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Effects of Cultural Conditions on High Temperature Tolerance of *Lentinula edodes* Mycelia

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Abstract: The effects of several cultural conditions on high temperature tolerance of vegetative mycelia of five *Lentinula edodes* strains were investigated. Mycelium of longer culture age (70 days) was significantly shown high temperature tolerance compared to mycelium of shorter culture ages (14 and 30 days) for four strains, whereas SA142 that showed reverse. When the culture plates were pretreated at 30 and 33°C for 48 h incubation before heat treatment (40°C, 8 h), mycelia of *L. edodes* strains were shown high temperature tolerance. Effects of nutritional factors in BM (basic medium) for the high temperature tolerance of *L. edodes* strains were also investigated. While yeast extract (as nitrogen source) and starch (as carbon source) were added to BM media, as a result cultured vegetative mycelia of *L. edodes* strains were significantly shown high temperature tolerance against heat treatment (40°C, 6 and 8 h). Further more, addition of bases (adenine + cytosine), vitamin (biotin) and organic acid (tartaric acid) to BM media, consequently vegetative mycelia of some *L. edodes* strains were also effective to increase high temperature tolerance.

Key words: *Lentinula edodes*, cultural conditions, high temperature tolerance, heat treatment, survival and growth rate

INTRODUCTION

Shiitake, *Lentinula edodes* (Berk.) Pegler is a wood-decaying fungus generally known as black oak mushroom with delicious taste, texture and polyporaceous affinities (Pegler, 1983). Its fruit bodies are rich in minerals, vitamins, essential amino acids (especially lysine and leucine), are high in fiber content but contain less than 10% crude fat (Ho *et al*., 1994; Mizuno, 1995). In addition, a polysaccharide called lentinan (Ikekawa *et al*., 1969) has been extracted from *L. edodes* fruit bodies and found to have pharmacologically useful immunomodulatory, anti-cancer and anti-viral effects (Chihara, 1993; Mizuno *et al*., 1995).

Concerning *L. edodes*, Nakanishi and Yoshitomi (1982) studied the effect of irradiation of direct sunlight on the mycelia of *L. edodes* in bed-logs and reported that the temperature of bed-logs became higher than 40°C as the result of sunlight that is damaging to the mycelium of *L. edodes*. Nakazawa *et al*. (1983) studied the effect of environmental factors, including high temperature, on 51 cultivated and wild *L. edodes* strains of Japan and other countries. Nakazawa and Mori (1988) studied high temperature tolerance of 16 *L. edodes* strains (Japanese: 15 and Papua New Guinea: 1) by two methods and found difference in the tolerance among strains. Shirasaka *et al*. (2006) examined the effect of trehalose on the heat tolerance of *L. edodes* mycelia and reported that trehalose functions were effective against heat treatment (40°C and 39 h).

High temperature stress is one of the most crucial factors that limit the growth and productivity of living organisms and plants (Frova, 1997; Wahid and Shabbir, 2005). Through the metabolic changes, living organisms respond to high temperature stress.

L. edodes is extensively cultivated in Japan, China and Korea and in recent years in USA and European countries (Chang, 1999). It has not been able to cultivate in tropical and sub-tropical countries, for example Bangladesh, because *L. edodes* is a mesophilic fungus and the high temperature limit of its growth is 30-32°C (Nakazawa and Mori, 1988). The average daily maximum

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temperature in Bangladesh is frequently higher than 30°C from March-October and some times the daily maximum temperature in April-May rises up to 40°C and above. To be able to cultivate *L. edodes* in tropical and sub-tropical countries, *L. edodes* has to be able to grow in temperatures near 30°C and have to have an ability to tolerate high temperature stress, like 40°C, for several hours in a day. Therefore, in this study we examined the effects of cultural conditions (culture age of mycelia, pretreatment at relatively high temperature at 30 and 33°C for 48 h incubation before heat treatment and constituents of media) on the survival of *L. edodes* mycelia after heat treatment (40°C for 4 ~ 8 h).

MATERIALS AND METHODS

Strains of *L. edodes*: Four stains of *L. edodes* from Japan (SA22, SA135, SA137, SA142) and one from Papua New Guinea (PNG; SA583, from NBRC: NITE Biological Resource Center) were used in the study. These strains were maintained in the laboratory on glucose-malt-yeast agar (GMYA: glucose 10 g; malt extract 10 g; yeast extract 4 g per liter distilled water and 2% agar).

