8	262.0	0.12
	12	7.2	212.0	0.10

TABLE 3 : Correlation between culture filtrate and phenol red agar plate method

		Zonediameter	Enzymeactivity
Zone diameter	Pearson correlation	1	.836(**)
	Sig. (2-tailed)	.	.000
Enzyme activity	Pearson correlation	.836(**)	1
	Sig. (2-tailed)	.000	.
	N	15	15

Correlation is significant at 0.01 level (2 tailed).

$CuNO_3.3H_2O$ trace, $ZnSO_4.7H_2O$ trace, $FeSO_4.7H_2O$ trace, Agar agar 20g and 1000ml of distilled water, pH was adjusted to 6.2[10,16] was employed for screening different thermophilic fungi for L-asparaginase production.

Modified Czepek Dox medium was supplemented with different concentrations (0.03 to 0.09m) of the dye. A stock solution of 2.5% (dissolving 2.5g of phenol red in 100 ml of ethanol) and the P^H was adjusted to 7.0 using 1M NaOH. The medium thus prepared was inoculated with 7 days old cultures and incubated at 45±2°C for 48 hours. Medium without dye and sodium nitrate as a nitrogen source in place of L-aspargine served as control.

At the end of incubation zone of pink coloration was measured in mm as positive for qualitative assessment and area of coloration was taken the quantitative measurement. Quantitative assay was carried out by inoculating the fungal strains in 250ml Ehrlen Mayer flask containing 50ml of medium. The inoculated flasks were incubated at 45 ±2°C for 4, 8 and 12 days respectively. Flasks containing sodium nitrate in place of L-aspargine served as control. At the end of incubation period the fungal cultures were harvested on preweighed whatmann No.1 filter paper for the assessment of growth. The filter paper along with mycelium was dried at 65 ±1°C for 48 hours till constant weight is obtained. Since the difference among the replicates was insignificant and average three replicates was taken as criteria for growth rate of the fungus. L-asparaginase activity was assayed as suggested by Imada et al.[11]. The reaction mixture consisting of 0.2ml of culture filtrate, 0.3 ml of sodium borate buffer (pH 8.5) and 1ml of 0.04M asparagine was incubated for 10 minutes at 45±2x°C. After incubation the reaction was terminated with the help of 1ml of 15% TCA. The reaction mixture was centrifuged at 300rpm for 10 min-

utes from that 0.1ml of supernatant taken and added 4ml of water, 1ml of Nesslers reagent and 1ml of 2M NaOH was added. After addition of all above the solution was leaved for 15 minute at 45 ± 2°C for the color development. The intensity of yellow color appeared measured at 500 nm and expressed as IU Ml^{-1} one international unit of L-asparginase activity is defind as that amount of enzyme catalyses the formation of 1 Umol of ammonia per min. under the conditions of the assay.

RESULTS AND DISCUSSION

From TABLE 1 it is clear that majority of isolates of different species of thermophilic fungi were potential of producing L-asparaginase. However, degree of production varied with in the same genera of different species followed by *Torula thermophila*, *Mucor pusillus* and *Mucor meihei*, *Malbranchea pulchella* was next good producers of L-asparaginase. *Myricoccum albomyces* failed to produce L-asparaginase. The amount of asparaginase secreted increased with increase in dye concentration. However, *Aspergillus* strains, *Humicola* strains and *Mucor* spp. the degree of coloration did not change with dye concentration of 0.09 and 0.1m. *A.nidulans*, *A.terreus*, *A.flavus* and *A.niger* were also poor in production of L-asparaginase.

TABLE 2 reveals that the L-asparaginase activity in culture filtrate on 8th day of incubation period *Torula thermophila* and *Mucor miehei*, *Humicola insolens* are producing high and equal amount of L-asparaginase among the all species. *Mucor pusillus* and *Humicola lanuginosa* are next good producers. This method also supports that the production of L-asparaginase is higher in thermophilic fungi when compared with thermotolerent fungi.

TABLE 3 explains the correlation between L-asparaginase activity in culture filtrate and phenol red method on agar plate. We have taken highest values in both methods. In culture filtrate the activity of L-asparaginase is found highest on 8th day of incubation period, diameter of pink color zone in phenol red agar plate method at dye concentration 0.1ml/100ml of media is found highest value. These two values taken as two variables and correlation are calculated by using SPSS 12.0 software version. The correlation is significant at the 0.01 level.

ACKNOWLEDGEMENT

We are thankful to the Head, Department of Microbiology Kakatiya University for providing necessary facilities and financial support given by UGC, New Delhi.

REFERENCES

[1] S.S.Ali, V.Rai, K.Soni, P.Kulashrestha, Waisk; Ind. J.Microbiol., **34**, 73-76 **(1994)**.
[2] K.Arima, T.Sakamoto, C.Araki, G.Tamura; Agric. Biol.Chem., **36**, 356-361 **(1972)**.
[3] A.M.Aslanian, M.S.Kilberg; Biochem.J., **358**, 59-67 **(2001)**.
[4] B.L.Asselin; Adv.Exp.Med.Biol., **457**, 621-629 **(1999)**.
[5] M.P.Curran, R.M.Daniel, R.G.Guy, H.W.Morgan; Arch.Biochem.Biophys., **241**, 571-576 **(1985)**.
[6] P.Dhevagi, E.P.Poorani; Indian Journal of Biotechnology, **5**, 514-520 **(2005)**.
[7] T.M.V.Elizabeth, V.Rai, S.S.Ali; Indian J.Microibiol. Ecol., **2**, 61-70 **(1991)**.
[8] M.A.Ferrara, N.M.B.Severino, J.J.Mansure, A.S.Martin, E.M.M.Oliveira, A.C.Siani Jr., F.A.G.Torres, E.P.S.Bon; Enzyme and Microbial Technology, **39**, 1457-1463 **(2006)**.
[9] H.Geckil, S.Gencer; Applied Microbiology and Biotechnology, **63**, 691-697 **(2004)**.
[10] R.Gulati, R.K.Saxena, R.Gupta; Lett.Appl. Microbiol., **24**, 23-26 **(1997)**.
[11] A.Imada, S.Igarasi, K.Nakahama, M.Isono; J.Gen.Microbiol., **76**, 85-99 **(1973)**.
[12] M.Jaskolski, M.Kozak, J.Lubkowski G.Palm, A.Wlodawar; Acta Crystallogr.D, **57**, 369-377 **(2001)**.
[13] L.Mashuburn, J.Wriston; Arch.Biochem.Biophys., **105**, 450-452 **(1964)**.
[14] H.J.Muller, J.Boos; Critical Reviews in Oncology/ Hematology, **28(2)**, 97-113 **(1998)**.
[15] C.T.Nakamura, R.Wilkinson, K.Woodruff; International Pedeatrics, **14**, 25-27 **(1999)**.
[16] R.K.Saxena, U.Sinha; Cur.Sci., **50**, 218-219 **(1981)**.
[17] M.I.Serquis, M.de, E.M.M.Oliveira, A.S.Santos, G.L.Costa; Memérias do Instituto Osvaldo Cruz., **99(5)**, 489-492 **(2004)**.
[18] W.A.G.Stams, M.L.den Boer, A.Holleman, I.M.Appel, H.B.Beverloo, E.R.Van Wering G.E.Janka-Schaub, W.E.Evans, R.Peters; Blood, **105**, 4223-4225 **(2005)**.
[19] H.E.Wade, K.Robinson, B.W.Phillips; Journal of General Microbiology, **65**, **(1971)**.