KMITL Sci. Tech. J. Vol. 12 No. 1 Jan. - Jun. 2012

The Production of 5´-nucleotides by Commercial Mushrooms Cultivated in Liquid Extracts of Water Hyacinth

Sakrin Boonlum[1]*, Oratai Sukcharoen[2] and Dusanee Thanaboripat[3]

[1]Scientific Instrument Centre, Faculty of Science, King Mongkut's Institute of Technology Ladkrabang, Bangkok, Thailand
[2]Department of Biotechnology, Faculty of Science, Ramkhamhaeng University, Bangkok, Thailand
[3]Department of Biology, Faculty of Science, King Mongkut's Institute of Technology Ladkrabang, Bangkok, Thailand

Abstract

Three specialty mushrooms produced commercially in Thailand, including Abalone mushroom No. 03 (*Pleurotus cystidiosus* O. K Miller), Hed Kon mushroom No. 02 (*Lentinus squarrosulus* Mont.) and Teenpok mushroom (*Lentinus sajor-caju*) were cultivated in four liquid media, i.e. PDB, media contained liquid extracts of water hyacinth leaves at 20, 30 and 40 g l^{-1} in order to select the most suitable medium for the production of flavor enhancing 5´-nucleotides. The results showed that the maximum 5´-nucleotides of 15.09 and 14.97 mg g^{-1} dry weight were produced from Teenpok mushroom cultivated in media containing liquid extracts of water hyacinth leaves at 30 and 40 g l^{-1}, respectively. The maximum 5´-nucleotides of 12.75 mg g^{-1} was produced from Hed Kon mycelia cultivated in PDB.

Keywords: *Lentinus sajor-caju, Lentinus squarrosulus, Pleurotus cystidiosus*, water hyacinth, 5´ nucleotide

1. Introduction

Five basic tastes have been identified as saltiness, sourness, sweetness, bitterness and savouriness (umami). In Japan, the term "umami" is used for savoury taste, whose character literally means delicious. The umami taste or palatable taste has characteristic qualities that differentiate it from other taste, including a taste-enhancing synergism between two umami compounds, L-glutamate and 5'-nucleotides such as inosinate and guanylate. Glutamate and nucleotides are present in many foods and play important roles in the taste, palatability and acceptability of foods. This distinctive taste was first discovered in 1908 by Ikeda [1]. In many foods, either a high concentration of glutamates or of the 5'-nucleotides such as inosine-5'-monophosphate (IMP) and guanosine-5'-monophosphate (GMP) are present [2-4]. However, both glutamate and nucleotides occur in some dried seaweed [3], in the cultivated mushroom *Agaricus bisporos* [5] and in dried shiitake mushrooms [3]. Fruits generally contains little glutamate, IMP or GMP [3, 4].

*Corresponding author: Tel: 662-329-8000 Fax: 662-329-8419
E-mail: kbsakrin@kmitl.ac.th

Water hyacinth (*Eichhornia crassipes*), a fast growing aquatic weed widely distributed throughout the world, can cause infestations over large areas of water resources and consequently lead to a series of problems such as reduction of biodiversity, blockage of rivers and drainage system, depletion of dissolved oxygen, alteration of water chemistry, and involvement in environmental pollution. Therefore, attempts have been made to prevent the spread of, or eradication of, water hyacinth. On the other hand, much attention has been focused on the applications of water hyacinth in industries and agriculture since the supply of water hyacinth is almost unlimited and cost-free[5,6]. It can be used as an alternative substrate for oyster mushroom cultivation [7-10].