Cultural condition before heat treatment: Mycelium is grown on 20 mL of potato dextrose agar (PDA: Eiken, Tokyo, Japan) or basic medium (BM: glucose 25 g, ammonium sulfate 1.44 g, $MgSO_4 \cdot 7H_2O$ 0.5 g, KH_2PO_4 1.0 g, Na_2CO_3 1.12 g, fumaric acid 1.32 g, $FeSO_4 \cdot 7H_2O$ 1 mg, $ZnSO_4 \cdot 7H_2O$: 1 mg, $MnSO_4 \cdot 4H_2O$: 0.8 mg, thiamine HCl: 200 μg, agar powder 21 g per liter distilled water and pH 4.0) (Leonian and Lilly, 1938) in a 90 mm petri plate culture at 25°C in the dark. GMY (GMYA without 2% agar) liquid medium (7 mL) containing test tube (18 mm diameter) was used in the water bath heat treatment.

Culture age and pre-treatment: In order to find out the effect of culture age of mycelia on high temperature tolerance of *L. edodes*, the strains were cultured on 20 mL of PDA medium in a 90 mm petri dish with three culture ages i.e. 14, 30 and 70 days. Then cultures were incubated in the dark at 25°C for respective culture ages. After incubation, mycelial plugs from each culture were subjected to heat treatment (40°C, 4, 6 and 8 h). Similarly, to survey the effect of pretreatment at relatively high temperature, strains were firstly cultured on PDA petri dishes at 25°C for 28 days after which cultures were divided into three temperature groups of (i) 25°C, (ii) 30°C and (iii) 33°C for additional 48 h incubation before heat treatment. Mycelial plugs were subjected to heat treatment (40°C, 8 h) in GMY liquid media.

Medium compositions (modified BM media were also prepared by changing carbohydrate or nitrogen sources): Carbohydrate sources such as sucrose, trehalose, glycerol, starch and cellulose (25 g L^{-1} each) were used instead of glucose to prepare modified basic media for testing the effects of carbohydrate sources under the heat treatment experiments. Consequently, casamino acids, yeast extracts, polypeptone, methionine, proline, glutamic acid and calcium nitrate tetrahydrate [$Ca(NO_3)_2 \cdot 4H_2O$] were used separately to replace ammonium sulfate in BM to test the effects of nitrogen substances in the heat treatment experiments at 40°C water bath. Instead of ammonium sulfate, other nitrogen sources were used in BM and the amount was determined by the equation of $X = 2 \times 1.44\ n^{-1} \times (Mx\ Mams^{-1})$ (X: amount of other nitrogenous substance in 1 L; Mx: mole. wt. of other nitrogen substance; Mams: mole. wt. of ammonium sulfate; n: number of nitrogen in other nitrogen substance).

Furthermore, bases, vitamins and organic acids were added to BM media as follows: Adenine (20 mg L^{-1}), Cytosine (20 mg L^{-1}), [Adenine (10 mg L^{-1}) + Cytosine (10 mg L^{-1})] were added to BM media individually. Similarly, ascorbic acid (2.5 g L^{-1}), biotin (10 μg L^{-1}) and riboflavin (200 μg L^{-1}) were added to BM separately. And each of 0.65 g L^{-1} of citric acid, tartaric acid and D-glucuronic acid were added to BM media individually.

The prepared media were autoclaved (Hirayama, HA-300M) at 121°C for 20 min. About 20 mL of above mentioned each modified BM media was poured in a 90 mm petri plate then inoculated the strains and incubated in the dark at 25°C for 11-15 days. After incubation, mycelial plugs from each culture were subjected to heat treatment at 40°C for 6 and 8 h. In every cases, glucose BM was used as control.

Heat treatment and investigation of mycelial survival and growth: From the periphery of petri dishes, mycelial colony of respective cultures (11-15 days at 25°C) were punched out by a cork borer (5 mm diameter) concentrically and immersed into the bottom of GMY liquid medium (7 mL) of test tubes (18 mm diameter). Then mycelium plugs containing test tubes were subjected to heat shock treatment at 40°C controlled water bath (Taitec, Personal-11) for 4-8 h depending on the kind of experiment. Then one mycelial plug was used for inoculation in 20 mL of PDA petri dish (90 mm diameter) with 5 replicates and incubated at 25°C in the dark for 30 days to observe mycelial growth. Visualization of continuous hyphal growth from the heat-treated mycelial plugs were determined as viable or survival. Growth measurements were performed at 3 days intervals. The initial growths of mycelial plugs were also checked and

recorded. Degrees of high temperature tolerance were determined by the survival rate and the growth rate.

