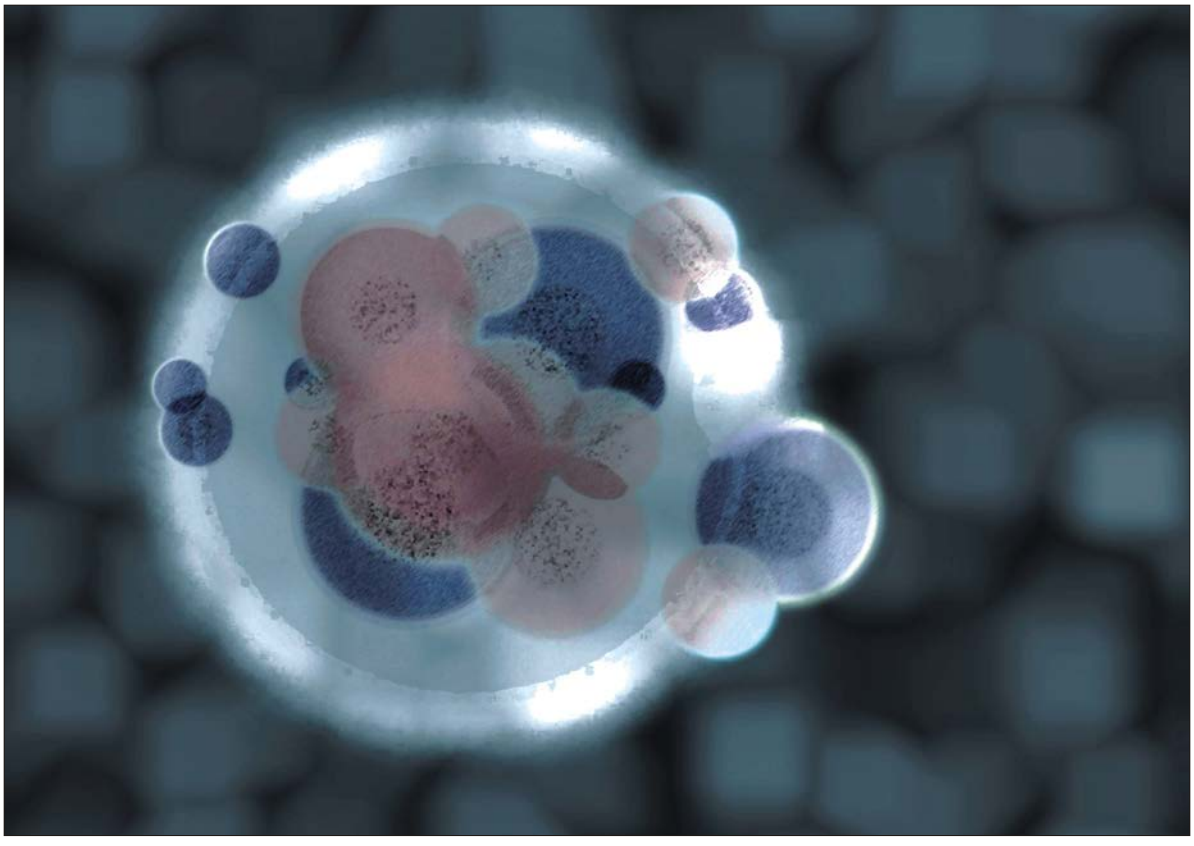

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Effective Microorganisms for Enhancing *Pleurotus ostreatus* (Fr.) Kummer Production

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Abstract: In the present study 0, 0.5, 1.0, 2.0, 4.0 and 8 mL of Effective Microorganisms (EM) concentrations were diluted with 1000 mL of tap-water and at each concentration was used to mix with *Pleurotus ostreatus* solid culture. Growth of *P. ostreatus* mycelia were scrutinized during 5 weeks of incubation period. Rapid growth of *P. ostreatus* mycelia were revealed in the EM mixed *P. ostreatus* solid culture. Twenty percent contamination were found in the EM unmixed *P. ostreatus* growth substrates while 0-5% were detected in the EM mixed *P. ostreatus* solid culture. Averages of total mushroom weight and number of oyster mushrooms (OMs.) were high when watering *P. ostreatus* with the tap-water instead of EM solution. 0.43-0.63 kg of the total mushroom weight and 69-110 of OMs. were detected per block. Mean total mushroom weight and number of OMs. per block was 2.0 to 4.0 times higher in the EM mixed than unmixed *P. ostreatus* growth substrates. However, it was found that irrigation of OM cultures using ordinary tap water and irrigation using EM dissolved in tap water, gave almost insignificant differences in average weight, width and height of OMs. at a confidence level of 95%, where the average weight, width and height of each OM was found to be approximately 6.26 g, 5.96 and 7.89 cm, respectively.

