EFFECT OF DIFFERENT TEMPERATURE AND GROWTH STAGES OF BLUE OYSTER MUSHROOM ON THE ACTIVITY OF ENZYMES*

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Abstract : The experiment on effect of different temperature and growth stages of blue oyster mushroom on the activity of enzymes was conducted under laboratory conditions at Department of Plant Pathology, Rajasthan College of Agriculture, Udaipur (Rajasthan) during 2007-08. The activities of different enzymes such as celluloytic and pectinolytic were determined in terms of loss in viscosity. The cellulase (Cx) and polygalacturonase transeliminase (PGTE) were detected in high quantity at 10 days (substrate colonization stage) after inoculation while polygalacturonase (PG) was maximum at 20 days (primordia initiation stage) and polymethyl galacturonase (PMG) and pectin transeliminase (PTE) were maximum at 30 days (young stage) after inoculation. Effect of temperature on the production of different enzymes such as cellulase, polygalacturonase, polymethyl galacturonase, pectin transeliminase and polygalacturonase transeliminase at different growth stages, temperature of 25°C was found better as compared to 20, 30 and 35°C temperature.

Keywords: Blue oyster mushroom, Enzymes, Growth stages, Temperature.

INTRODUCTION

Mushroom is a cash crop grown world wide on small as well as commercial scale for domestic consumption and export. It is a rich source of proteins, minerals and vitamins with low calorie value with no cholesterol. The digestibility of mushroom protein is as high as 72 to 83 per cent (Chang, 2007). Total mushroom production of world is around 7.2 MT (Thakur, 2005). In India, major mushroom growing states are Himachal Pradesh, Haryana, Punjab, Jammu & Kashmir, Uttar Pradesh, Uttaranchal, Rajasthan, Jharkhand and Maharashtra. The major mushroom growing districts of Rajasthan are Udaipur, Jaipur, Ajmer, Bikaner, Bhilwara, Kota, Banswara, Sriganganagar and Sirohi. According to Weil (2006) *Hypsizygus ulmarius* the name itself is a mouthful and over the years. Therefore, introduction of some potential mushrooms like the blue oyster mushroom *Hypsizygus ulmarius* which occupy a prime position can be cultivated easily under the agroclimatic conditions of Rajasthan. Thus, very meagre work has been carried out on the blue oyster mushroom, *Hypsizygus ulmarius* which has a great potential for cultivation in tropical area like Rajasthan. The physical environmental factor, nutrition, quality and media are known to have direct effect on the mycelial growth and quality which can be exploited for the commercial cultivation of blue oyster mushroom. Therefore, the present study was carried out on the effect of different temperature and growth stages of blue oyster mushroom on the activity of enzymes.

MATERIAL AND METHOD

The studies on effect of different temperature (20, 25, 30 and 35°C) on the production of cellulolytic and pectinolytic enzymes at various growth stages *viz.*, 10 (substrate colonization stage), 15 (spawn run stage), 20 (primordia initiation stage), 25 (button stage) and 30 (young stage) days was carried out under laboratory condition at Department of Plant Pathology, Rajasthan College of Agriculture, Udaipur (Rajasthan) during 2007-08.

Twenty five gms of each sample was collected for enzyme cellulolytic extraction and transferred to a blender and 50 ml distilled water was added. It was then blended for 10 minutes and filtered trough two layers of cheese cloth. The filtrate thus obtained was centrifuged at 2000 rpm for 20 minutes. The supernatant was removed and dialysed against several volumes of double glass distilled water at 4°C for 24 hours by changing the water at least two times. The dialysed solution was used as enzyme source. Few drops of toluene was added in each enzyme extract to check the activity of the enzyme. For pectinolytic enzyme extraction twenty five gms of each sample was transferred to a blender and 50 ml of 0.15M NaCl solution was added. It was then blended for 5 minutes and filtered through two layers of cheese cloth. The filtrate was centrifuged at 2000 rpm for 20 minutes. The supernatant was removed and dialysed against several volumes of double glass distilled water for 24 hours at 4°C by changing the water at every 8 hours. The dialysed solution was used as the crude pectin enzyme extract. Few drops of toluene were added to check the enzyme extract.

Measurement of celluloytic and pectinolytic enzyme activity

Cellulase (Cx)

Cellulase (Cx) activity was measured by loss in viscosity of cellulolytic substrate. In a viscometer 4 ml Carboxy methyl cellulose (CMC) solution, 1 ml buffer and 2 ml of enzyme extract were pipetted out. The contents were mixed by drawing air gently through the large arms of the viscometer. The efflux time of the mixture was determined at prefixed intervals. The percentage loss in viscosity was calculated by employing the following formula:

$$V = \frac{T_0 - T}{T_0 - T_w} \times 100$$

Where,

V = Per cent loss in viscosity

T_0 = Flow time in seconds at zero time

T = Flow time of the reaction mixture at ' T ' time.

T_w = Flow time of distilled water

Polygalacturonasae (PG):

Four ml of sodium polypectate solution was taken in to viscometer and added 1 ml of acetate buffer + 2 ml of enzyme extract. The efflux time of the reaction mixture was determined after gently mixing the content. The per cent reduction in viscosity was calculated by employing the formula given in previous assay.