Statistical analysis: All the obtained data were analyzed by one way analysis of variance (ANOVA) by using the statistical package program (SPSS 12.0 for Windows). A test of significant differences was determined by LSD (least-significant difference) at ($p<0.05$).

RESULTS AND DISCUSSION

Effect of the culture age of mycelium: Effect of culture ages (14, 30 and 70 days) on the survival of *L. edodes* mycelia after heat treatment (40°C for 4, 6 and 8 h) is shown in Fig. 1. In the case of culture age of mycelium (14 days) and heat treatment (40°C, 4 h), the survival rates of all strains were 100%, except SA583 (0%) indicating that SA583 was the weakest strain among the five strains of *L. edodes*. Only strain with sufficient survival rate in 8 h heat treatment was SA142 (survival rate of 80%). Considering growth rate, SA135 was the highest (5.69 mm), after which SA142, SA22 and SA137 were followed, respectively (Table 1).

After 8 h heat treatment, survival rates of longer culture age (70 days) mycelia of SA22, SA135 and SA137 were 100%. But survival rates of above-mentioned mycelia on younger culture ages (14 and 30 days) were lower (0-80%). Since 8 h heat treatment was severe for every culture ages of mycelia (SA583), therefore, 6 h heat treatment was used to determine the effect of culture ages of mycelia on high temperature tolerance. So, longer culture age (70 days) of SA583 mycelia could survive 60%, whereas survival rates of younger culture ages of mycelia had no survival. On the contrary, after 8 h heat treatment survival rate of younger culture age (14 days) of SA142 was 80%, while longer culture ages (30 and 70 days) were 0-20%.

Test of significance indicated that longer culture age of mycelia (70 days) for four *L. edodes* strains showed significant high temperature tolerance compared to shorter culture ages of mycelia whereas only SA142 mycelia of 14 days culture age showed significant high temperature tolerance than longer culture ages of mycelia (Fig. 1).

High temperature stress limits the growth rate, wherein growth rates are higher in short period heat treatment strains compared to long 8 h strains in two youngest culture period groups (14 and 30 days). In case of older culture (70 days) growth rates of 3 strains (SA22, SA135 and SA137) showed similar rates between shorter to longer time length heat treatments, whereas SA142 and SA583 mycelia failed to show any similarities between lengths of heat treatment (Table 1).

The effect of culture age was different between the strain SA142 and other four strains of *L. edodes*. SA142 was more high temperature tolerant during early culture stage, whereas other four strains were more tolerant during late culture stage. One possible explanation for the result is that different mechanisms may be involved between the two groups, but specific mechanisms are unknown.

Effect of pre-treatment of mycelia: After heat treatment (40°C, 8 h), the survival rates of 4 strains (SA137, SA142, SA135 and SA22) were 100% those cultures were pre-treated at 30 and 33°C temperature. On the other hand, survival rates of SA583 mycelia were 60 and 40% while cultures were pre-treated at 30 and 33°C temperature, respectively. But all strains of 25°C pre-treatment (control) showed no survival after heat treatment (Table 2). While checking the growth of mycelial plugs, after heat treatment (40°C, 8 h), all strains of 33°C pre-treated mycelia were started growth earlier than the pre-treated mycelia of 30°C (Table 2).

Test of significance showed that there was no significant difference between the survival rate of the strains under 30 and 33°C pre-treatment, whereas growth rate showed significant differences ($p<0.01$) among the four *L. edodes* strains under different pretreatments, whereas SA583 strain had no significant difference (Table 2). The effect of pretreatment at relatively high temperature (30 or 33°C) was very clear for all five strains of *L. edodes*. Forty-eight hours of incubation at 30 and 33°C temperatures was sufficient to induce high temperature tolerance, even that the survival rate of the weakest strain (SA583) was only 40-60% after the heat treatment. Observed results are consistent with the previously reported results of the influence of heat treatment in *S. cerevisiae* (Lewis *et al*., 1995) and *Lactobacillus delbrueckii* ssp. *bulgaricus* (Gouesbet *et al*., 2001).