Key words: Effective microorganisms, contamination, irrigation, *Pleurotus ostreatus*

INTRODUCTION

The Oyster Mushroom (OM) is a mushroom that Thai people know very well and is becoming increasingly popular for consumption, because it is clean and white, has high nutritional value, has a delicious flower, the flesh is not chewy like other types of mushroom and importantly, it is a mushroom with medicinal properties comparable to other types and species. The OM has high protein levels, second only to beans and has many other constituents such as Vitamin B1 and B2 (higher than other mushrooms); and it has higher levels of folic acid than vegetables and meat. Folic acid has medicinal properties in helping protect against anaemia, diabetes and high blood pressure, for example[1]. Apart from this, oyster mushrooms contain the chemical "Retime" that is claimed to be able to cure cancer[2]; has low levels of sodium, which makes it suitable for people with heart disease and kidney infections. Additionally, OMs. are found to have low calory levels and so are popular for consumption by people who are dieting[3].

In OM cultivation there is a problem with the breakdown of raw materials used in mushroom culture and contamination of pure mushroom culture from foreign micro-organisms that cause effects on the growth of mycelia, which grow at sub-optimum levels and give low yields. Hence, in this study, effective micro-organisms (EM; pH 3.49) are use to help in mushroom cultivation, because the main micro-organism found in EM, are the group of bacteria that produce lactic acid, yeasts, photosynthetic bacteria, actinomycetes and fungi. Because EM are able to reduce the time taken to breakdown the waste products of animals, plants and agricultural residues and are able to produce lactic acid during their growth phase[4], it is therefore expected that EM can be used to breakdown materials used in OM cultivation into minor components. This should have an effect on OM pure culture being able to utilise those products of material breakdown, resulting in better growth, or EM may be able to check and destroy the impure micro-organisms in the pure OM culture. Therefore, it is expected that introducing EM for breaking down OM culture medium before autoclaving to sterilise it and then inoculating with pure OM culture will be effective in helping to improve the growth rate of the mycelia. This in turn will help to reduce the period of OM culture and it should increase production of OM to meet demand. Therefore, this experiment has the objective of

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studying the production and growth of oyster mushrooms when EM is used to help breakdown various raw materials used in the cultivation of mushrooms.

MATERIALS AND METHODS

Preparation of culture medium of OM: The preparation of a culture medium for OM used a mixture of rubber tree sawdust (1000 kg); soft rice bran (100 kg); pumice (10 kg); lime (10 kg); gypsum (2 kg) and salt (2 kg). These were mixed thoroughly together and then separated into six piles.

Pile No. 1: Mixed with ordinary tap water (pH 7.55) that has been left to stand for 7 days (for dechlorination)-Control

Pile No. 2-6: Mixed with EM dissolved in water at concentrations of 0.5 (pH 7.51), 1.0 (pH 7.23), 2.0 (pH 6.89), 4.0 (pH 6.22) and 8.0 (pH 5.95) mL per 1000 mL, respectively

Each pile was mixed thoroughly together until it had a moisture content of around 50%. Each pile was taken and put in cylindrical plastic blocks at a rate of 1.2 kg per block. The OM blocks that did not contain EM were sterilised, while those containing EM were left for 7 days in the shade to allow the EM to breakdown the culture medium. Cotton wool was used to block the entrance to the OM blocks and then tightly sealed with paper before the blocks were sterilised.

Method of OM cultivation: Take the OM culture blocks that had been sterilised and then spawning with pure OM culture using a sterile method. Acclimatise at room temperature until the mycelia are widespread and then move to the mushroom fruiting house, open up the block entrance; pull out sorghum seeds and leave for the large mycelia to develop into OMs. In laying out the cultivation blocks in the mushroom fruiting house, the blocks should be arranged in a Randomized Complete Block Design (RCBD).

Method of irrigation: Each culture medium block was irrigated using tap water that has been left to stand for 7 days (pH 7.55), with irrigation being done every morning and evening. In the experimental group using EM, the blocks were irrigated with water containing EM dissolved in water at a ratio of 1: 100 (pH 4.61) and 2: 100 (pH 4.17) just once a week on Saturday.