Polymethyl galacturonase (PMG):

The basic method of assay of PMG was the same as that of PG, except 1 per cent pectin was used as the substrate which was prepared in following manner.

Pectin (1 g) dissolved in 100 ml of acetate buffer (pH 5.2) kept in blender. Then blended for 2-3 minutes at low speed and then high speed for another 3 minutes filtered through 2 layers of cheese cloth.

Pectin transeliminase (PTE):

Four ml of 1% pectin solution + 1 ml of buffer (boric acid borax buffer pH 8.2) + 2 ml of enzyme extract were taken into the viscometer and the contents were mixed. The efflux time of the mixture was worked out and per cent loss in viscosity was calculated as per standard method given earlier.

Polygalacturonase transeliminase (PGTE):

PGTE also referred to pectic acid transeliminase, trans eliminatively splits pectic acid releasing unsaturated galacturonic acids. The rate of release of galacturonic acids can be followed by viscosity assay using sodium polypectate as substrate. The method of assay of PGTE was the same as that of PTE except 1.2 per cent sodium polypectate was used in place of pectin 1%.

RESULT AND DISCUSSION

Effect of different temperature (20, 25, 30 and 35°C) on the production of cellulolytic (Cx) and pectinolytic enzymes (PG, PMG, PTE, and PGTE) at various growth stages *viz.,* 10 (substrate colonization stage), 15 (spawn run stage), 20 (primordia initiation stage), 25 (button stage) and 30 (young stage) days presented in Table 1.

Enzymatic activity at 20±1°C

The activity of cellulase (Cx) in terms of reduction in the viscosity was maximum (66.83 %) at 10 days (substrate colonization stage) of mycelial growth followed by 15 days (57.75 % at spawn run stage). The activity of polygalacturonase (PG) was highest (51.53 %) at 20 days (primordia initiation stage) and it was quite similar on 25 days (50.58 % at button stage) of mycelial growth in *H. ulmarius.* The activity of polymethyl galacturonase (PMG) in terms of reduction in the viscosity was maximum (64.62 %) at 30 days (young stage) of mycelial growth followed by 25 days (60.99% at button stage). In case of pectin transeliminase (PTE) the per cent loss in viscosity was also maximum (58.45 %) at 30 days (young stage) of mycelial growth followed by 25 days (55.20 % at button stage). Maximum activity of polygalacturonase transeliminase (PGTE) was observed at 10 days (49.71 % at substrate colonization stag) followed by 15 days (49.32 % at spawn run stage) of mycelial growth.

Enzymatic activity at 25±1°C:

The activity of cellulase (Cx) in terms of reduction in the viscosity was maximum (75.44 %) at 10 days (substrate colonization stage) of mycelial growth followed by 15 days (59.59 % at spawn run stage). The activity of polygalacturonase (PG) was highest (53.02 %) at 25 days (primordia initiation stage) and it was quite similar on 20 days (52.83 % at button stage) of mycelial growth in *H. ulmarius.* The activity of polymethyl galacturonase (PMG) in terms of reduction in the viscosity was maximum (65.53 %) at 30 days (young stage) of mycelial growth followed by 25 days (63.68 % at button stage). In case of pectin transeliminase (PTE) the per cent loss in viscosity was also maximum (60.32 %) at 30 days (young stage) of mycelial growth followed by 25 days (57.06 % at button stage). Maximum activity of polygalacturonase transeliminase (PGTE) was observed at 10 days (56.68 % at substrate colonization stag) followed by 15 days (52.19 % at spawn run stage) of mycelial growth.

Table 1: Cellulolylic and pectinolytic enzymes activity in various growth stages of *H. ulmarius* at different temperature levels