Effect of carbohydrate source: Effect of carbohydrate source in BM media on the survival of *L. edodes* mycelia after heat treatment (40°C, 6 and 8 h) is shown in Fig. 2. Mycelia cultured on starch containing BM media and after 8 h heat treatment, the survival rates of SA137, SA22 and SA142 were 80% and above, while survival of SA135 and SA583 had 20 and 0%, respectively. The growth rate of SA22 was the highest (2.59 mm) and SA135 was the lowest (0.69 mm) (Table 3). And growths of above mentioned strains were started between 8 to 9.5 days after incubation followed by heat treatment.

On high temperature tolerance of *L. edodes* mycelia, after 8 h heat treatment, significant differences were observed between starch containing and control medium

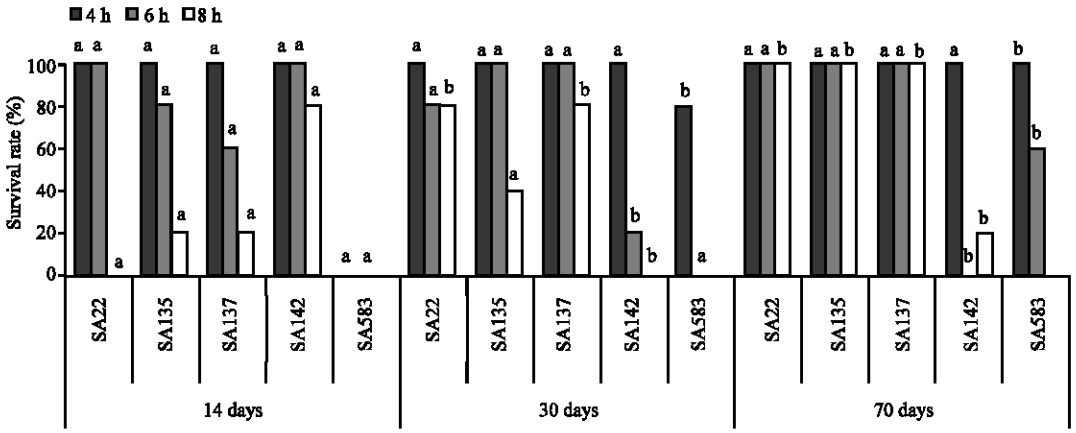

Fig. 1: Effect of culture ages (14, 30 and 70 days) on the survival of *L. edodes* mycelia after heat treatment (40°C, 4, 6 and 8 h), Significant differences among the survival rates of same strain on different culture ages of mycelia were analyzed using one way ANOVA followed by LSD (least-significant difference) multiple comparison. Values with different letters are significantly different at $p<0.05$

Table 1: Effect of culture ages (14, 30 and 70 days) on the mycelial growth of *L. edodes* after heat treatment (40°C for 4, 6 and 8 h)

	Growth rates after heat treatment								
	Culture age 14 days			Culture age 30 days			Culture age 70 days		
Strains	4 h	6 h	8 h	4 h	6 h	8 h	4 h	6 h	8 h
SA22	5.14±0.14	3.89±0.07	-	5.49±0.27	3.95±1.03	3.26±0.77	5.78±0.27	5.15±0.15	4.49±0.15
SA135	5.69±0.30	3.52±0.89	0.63±0.64	5.20±0.01	4.99±0.06	1.82±1.12	4.67±0.20	5.15±0.10	4.80±0.03
SA137	4.21±0.16	2.44±1.00	0.70±0.70	4.71±0.22	3.68±0.12	2.64±0.67	5.04±0.12	4.44±0.20	3.67±0.12
SA142	5.57±0.36	3.79±0.83	3.00±0.76	3.88±0.26	0.53±0.53	-	6.24±0.18	-	1.03±1.03
SA583	-	-	-	2.78±0.80	-	-	4.28±0.01	1.15±0.59	-

Values are expressed as the mean±SE of mycelial growth measurements (mm) after heat treatment, - : No growth

Table 2: Effect of pretreatment at relatively high temperature on the growth and survival of *L. edodes* mycelia after heat treatment (40°C, 8 h)