Mushrooms are commonly used as food and food flavoring substance and also as traditional medicines [11]. The typical flavor of mushrooms consists of nonvolatile components including 5' - nucleotides, free amino acids and soluble sugars and sugar alcohols (polyols) [12]. Common mushroom or button mushroom (*Agaricus bisporus* (Lange) Imbach) and paddy straw mushroom (*Volvariella volvacea*) were the two mushroom with the most umami taste [13]. Nonvolatile test components of several specialty mushrooms, commercial mushrooms, medicinal mushrooms and canned mushrooms were examined [14-17]. It was observed that some mushrooms contained higher amount of taste components than the others. In Thailand, many edible mushrooms including abalone mushroom (*Pleurotus cystidiosus* O.K Miller), Hed Kon mushroom (*Lentinus squarrosulus* Mont.) and Teenpok mushroom (*Lentinus sajor-caju*), are produced commercially as food. In order to add more value to edible mushroom, cultivating them as filamentous fungi for food enhancer is another option. Therefore, the objective of this research was to cultivate edible mushrooms as filamentous fungi in liquid media using extracts of water hyacinth leaf as substrate for nucleotide production.

2. Materials and Methods

2.1 Mushrooms

Pure cultures of Abalone mushroom no. 03 (*Pleurotus cystidiosus* O.K Miller), Hed Kon mushroom isolate no. 02 (*Lentinus squarrosulus* Mont.) and Teenpok mushroom (*Lentinus sajor-caju*) were purchased from Thailand Mushroom Culture Collection Centre, Bangkok, Thailand. The mycelia were maintained on Potato Dextrose Agar (PDA, Difco Laboratories, Sparks, MD) at 30±2°C and subcultured every 7 days. Mycelial broth was prepared by inoculating mycelial culture into 5 ml sterile distilled water.

2.2 Preparation of liquid extracts of water hyacinth leaves

Fresh water hyacinth plants were sampled from large floating masses in Khlong Prawet Buri Rom, Ladkrabang district, Bangkok, Thailand. The plants were thoroughly washed with distilled water; mature leaves were dried at 60 °C in hot air oven for 16 h, and then ground to powder (100 mesh) using a blender. Three concentrations of water hyacinth leaf extracts were prepared using the powder: 20 g l^{-1}, 30 g l^{-1} and 40 g l^{-1}. Each preparation was heated at 100°C for 10 minutes, and then filtered through whatman paper no.1. After filtration, 0.5 g l^{-1} of $MgSO_4$ and 2.5 g l^{-1} of KH_2PO_4 were added to each of the preparations and final pH was adjusted to 5.5 using HCl; 100 ml of each concentration were added into 250 ml flasks, and then autoclaved at 121 °C for 15 min [18].

2.3 Mycelial cultivation

One ml of mycelial broth of each isolate was inoculated into 250 ml flasks containing 100 ml of four liquid media i.e. PDB, liquid extracts of water hyacinth leaves at 20, 30 and 40 g l^{-1} and incubated at 30±2°C for 7 days at 120 rpm. After 7 days of incubation, these mycelia were harvested by filtering through whatman paper no.1. The mycelial mass were washed twice with distilled water and filtered before freezing at -20 °C prior to further analysis.

2.4 Assay of 5′-nucleotides

5′-nucleotides were analysed using a method modified from Taylor *et al*. [19]. The mycelial samples kept at -20°C were frozen with liquid nitrogen, and then ground using an agate mortar and pestle, with the addition of 10 ml distilled water; then 1 ml of aqueous extract was centrifuged at 12,000 rpm for 15 min. The aqueous extract was filtered through 0.45µm filter paper prior to injection into a high-performance liquid chromatograph (HPLC) (Platinum EPS C18, 5µm, 150 x 4.6 mm Column) using the following conditions for analysis: mobile phase (2.0 M KH_2PO_4/H_3PO_4), pH 4.0, flow rate of 0.7 ml/min, and UV detection at 254 nm. Each 5′-nucleotide was quantified by the calibration curve derived from the standard 5′-nucleotide: disodium-5′-guanosine monophosphate (5′-GMP), disodium-5′-inosine monophosphate (5′-IMP) and disodium-5′-xanthosine monophosphate (5′-XMP).