Method of harvesting OM flowers: Bunches per clusters of OM flowers were harvested by pulling them off from the block after 15 days of the experiment, counted from the day the mouth of the blocks were opened. Harvesting was done until full OM culture medium consumption.

Method of analysis: Analyse to find the percentage contamination of the OM culture; compare the rate of growth of the mycelia; compare the mean weights; number of OMs.; height of the OMs. and the width of the OM cap and then data groups were analyzed using SPSS for windows 7.5.2. Treatment means were compared using Ducan's multiple range test.

RESULTS

pH and contamination of the OM cultures: The OM cultures which were mixed with EM in the culture medium at ratios of 0.5, 1.0, 2.0, 4.0 and 8.0 mL per 1000 mL of tap water had a percentage contamination of between 0-5.0%. The Control group showed contamination of 20%, as shown in Table 1, such that the contamination of the OM culture will occur when the culture is acclimatising in week 1 and it was found that the group of micro-organisms and the OM culture were black in colour, with different characteristics from the normal white colour of OM Initial pH of solutions mixed in culture medium for OM was varied by amount of EM solution added. The pH values ranged between 5.95 to 7.55 however when these solutions were supplemented in OM culture medium the initial pH of OM cultures were slightly different (5.64-6.32; Table 1).

Growth of OM mycelia: The experiment found that the growth of the OM mycelia which were mixed with EM in the culture medium at a ratio of 0.5. 1.0, 2.0, 4.0 and 8.0 mL per 1000 mL of tap water, were similar. The OM cultures mixed with tap water only (Control) showed slower growth (Fig. 1).

Analysis and comparison of different characteristics of the OM flower when watering with tap-water only: The experiment found that the OM culture mixed with EM in the culture medium had a total OM weight and average number of OMs. per block, that was higher than the OM cultures mixed only with tap water, by a factor of 2.0-4.0. However, average weight and average height of the OMs. in all experimental groups were ranged between 5.74-6.80 g and 7.68-8.63 cm, respectively, such that there was no significant difference between groups. While, the average width of the OM cap was significant different between OM culture mixed (5.90-6.28 cm) and unmixed with EM (7.48 cm) in the culture medium (Table 2).

Table 1: pH and percentage of *Pleurotus ostreatus* contamination

	EM concentration (mL) per 1000 mL of tap-water					
Pleurotus ostreatus sample	0 (pH 7.55)	0.5 (pH 7.51)	1.0 (pH 7.23)	2.0 (pH 6.89)	4.0 (pH 6.22)	8.0 (pH 5.95)
Initial pH of OM culture	6.32	6.30	6.27	6.20	5.99	5.64
Blocks contaminated	20.00	4.00	0.00	5.00	2.00	0.00
%contamination	20.00	4.00	0.00	5.00	2.00	0.00

Table 2: Mean±SD of *Pleurotus ostreatus* yields when watering with tap-water only

	Pleurotus ostreatus yield analysis				
EM concentration (mL) per 1000 mL of tap-water	Total weight per 100 blocks (kg)	OM weight (g)	Number of OMs. per block	OM height (cm)	OM cap width (cm)
0	17.80±6.88^{c}	6.80±1.28^{NS}	28.16±14.83^{c}	8.20±1.25^{NS}	7.48±1.51^{a}
0.5	43.31±11.75^{b}	6.46±1.33	69.00±22.08^{b}	7.68±1.07	6.28±1.00^{b}
1	63.49±12.51^{a}	6.52±2.94	108.00±34.51^{a}	8.63±1.37	5.90±1.83^{b}
2	59.31±18.29^{a}	5.74±1.40	110.50±41.68^{a}	8.32±1.16	5.92±1.28^{b}
4	58.89±16.39^{a}	5.82±1.13	105.50±35.12^{a}	7.83±1.06	5.97±1.39^{b}
8	50.44±20.96^{a}	5.59±0.59	109.00±35.80^{a}	8.10±0.57	5.92±0.64^{b}

NS = not significantly different

Table 3: Comparison of the yield of *Pleurotus ostreatus* when irrigated with tap water and water containing dissolved EM