	Growth								
	25°C pretreatment			30°C pretreatment			33°C pretreatment		
Strains	Rate (mm)	Started after (days)	Survival rate (%)	Rate (mm)	Started after (days)	Survival rate (%)	Rate (mm)	Started after (days)	Survival rate (%)
SA22	-	-	0	4.47±0.16[a]	5.4±0.40	100	5.18±0.05[b]	2.4±0.24	100
SA135	-	-	0	5.24±0.26[a]	3.6±0.24	100	6.44±0.03[b]	2.0±0.00	100
SA137	-	-	0	3.37±0.29[a]	9.0±1.26	100	4.26±0.05[b]	4.4±0.24	100
SA142	-	-	0	4.36±0.22[a]	7.4±0.98	100	5.77±0.13[b]	2.8±0.20	100
SA583	-	-	0	1.18±0.62[a]	18.7±3.84	60	1.33±0.81[b]	11.0±0.00	40

Values are expressed as the mean±SE of mycelial growth measurements (mm) and growth initiated after incubation (days) followed by heat treatment. Values followed by the different letter(s) in rows are significantly different ($p<0.01$), - : No growth

Table 3: Effect of carbohydrate sources BM media on the growth of *L. edodes* mycelia after heat treatment (40°C, 6 h and 8 h)

		Growth rate after heat treatment					
Strain	HT[c]	Glucose[b]	Cellulose	Glycerol	Starch	Sucrose	Trehalose
SA22	6 h	2.39±0.42	3.64±0.06[a]	-[a]	3.60±0.12[a]	3.30±0.24[a]	3.41±0.19[a]
SA135		-	0.44±0.44	-	2.83±0.85[a]	-	-
SA137		0.37±0.37	1.56±0.54[a]	-	3.10±0.06[a]	0.97±0.48	0.18±0.18
SA142		2.19±0.89	-[a]	0.70±0.70[a]	3.52±0.09[a]	3.20±0.09	-[a]
SA583		-	-	-	-	-	0.70±0.70
SA22	8 h	-	1.62±0.72[a]	-	2.59±0.65[a]	-	-
SA135		-	-	-	0.69±0.69	-	-
SA137		-	-	-	2.42±0.18[a]	-	-
SA142		-	-	-	2.27±0.60[a]	-	-
SA583		-	-	-	-	-	-

Values are expressed as the mean±SE of mycelial growth measurements (mm); [a]: Significantly different from control: $p<0.05$; [b]: Carbohydrate source was used in control BM media; [c]: Heat treatment (hour), - : No growth

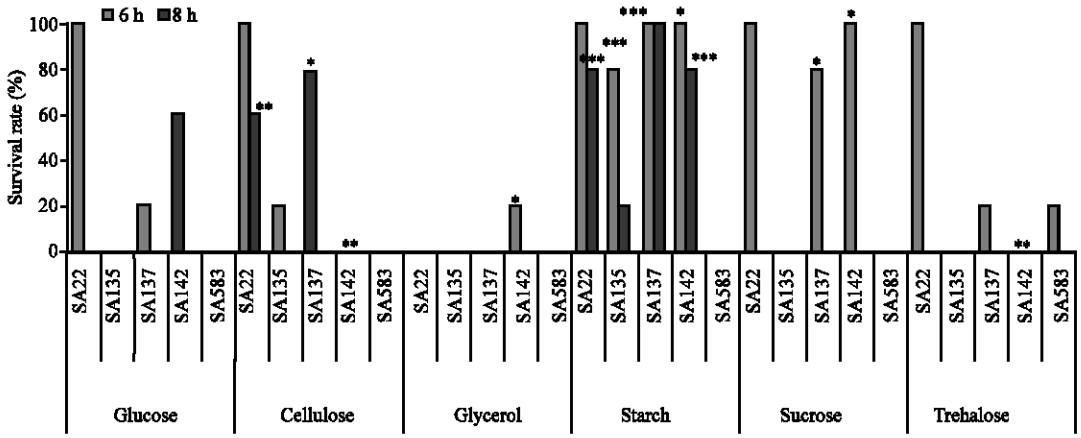

Fig. 2: Effect of carbohydrate supplemented media on the survival of *L. edodes* mycelia after heat treatment (40°C, 6 and 8 h), Significance level against glucose (control), *: Significant at 5%; **: Significant at 1%; ***: Significant at 0.1%