Growth stages (in days)	Per cent loss in viscosity																			
	20±1°C					25±1°C					30±1°C					35±1°C				
	Cx	PG	PMG	PTE	PGTE	Cx	PG	PMG	PTE	PGTE	Cx	PG	PMG	PTE	PGTE	Cx	PG	PMG	PTE	PGTE
Substrate colonization (10)	66.83 (84.52)	45.45 (50.78)	50.87 (60.17)	38.94 (39.50)	49.71 (58.18)	75.44 (93.68)	47.37 (54.13)	52.31 (62.62)	40.78 (42.66)	56.68 (66.57)	70.42 (88.77)	46.83 (53.19)	51.02 (60.43)	39.29 (40.11)	53.43 (64.51)	63.56 (80.17)	43.31 (47.06)	50.08 (58.82)	37.06 (36.32)	51.10 (60.57)
Spawn run (15)	57.75 (71.53)	48.26 (55.67)	53.20 (64.11)	44.23 (48.66)	49.32 (57.51)	59.59 (74.38)	50.16 (58.95)	55.35 (67.67)	45.44 (50.76)	52.19 (62.42)	56.60 (69.70)	50.45 (59.46)	53.43 (64.51)	43.89 (48.07)	51.99 (62.08)	53.43 (64.51)	47.66 (54.63)	52.41 (62.79)	43.55 (47.47)	49.15 (57.22)
Primordia initiation (20)	49.90 (58.50)	51.53 (61.30)	55.60 (68.08)	49.48 (57.79)	48.75 (56.53)	47.97 (55.17)	52.83 (63.49)	58.36 (72.48)	49.96 (58.61)	49.71 (58.18)	45.10 (50.18)	53.91 (65.30)	56.28 (69.19)	48.62 (56.31)	50.51 (59.56)	44.19 (48.58)	51.61 (61.44)	54.74 (66.67)	49.77 (58.29)	47.59 (54.52)
Button stage (25)	44.14 (48.50)	50.58 (59.68)	60.99 (76.49)	55.20 (67.43)	35.26 (33.32)	45.52 (50.91)	53.02 (63.81)	63.68 (80.35)	57.06 (70.43)	44.19 (48.58)	43.12 (46.72)	52.42 (62.81)	62.89 (79.24)	54.66 (66.54)	37.33 (36.77)	39.33 (40.16)	50.58 (59.68)	58.29 (72.37)	54.95 (67.02)	39.62 (40.66)
Young stage (30)	41.11 (43.22)	48.31 (55.76)	64.62 (81.62)	58.45 (72.62)	32.70 (29.18)	42.50 (45.64)	50.69 (59.86)	65.53 (82.84)	60.32 (75.48)	42.11 (44.96)	40.07 (41.44)	50.12 (58.89)	66.86 (84.55)	56.72 (69.89)	35.46 (33.65)	37.41 (36.91)	47.74 (54.78)	62.86 (79.19)	59.40 (74.09)	37.06 (36.31)
Control	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)
CD at 5%	2.123	2.316	2.580	1.922	2.489	2.672	2.922	3.760	2.774	2.677	2.931	3.169	3.784	2.968	2.778	2.526	2.751	3.942	3.102	3.126

Figures in parentheses are angular retransformed values.

Enzymatic activity at 30±1°C:

The activity of cellulase (Cx) in terms of reduction in the viscosity was maximum (70.42 %) at 10 days (substrate colonization stage) of mycelial growth followed by 15 days (56.60 % at spawn run stage). The activity of polygalacturonase (PG) was highest (53.91 %) at 20 days (primordia initiation stage) followed by 25 days (52.42 % at button stage) of mycelial growth in *H. ulmarius.* The activity of polymethyl galacturonase (PMG) in terms of reduction in the viscosity was maximum (66.86 %) at 30 days (young stage) of mycelial growth followed by 25 days (62.89 % at button stage). In case of pectin transeliminase (PTE) the per cent loss in viscosity was also maximum (56.72 %) at 30 days (young stage) of mycelial growth followed by 25 days (54.66 % at button stage). Maximum activity of polygalacturonase transeliminase (PGTE) was observed at 10 days (53.43 % at substrate colonization stag) followed by 15 days (51.99 % at spawn run stage) of mycelial growth.

Enzymatic activity at 35±1°C:

The activity of cellulase (Cx) in terms of reduction in the viscosity was maximum (63.56 %) at 10 days (substrate colonization stage) of mycelial growth followed by 15 days (53.43 % at spawn run stage). The activity of polygalacturonase (PG) was highest (51.61 %) at 20 days (primordia initiation stage) followed by 25 days (50.58 % at button stage) of mycelial growth in *H. ulmarius.* The activity of polymethyl galacturonase (PMG) in terms of reduction in the viscosity was maximum (62.86 %) at 30 days (young stage) of mycelial growth followed by 25 days (58.29 % at button stage). In case of pectin transeliminase (PTE) the per cent loss in viscosity was also maximum (59.40 %) at 30 days (young stage) of mycelial growth followed by 25 days (54.95 % at button stage). Maximum activity of polygalacturonase transeliminase (PGTE) was observed at 10 days (51.10 % at substrate colonization stag) followed by 15 days (49.15 % at spawn run stage) of mycelial growth.

It is concluded that the role of cellulase (Cx) enzyme may be in the substrate colonization, whereas, polygalasturonase and polygalacturonase transeliminase may be in the primordia initiation. Subsequently the role of polymethyl galacturonase and pectin transeliminase may be in the multiplication and enlargement of tissues. It was found that activity of Cx and PGTE enzyme was maximum at 10 days after inoculation while PG at 20 days and PMG and PTE was maximum at 30 days after inoculation in 25°C temperature as compared to 20, 30 and 35 °C.

Nakazawa *et al.* (1974) investigated the cellulolytic enzymes activity in various edible and non-edible fungi and recorded that the enzymes activity was high in culture medium than the fruit bodies. Sharma and Doshi (1994) studied the production of pectinolytic and cellulolytic enzymes during different growth stages of *Phellorinia inquinans* and found that that the activity of polygalacturonase (PG) and polymethyl galacturonase (PMG) was higher in mature fruit bodies while the high activity of pectin-transeliminase (PTE), polygalacturonase transeliminase (PGTE), pectin transeliminase (PTE), polygalacturonase transeliminase (PGTE) and cellulase (Cx) was found at button stage.

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