		Pleurotus ostreatus yield analysis				
EM concentration (mL) per 1000 mL of tap-water		Total weight per 100 blocks (kg) x̄±SD	OM weight (g) x̄±SD	Number of OMs. per block x̄±SD	OM height (cm) x̄±SD	OM cap width (cm) x̄±SD
Watering with Tap water	0	17.80±6.88^{de}	6.80±1.28^{abcd}	28.16±14.83^{fg}	8.20±1.25^{a}	7.48±1.51^{ab}
	0.5	43.31±11.75^{bc}	6.46±1.33^{abcd}	69.00±22.08^{abcdef}	7.68±1.07^{a}	6.28±1.00^{abc}
	1	63.49±12.51^{a}	6.52±2.94^{abcd}	108.00±34.51^{ab}	8.63±1.37^{a}	5.90±1.83^{abc}
	2	59.31±18.29^{ab}	5.74±1.40^{cd}	110.50±41.68^{a}	8.32±1.16^{a}	5.92±1.28^{abc}
	4	58.89±16.39^{ab}	5.82±1.13^{cd}	05.50±35.12^{ab}	7.83±1.06^{a}	5.97±1.39^{abc}
	8	50.44±20.96^{ab}	5.59±0.59^{cd}	09.00±35.80^{ab}	8.10±0.57^{a}	5.92±0.64^{abc}
Watering with EM (1:100)	0	17.56±8.16^{de}	8.63±8.79^{abc}	28.50±11.35^{fg}	8.23±0.70^{a}	6.00±2.18^{abc}
	0.5	29.07±16.19^{cd}	6.68±1.82^{abcd}	45.00±27.05^{defg}	8.92±2.15^{a}	6.12±0.65^{abc}
	1	40.55±6.64^{c}	6.03±0.66^{bcd}	67.50±10.24^{bcdef}	7.92±0.46^{a}	5.80±0.31^{abc}
	2	41.98±10.00^{ab}	5.23±1.39^{cd}	81.00±10.39^{abcd}	8.55±0.84^{a}	6.01±0.56^{abc}
	4	43.40±9.28^{bc}	4.87±0.82^{cd}	90.00±21.35^{abc}	7.60±0.46^{a}	5.32±0.19^{c}
	8	41.07±8.51^{c}	5.73±0.20^{cd}	72.00±16.97^{abcde}	7.52±0.60^{a}	5.58±0.25^{bc}
Wateringwith EM (2:100)	0	2.40±4.81^{e}	1.00±2.00^{e}	6.00±12.00^{g}	1.72±3.45^{b}	1.26±2.52^{d}
	0.5	25.11±12.74^{cd}	4.45±0.94^{d}	54.00±17.66^{cdef}	8.39±0.44^{a}	6.00±0.07^{abc}
	1	26.37±13.47^{cd}	9.80±9.24^{ab}	39.00±25.21^{efg}	9.13±1.32^{a}	6.86±0.49^{abc}
	2	25.83±5.03^{cd}	7.53±2.19^{abcd}	36.00±10.95^{efg}	7.70±0.60^{a}	6.90±0.22^{abc}
	4	27.12±9.93^{cd}	5.61±1.40^{cd}	48.00±12.00^{def}	8.55±1.32^{a}	6.33±3.20^{abc}
	8	26.90±10.94^{cd}	10.17±2.98^{a}	30.00±17.66^{fg}	9.08±0.12^{a}	7.70±0.14^{a}

Means±SD in each column with different superscripts indicate statistical differences ($p<0.05$)

14 days 28 days

Fig. 1: *Pleurotus ostreatus* **mycelium growth**
Various EM concentrations were diluted in 1000 mL water and mixed with *Pleurotus ostreatus* **solid culture. From left to right, EM concentrations were 0, 0.5, 1, 2, 4 and 8 mL, respectively**

Comparison of the yield of OM when irrigated with tap water and water containing dissolved EM: From the experiment it was found that irrigation with tap water only, caused the total weight and numbers of OMs. to be higher than the OM cultures irrigated by dissolved EM in tap water (at a rate of 1 : 100 and 2 : 100 mL; Table 3). When ordinary tap water is used for irrigation, OM culture mixed with EM in the culture medium gave total weight of OMs. and number of OMs. approximately equivalent to 0.43-0.63 kg per block and 69-110 OMs. per block, respectively (Table 2). Apart from OM culture mixed with tap water in the culture medium, for the total weight of OMs. and number of OMs. that were irrigated with dissolved EM mixed in tap water at both concentrations, they were approximately equivalent to 0.25-0.43 kg per block and 30-90 OMs. per block, respectively (Table 3).

However, it was found that irrigation of OM cultures using ordinary tap water and irrigation using EM dissolved in tap water, gave almost insignificant differences in average weight, width and height of OMs. at a confidence level of 95% (Table 3), where the average weight, width and height of each OM flower was found to be approximately 6.26 g, 5.96 and 7.89 cm, respectively. Average weight, width and height of OMs. obtained in this experimental were coincided to the Thai market standard.