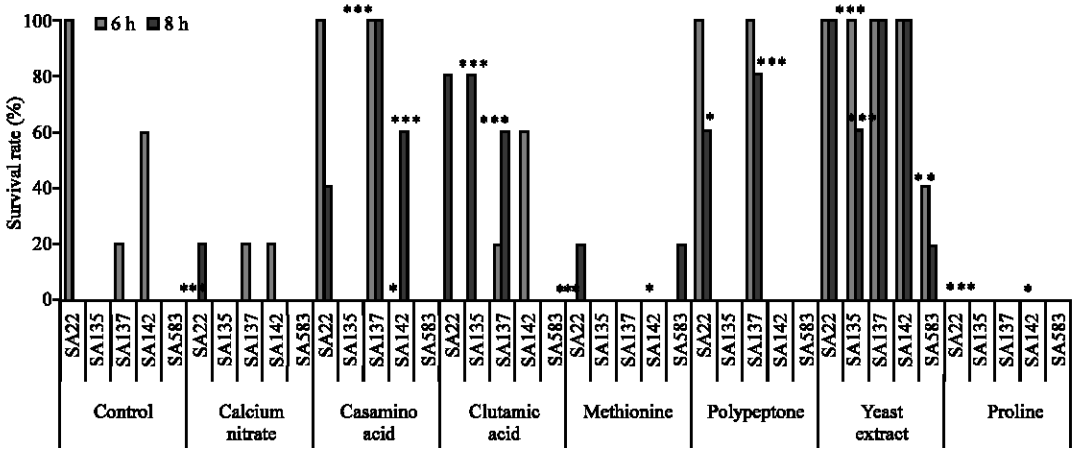

Fig. 3: Effect of nitrogen supplemented media on the survival of *L. edodes* mycelia after heat treatment (40°C, 6 and 8 h), Significance level against control (ammonium sulfate), *: Significant at 5%; **: Significant at 1%; ***: Significant at 0.1%

for SA137, SA22 and SA142 strains, whereas SA583 failed to survive. Consequently, mycelia of SA137 and SA142 cultured on sucrose containing media, significantly enhanced high temperature tolerance up to 6 h and mycelia (SA137) cultured on cellulose medium significantly enhanced high temperature tolerance up to 6 h compared to the glucose BM (control). Trehalose and glycerol containing media were not effective to improve high temperature tolerance of *L. edodes* in this study. But Shirasaka *et al.* (2006) reported that trehalose functions had positive role to protect *L. edodes* mycelia against heat treatment (39°C, 48 h), since glass transition of the trehalose never occurs below 65°C (Akao *et al.*, 2001), so the sugar remained in the glassy state during the 39°C incubation. One factor may be the difference of our result that is cultural medium or different condition. On the other hand, Matsuo (1950) reported that starch was the best carbon source for the growth of *L. edodes*. Similarly, Khan *et al.* (1991) reported that *L. edodes* grew best with starch among four carbon sources tested. Ohta (1997) reported that several mychorrhizal fungi including *Lyophyllum shimeji* (Kawamura) Hongo showed good growth in a medium containing starch. These results indicate that starch is a good component of media for growth of mushrooms. The result of this study shows further that starch containing basic media is effective to increase high temperature tolerance of *L. edodes*.

Effect of nitrogen source: Effect of nitrogen sources media on the survival of *L. edodes* mycelia after heat treatment is shown in Fig. 3. Considering yeast extract

Table 4: Effect of nitrogen sources BM media on the growth of *L. edodes* mycelia after heat treatment (40°C, 6 h and 8 h)

		Growth rate after heat treatment							
Strain	HT[c]	Ammonium sulfate[b]	Calcium nitrate tetrahydrate	Casamino acids	Glutamic acid	Methionine	Polypeptone	Proline	Yeast extract
SA22	6 h	2.29±0.42	-	3.42±0.20[a]	2.49±0.67	-[a]	3.94±0.10[a]	-[a]	4.12±0.03[a]
SA135		-	-	-	3.08±0.84[a]	-	-	-	3.80±0.35[a]
SA137		0.37±0.37	0.02±0.02	3.38±0.12[a]	0.56±0.56[a]	-	2.95±0.16[a]	-	3.16±0.05[a]
SA142		2.19±0.89	0.72±0.72	-[a]	1.24±0.63	-	0.74±0.74	-[a]	4.39±0.26[a]
SA583		-	-	-	-	-	-	-	1.73±1.06[a]
SA22	8 h	-	0.60±0.60	0.98±0.65	-	0.73±0.73	1.81±0.74[a]	-	4.18±0.05[a]
SA135		-	-	-	-	-	-	-	2.26±0.94[a]
SA137		-	-	3.06±0.06[a]	1.51±0.68[a]	-	1.77±0.59[a]	-	2.91±0.18[a]
SA142		-	-	1.82±0.75[a]	-	-	0.57±0.57	-	3.84±0.19[a]
SA583		-	-	-	-	0.29±0.29	0.59±0.59	-	0.85±0.85