2.5 Statistical analysis

Three replicates of samples were used and the experimental data were subjected to an analysis of variance (ANOVA) using a completely random design. Duncan's New Multiple Range Test (DMRT) was used for mean comparison to determine the least significant difference at 0.05 ($P \leq 0.05$).

3. Results and Discussion

3.1 Growth of mycelial mushroom in four liquid media under shake flask conditions

Abalone mushroom no. 03, Hed Kon mushroom no.02 and Teenpok mushroom were cultivated in four liquid media i.e. PDB, liquid extracts of water hyacinth leaves at different concentrations (20, 30 and 40 g l^{-1}) under shake flask condition for 7 days. The results showed that these mushrooms cultivated in PDB gave higher dry weight (g l^{-1}) than in liquid extracts of water hyacinth leaves (Table 1).

3.2 Production of 5′-nucleotides

The amount of total 5′-nucleotides (5′-GMP, 5′-IMP and 5′-XMP) produced by three mushrooms were calculated in both mg g^{-1} dry weight and mg l^{-1} medium. From Table 2, the maximum production of 5′-nucleotides in mg g^{-1} dry weight was produced from Teenpok mushroom cultivated in liquid extracts of water hyacinth leaves at 30 and 40 g l^{-1} followed by Hed Kon no.02 cultivated in PDB with the amounts of 15.09, 14.97 and 12.75 mg g^{-1} dry weight, respectively. When the amount of 5′-nucleotides were calculated in mg l^{-1} medium, it was found that Hed Kon no.02 cultivated in PDB gave the highest yield of 51.43 g l^{-1} followed by Teenpok mushroom cultivated in liquid extracts of water hyacinth leaves at 40 g l^{-1} and in PDB with the amounts of 37.11 and 32.01 mg l^{-1}, respectively (Table 3).

Flavour 5′-nucleotides were found to be disodium-5′-guanosine monophosphate (5′-GMP), disodium-5′-inosine monophosphate (5′-IMP) and disodium-5′-xanthosine monophosphate (5′-XMP) [20]. Liquid fermentation methods have been reported to be an important means of producing more uniform mycelia biomass from several types of medicinal mushroom for product extraction and purification [21]. Contents of flavour 5′-nucleotides could be divided into three

ranges: low (<1 mg g^{-1}), middle (<1-5 mg g^{-1}) and high ranges (<5 mg g^{-1}) and the content of 5′-nucleotides in mushroom mycelia was in the high range whereas that in fruit bodies was in the low range [22]. Huang *et al*. [23] found that the contents of total and flavor 5′-nucleotides were high in mycelia *of Cordyceps militaris* (26.27 and 9.34 mg g^{-1}). In addition, Nageswaran *et al*. [24] reported that the substrate of 25% water hyacinth in combination with 74% paddy straw gave higher oyster mushroom production than paddy straw alone. Marcelo *et al*. [25] found that wood waste and agricultural waste are important inputs in the production of mushroom especially the edible mushroom. These findings are similar to our finding that mushroom mycelia produced in liquid extracts of water hyacinth gave high amount of 5′-nucleotides.

Table 1 The mycelial dry weights (g l^{-1}) of Abalone mushroom no. 03 (*Pleurotus cystidiosus* O.K Miller), Hed Kon mushroom no. 02 (*Lentinus squarrosulus* Mont.) and Teenpok mushroom (*Lentinus sajor-caju*) under shake flask conditions.

Mushrooms	Dry weight (g l^{-1})*			
	PDB	Liquid extract of water hyacinth (20 g l^{-1})	Liquid extract of water hyacinth (30 g l^{-1})	Liquid extract of water hyacinth (40 g l^{-1})
Abalone no. 03	1.51±0.06^{a}	0.45±0.02^{c}	0.57±0.00^{b}	0.62±0.02^{b}
ed Kon no. 02	4.03±0.03^{a}	1.36±0.06^{d}	1.75±0.05^{c}	2.22±0.02^{b}
Teenpok	3.70±0.08^{a}	1.07±0.07^{d}	1.92±0.05^{c}	2.48±0.09^{b}

* Means with different letters differ significantly at $P \leq 0.05$

Table 2 Content of total 5′-nucleotides (mg g^{-1} dry weight) in mycelia of Abalone mushroom no. 03, Hed Kon mushroom no. 02, and Teenpok mushroom under shake flask conditions.