DISCUSSION

By using EM to breakdown the OM culture medium for seven days before sterilisation of the mushroom culture, it was found that the culture mixed with EM showed faster growth of mycelia than those culture without EM. The rate of culture contamination and wastage of the OM cultures were also less than in those cultures not mixed with EM by a factor of about four. This might be caused by substances dissolved in EM, mainly from the group that produce lactic acid, (including *Lactobacillus casi*, *Lactobacillus brugericus* and *Streptococcus lactis*). Hence, the quantity of lactic acid produced in EM perhaps causes conditions that are acidic which is not suitable for the growth of micro-organisms which are responsible for contaminating OM cultures.

The total weight and number of OMs. of OM cultures that were mixed with EM, were higher than those cultures not mixed with EM, such that the average value was ranged between a factor of 2.0-4.0. This may be caused by groups of micro-orgamisms in EM that are able to digest organic substances, such as *Tricoderma* sp., *Aspergillus* sp., *Pennicillium* sp., *Streptomyces* sp., *Micromonospora* sp., *Streptosporangium* sp. and *Nocardia* sp., mixed together. These micro-organisms are able to produce enzymes in the cellulytic enzyme group, such as hemicellulose and cellulase, which can digest rubber tree sawdust and soft rice bran which are the main raw materials used in OM culture. The components of sawdust and rice bran include cellulose and hemicellulose mostly. Digestion of cellulose produces glucose and cellobiose, while digestion of hemicellulose produces xylose mostly and other sugars, such as galactose, mannose, arabinose, pyranose, plus glucoronic acid and galacturonic acid as secondary products[5,6,7]. Mostly the source of carbon that the mushroom culture uses for growth is carbohydrate, by utilising hexose molecules, such as glucose, sucrose and lactose. Hexose is mostly converted into glucose-6-phosphate or fructose-6-phosphate before being digested through the process of glycolysis and the tricarboxylic acid cycle (TCA) using energy from carbon dioxide and water[8].

When EM digests the OM culture medium, different sugars are released which are converted into sources of carbon that the OM culture can easily utilise. Therefore, when the mushroom culture is able to use these sugars, good growth of mycelia results and there will be increased production of mushroom flowers. Hence, using EM to digest the OM culture medium before introducing the pure OM culture, is one approach that has potential and the ability to increase yields of OM in the future. The results from this experiment confirm those of Hleklai, who used microbial activator (LLD.-1) with 0.5% urea together to digest rubber sawdust for a period of 9 days before culturing OM He found that adding the catalyst with 0.5% urea caused production of mushrooms in 6 flushes to have a total weight of 499.1 kg per 1000 blocks of culture medium. For the culture medium which did not undergo digestion, gave a lower yield in 5 flushes and a total weight of 344.6 kg[9].

In cultivation of OM, it was found that the cultures which were irrigated by ordinary tap water (pH 7.55) gave approximately of total weight 0.40-0.63 kg and a total number of OMs. of 69-110 OMs. per bunch, which was higher in comparison to the cultures that were irrigated by EM dissolved in water at a rate of 1:100 (pH 4.61) and 2:100 (pH 4.17) mL. This may have been caused by when the culture is sterilised and inoculated by pure OM culture, the OM culture is able to easily use different sugars that are produced from the digestion of the culture medium. Hence, growth and mushroom yield will be increased. But when dissolved EM (that is compose of a mixture of over 80 spp.) is introduced on the pure OM cultures with the irrigation water, it will cause contamination by the various micro-orgamisms in the mushroom culture. When the contaminating micro-organisms use the various nutrients in the culture medium

\for growth it will cause the growth of OM to decline and will cause overall yield of OM to decrease too. Additionally, in the dissolved EM (pH 4.17 and 4.61), there are major groups of micro-organisms that produce lactic acid, including *Lactobacillus casi*, *Lactobacillus brugaricus* and *Streptococcus lactis*. Therefore, the quantity of lactic acid that EM produces may have an effect on the acidity (as low pH was detected in the dissolved EM: pH 4.17 and 4.61), which is not suitable for the growth of OM cultures. Because the pH value and temperature effects the growth of *Pleurotus sp.* through effecting the enzyme activity in the cells. Generally, the pH value which is suitable for mycelia growth is in the range of pH 5.5-6.0, while suitable temperatures are between 28-30°C[10,11].

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