Values are expressed as the mean±SE of mycelial growth measurements (mm); [a]: Significantly different from control ($p<0.05$); [b]: Nitrogen source was used in control BM media; [c]: Heat treatment (hour); - : No growth

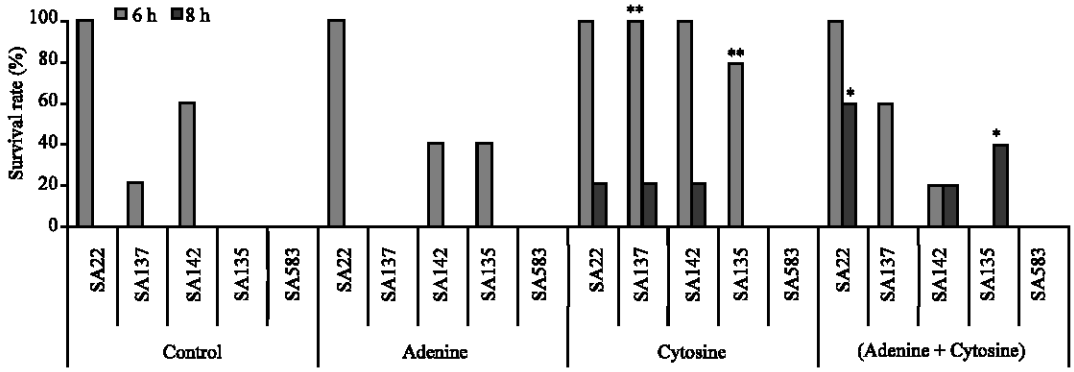

Fig. 4: Effect of nucleic acid supplemented media on the survival of *L. edodes* mycelia after heat treatment (40°C, 6 and 8 h), Significance level against control (basic medium), *: Significant at 5%; **: Significant at 1%

containing media and heat treatment (40°C, 6 and 8 h), the survival rates of 3 strains SA142, SA22 and SA137 were 100%, whereas SA135 and SA583 strains had 60 and 20%, respectively. The growth rate of SA142 was the highest (4.39 mm) followed by SA22 after 6 h heat treatment, but after 8 h heat treatment the highest growth rate (4.18 mm) was observed for SA22 followed by SA142 (Table 4). After 6 and 8 h heat treatment, mycelial growths of SA22 were started about 3 days earlier than the mycelial growths of SA142.

Test of significance showed that after 6 h heat treatment, yeast extract containing medium significantly enhanced high temperature tolerance of SA137, SA142 and SA583 strains compared to control, while there were no significant differences for SA22 and SA135 strains. On the other hand, after 8 h heat treatment, except SA583, other four strains of *L. edodes* mycelia significantly promoted high temperature tolerance compared to control. Similarly, mycelia (SA137 and SA142) cultured on casamino acids and mycelia (SA22 and SA137) cultured on polypeptone supplemented BM media significantly demonstrated high temperature tolerance compared to control after 8 h heat treatment. Considering glutamic acid supplemented media, mycelia of SA137 significantly showed high temperature tolerance compared to control after 8 h heat treatment. On the other hand, after 6 h heat treatment, SA142 and SA22 mycelia cultured on methionine and proline supplemented media had significant lower effect compared to the control. Similarly, mycelia of SA22 cultured on calcium nitrate tetrahydrate supplemented BM media also had significantly lower effect on high temperature tolerance compared to the control. The results indicated that different nitrogen sources might have different effects on metabolic activities during fungal growth and high temperature stress condition.