Mushrooms	5′-nucleotides (mg g^{-1} dry weight)*			
	PDB	Liquid extract of water hyacinth (20 g l^{-1})	Liquid extract of water hyacinth (30 g l^{-1})	Liquid extract of water hyacinth (40 g l^{-1})
Abalone no. 03	0.58±0.02^{d}	10.93±0.57^{a}	8.34±0.10^{c}	9.75±0.31^{b}
Hed Kon no. 02	12.75±0.08^{a}	5.35±0.25^{c}	6.47±0.18^{b}	6.54±0.05^{b}
Teenpok	8.65±0.19^{b}	7.57±0.51^{c}	15.09±0.47^{a}	14.97±0.55^{a}

* Means with different letters differ significantly at $P \leq 0.05$

Table 3 Content of total 5'-nucleotides (mg l^{-1}) in mycelia of Abalone mushroom no. 03, Hed Kon mushroom no. 02, and Teenpok mushroom under shake flask conditions.

Mushroom	5'-nucleotides (mg l^{-1})			
	PDB	Liquid extract of water hyacinth (20 g l^{-1})	Liquid extract of water hyacinth (30 g l^{-1})	Liquid extract of water hyacinth (40 g l^{-1})
Abalone no. 03	0.89±0.00^{k}	4.94±0.01^{j}	4.81±0.01^{j}	6.10±0.00^{i}
Hed Kon no. 02	51.43±0.18^{a}	7.27±0.02^{h}	11.35±0.04^{f}	14.57±0.01^{e}
Teenpok	32.01±0.03^{c}	8.10±0.02^{g}	28.9±0.13^{d}	37.11±0.01^{b}

* Means with different letters differ significantly at $P \leq 0.05$

4. Conclusions

It can be concluded that dried water hyacinth leaves at 30-40 g l can be used as liquid medium for the production of 5´nucleotide by mycelia of Teenpok mushroom. Water hyacinth has an advantage over other substrates for it is plentiful and cost-free. The use of water hyacinth could also reduce the aquatic weed problem to some extent.

References

[1] Yamaguchi, S., and Ninomiya. K. **2000**. Umami and Food Palatability. *Journal of Nutrition,* 130 (suppl. 4S), 921S-926S.

[2] Erilsen, S. and Fagerson, I. S. **1976**. Flavours of amino acid and peptides. *International Flavors and Food Additives,* 7(1), 13-16.

[3] Ninomiya, K. **1998**. Natural occurrence. *Food Reviews International*. 14, 177-212.

[4] Solms, J. **1971**. Nonvolatile compounds and flavor. In: Gustation and Olfaction (Olhloff, G. & Thomas, A. F., eds.), pp 92-110. Academic Press, London, England.

[5] Gajalakshmi, S. and Abbasi, S. A. **2002**. Effect of the application of water hyacinth compost vermicompost on the growth and flowering of *Crossandra undulaefolia*, and on several vegetables. *Bioresource Technology*. 85, 197-199.

[6] Gunnarsson, C. C. and Petersen, C. M. **2007**. Water hyacinths as a resource in agriculture and energy production: a literature review. *Waste Management*, 27, 117-129.

[7] Osemwegie, O. O., Akpaja, E., Okhuoya, J. A. and Anedu, C. **2003**. Waterhyacinth, an aquatic weed as a growth substrate for *Pleurotus pulmonarius* and *Pleurotus tuberregium* (Pt-Omon 13). *Nigerian Journal of Applied Science*, 21, 9-14.