Since yeast extract containing BM media showed very good result for enhancement of high temperature tolerance of *L. edodes* mycelia, but methionine was not effective. Fries (1953) reported that yeast extract and methionine were effective to increase growth in high temperature for *C. fimetarius*. Kurtz (1958) reported that

climatic ills of temperature sensitive mutants of *Neurospora* were cured by the addition of adenine. As yeast extract contains sufficient nucleic acids, it supports our observation.

Effect of base: After 8 h heat treatment, mycelia (SA22 and SA135) cultured on (adenine + cytosine) containing BM media significantly ($p<0.05$) enhanced high temperature tolerance compared to control. Similarly, after 6 h heat treatment, mycelia (SA137 and SA135) cultured on cytosine supplemented BM media significantly ($p<0.01$) enhanced high temperature tolerance compared to control (Fig. 4).

Purine and pyrimidine are important compounds that enter into the composition of nucleic acids. Haruhiko (1967) reported that alone or in any combination of two substances purine-adenine and pyrimidine-cytosine enhanced the growth of *L. edodes* mycelium very much. It is possible that these substances increase growth and vigority of mycelia, which further promote the resistance to high temperature stress.

Effect of vitamin source: After heat stress (40°C, 6 h), survival rates of mycelia cultured on biotin-supplemented BM media of SA22, SA135 and SA142 were 100%, whereas SA137 and SA583 had no survival. But after 8 h heat stress, only SA22 and SA142 mycelia were survived 40%, whereas rest of 3 strains failed to survive. Mycelia cultured on ascorbic acid supplemented BM media of SA135, SA142 and SA137 were survived 20% each after 8 h heat stress, whereas SA137 and SA142 failed to survive (data not shown). Mycelia of SA137 and SA142 had no survival rate after 6 h heat tress too.

Test of significance showed that after 6 h heat treatment, mycelia of SA135 cultured on biotin and ascorbic acid supplemented BM media significantly enhanced high temperature tolerance compared to control. And mycelia of SA142 cultured on ascorbic acid containing media had significant lower effect compared to control. Haruhiko (1967) clarified and reported that *L. edodes* required thiamine for the growth of mycelium. Addition of vitamin mixture supported growth only when thiamine was present. On the effect of vitamin mixtures, he also reported that only ascorbic acid worked together for the increasing growth in the presence of thiamine, but also biotin was effective. The result of this study shows further that biotin and ascorbic acid are effective to increase high temperature tolerance of *L. edodes*.

Effect of organic acid source: After 6 h heat stress, mycelia of SA22 cultured on each organic acid added BM media were survived 100%, but after 8 h heat stress only mycelia of SA22 cultured on tartaric acid significantly survived (80%) compared to control (data not shown).

The effects of organic acids on the growth of fungi have been studied by several researchers (Burkholder and Mcveigh, 1940; Leonian and Lilly, 1940; Jennison *et al.*, 1955). Jennison *et al.* (1955) reported that growth of wood rotting basidiomycetes grew in ammonium chloride medium with succinic acid, but not without it. Haruhiko (1967) worked on *L. edodes* using 12 organic acids and reported that organic acids were hardly utilized on the mycelial growth of *L. edodes* and growth was increased when fumaric acid, tartaric acid and citric acid were added to a medium with glucose. The results indicate that tartaric acid is a good component of media for the growth of mushrooms. The result of this study shows that tartaric acid containing media is effective to increase high temperature tolerance of *L. edodes*.

From this study, it was clearly shown that cultural conditions significantly enhanced high temperature tolerance on the survival of *L. edodes* vegetative mycelia after heat treatment. Carbohydrates are important carbon and energy sources for cultured cells and nitrogen sources might have various effects on metabolic activities during fungal growth. By means of metabolic changes, living organisms respond to high temperatures. Under stress condition accumulation of compatible solutes of low molecular weight, such as glycinebetaine, sugars, polyols and amino acids have been suggested to be a major mechanism by which plants acclimate to various stresses (Papageorgiou and Murata, 1995; Yancey *et al.*, 1982). Similarly, production of HSP (heat shock proteins) (Lindquist, 1986; Parsell and Lindquist, 1993), sugars and polyols (Managbanag and Torzilli, 2002) have been reported to be the mechanism of high temperature tolerance of many organisms including fungi. Therefore, accumulation of above compounds may be involved in vegetative mycelia as nutrients of carbon and nitrogen sources directly linked with cell proliferation and metabolite biosynthesis (Zou, 2005) by which *L. edodes* strains adjusted to high temperature tolerance.

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