[8] Murugesan, A. G., Vijayalakshmi, G. S.,Sukumaran, N. and Mariappan, C. **1995**. Utilization of water hyacinth for oyster mushroom cultivation. *Bioresource Technology* 51, 97-98.

[9] Limsoonton, T. **2000**. Water hyacinth utilization in oyster mushroom cultivation. *Master's Thesis*, Gradute School, Mahidol University, Bangkok, Thailand.

[10] Nageswaran, M., Gopalakrishnan, A., Ganesan, M., Vedhamurthy, A. and Selvaganapathy, E. 2003. Evaluation of waterhyacinth and paddy straw waste for culture of oyster mushrooms. *Journal of Aquatic Plant Management*, 41, 122-123.

[11] Wasser, S. P., and Weis, A. L. **1999**. Medicinal properties of substances occurring in higher Basidiomycetes mushrooms: current perspective (review). *International Journal of Medicinal Mushroom*. 1, 31-62.

[12] Litchfield, J. H. **1967**. Morel mushroom mycelium as a food flavoring material. *Biotechnology and Bioengineering*, 9, 289-304.

[13] Mau, J.-L., **2005**.The umami taste of edible and medicinal mushrooms. *International Journal of Medicinal Mushrooms*, 7, 113-119.

[14] Mau, J.-L., Lin, H.-C., Ma, J.-T. and Song, S.-F. **2001**. Non-volatile taste components of several specialty mushrooms. *Food Chemistry*, 73, 461-466.

[15] Yang, J.-H., Lin, H.-C. and Mau, J.-L. **2001**. Non-volatile taste components of several commercial mushrooms. *Food Chemistry*, 72, 465-471.

[16] Mau, J.-L., Lin, H.-C. and Chen, C.-C. **2001**. Non-volatile components of several medicinal mushrooms. *Food Research International*, 34, 521-526.

[17] Chiang, P.-D., Yen, C.-T. and Mau, J.-L. **2006**. Non-volatile components of canned mushrooms. *Food Chemistry*, 97, 431-437.

[18] Huang, S.-J., Tsai, S.-Y., Lee, Y.-L. and Mau, J.-L. **2006**. Nonvolatile taste components of fruit bodies and mycelia of *Cordyceps militaris*. *LWT-Food Science and Technology*, 39, 577-583.

[19] Taylor, M. W., Hershey, R.A., Coy, K. and Olivelle, S. **1981**. Improved method of resolving nucleotides by reverse-phase high performance liquid chromatography. *Journal of Chromatography*, 219, 133-139.

[20] Chen, H.-K. **1986**. Studies on the characteristics of taste-active components in mushroom concentrate and its powderization. *Master's Thesis*, National Chung-Hsing University, Taichung, Taiwan.

[21] Smith, J., Rowan, N. and Sullivan, R. **2002**. *Medicinal Mushrooms. Their Therapeutic Properties and Current Medical Usage with Special Emphasis on Cancer Treatments*. Cancer Research, U.K.

[22] Yang, J.-H., Lin, H.-C. and Mau, J.-L. **2001**. Non-volatile taste components of several commercial mushrooms. *Food Chemistry* 72, 465-471.

[23] Huang, S.-J., Tsai, S.-Y., Lee, Y.-L. and Mau, J.-L. **2006**. Nonvolatile taste components of fruit bodies and mycelia of *Cordyceps militaris*. *LWT-Food Science and Technology* 39, 577-583.

[24] Nageswaran, M., Gopalakrishnan, A., Ganesan, M., Vedhamurthy, A. and Selvaganapathy, E. **2003**. Evaluation of waterhyacinth and paddy straw waste for culture of oyster mushrooms. *Journal of Aquatic Plant Management* 41, 122-123.

[25] Marcelo, J. S. R., Samia, M. T. T., Vera, L. R. B. and Marina, C. **2001**. Cultivation of the edible mushroom *Oudemansiella canarii* (Jungh.) Hohn. in lignocellulosic substrates. *Brazilian Journal of Microbiology*, 32, 211-214.