Journal of Agricultural Technology 2011 Vol. 7(4): 957-965
Available online http://www.ijat-aatsea.com
ISSN 1686-9141

Effect of plant oils, surfactants and organic acids on the production of mycelial biomass and exopolysaccharides of *Trametes* spp.

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Bolla, K., S.V.S.S.S.L. Hima Bindu N, Samatha Burra, and M.A. Singara Charya (2011) Effect of plant oils, surfactants and organic acids on the production of mycelial biomass and exopolysaccharides of *Trametes* spp. Journal of Agricultural Technology 7(4):957-965.

Effect of plant oils, surfactants and organic acids on the production of biomass and exopolysaccharides from *Trametes elegans and Trametes gibbosa* were studied. Plant oils (castor oil, soyabean oil and peppermint oil), surfactants (tween 20, tween 80 and triton x 100) and organic acids (propionic acid, lactic acid and valeric acid) were taken in the concentrations of 0.5% and 1%. The addition of plant oils, surfactants and organic acids was evaluated with the increasing the production of exopolysaccharide (EPS). The addition of soybean oil gave the best stimulatory effect on the polysaccharide and biomass production by *Trametes* spp. 0.5% of soybean oil that showed maximum production over 1%. The maximum production of EPS was 6.9 and 7.2 g. dr. w/L with soybean oil (0.5%) after 7 and 14 days of incubation in *T. elegans.* EPS production showed a maximum with soybean oil (0.5%) of 7.1 and 7.7 g. dr. w/L after 7 and 14 days of incubation in *T. gibbosa*, respectively.

Key words: *Trametes* spp., exopolysaccharides, plant oils, surfactants and organic acids

Introduction

Trametes spp belongs to the Polyporales, Basidiomycetes and widely known that represents a major source of new pharmaceutical products. In particular, polysaccharides and polysaccharide-protein complexes from these organisms have received the most extensive attention of researchers (Ooi and Liu, 1999; Wasser and Weis, 1999 and De baets, 2001). Although a number of reports have attempted to obtain the best culture conditions and EPS characterization from different fungi, the effect of medium composition on fermentations and cultivation kinetics, which are important parameters to EPS production, remain relatively unexplored (Krishna *et al.* 2010). *Trametes elegans* consists of a definite cap with a fertile surface consisting of gills. The

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fruiting body usually also has a stem, although that may be lateral or absent (usually, then, the mushroom is growing from wood). This species is usually described as southeastern in distribution. The pore surface of *Trametes elegans* does not bruise pinkish-brown. This polypore is thoroughly confused. It can't make up its mind what kind of pore surface it wants to have: one with normal-looking, angular to roundish pores, or one with pores that are "daedaloid" or nearly "lamellate," to use the official terms in Mycologese that mean "maze-like" and "gill-like," respectively. In fact we find all three conditions represented on the same mushroom which turns out to help, rather than hinder, the identification process. *Trametes gibbosa* is commonly known as the 'lumpy bracket', is a polypore mushroom that causes a white rot. It is found on beech stumps and the dead wood of other hardwood species. Fruit bodies are 8-15 cm in diameter and semicircular in shape. The upper surface is usually gray or white, but may be greenish in older specimens due to algal growth. Elongated pores are located on the under-surface.

Plant oil plays a role and a function of antifoam agent in fermentation that has been reported to be favorable for the mycelial growth in several medicinal mushrooms and fungi, and to increase the production of bioactive metabolites (Yang *et al* 2000, Peacock *et al* 2003 and Park *et al* 2002). In this research, effects of the additives of plant oils like castor oil, soybean oil and peppermint oil were used additionally in the media as 0.5% and 1% concentration that added in *Trametes* spp in the submerged fermentation. The mycelial growth of *Trametes* was found to increase when the additives oil concentration increased. The stimulation of cell growth by oil in this study was caused by the partial incorporation of lipids in the cell membrane, thereby facilitating the uptake of nutrients from the medium as report by Yang *et al*.,(2000). Volume fractions of 0.5 and 1% of two hydrophilic Tween series and Triton X 100 surfactants and organic acids were studied in the submerged culture of *Trametes* spp in glucose media. The cell growth and polysaccharides production were also studied.

Materials and methods

Thirty strains of native south Indian Basidiomycetes were collected and cultured as per the method suggested by Rosana Maziero *et al.,* (1999). These are maintained in Microbial Culture Collection Lab, Kakatiya University, Warangal and were used in the present research work. The strains of white rot fungi were grown on Malt extract agar medium. The composition of the medium was followed Chang and Chou (1995). Malt extract medium conststed of 15.0, K_2HPO_4 1.0, NH_4Cl 1.0, Citric acid (1N) 15.0 ml and agar 20.0g. Mushroom complete medium (g/L) consisted of peptone 1.0, yeast extract 2.0, K_2HPO_4 1.0, $MgSO_4$.7H2O 0.2, $(NH_4)_2SO_4$ 5.0, glucose 20.0, pH 6.0 and

distilled water1.0 L. This medium was selected for exopolysaccharide production by Basidiomycetes. Erlenmeyer flasks containing 100 ml of sterilized culture medium were inoculated with the suspension in sterile water of fungal mycelium grown on malt extract agar slants. Incubation was done at 27°C.

Effect of plant oils

The effects of plant oils supplement in the media for the production of exopolysaccharides were examined by adding three different plant oils at 0.5% and 1% including castor oil, soyabean oil and peppermint oil to each flask culture medium. The fermentation was carried out at 28 ± 2 ^{o}C with initial pH 6 for 7 and 14 days of incubation period.

Effect of surfactants

The effects of surfactants supplement in the media for the production of exopolysaccharides were examined by adding three different plant oils at 0.5% and 1% including tween 20, tween 80 and triton x 100 to each flask culture medium. The fermentation was carried out at 28 ± 2 ^{o}C with initial pH 6 for 7 and 14 days of incubation period.

Effect of organic acids

The effects of organic acids supplement in the media for the production of exopolysaccharides were examined by adding three different plant oils at 0.5% and 1% including propionic acid, lactic acid and valeric acid to each flask culture medium. The fermentation was carried out at 28 ± 2 ^{o}C with initial pH 6 for 7 and 14 days of incubation period.

Results and discussion

The biomass and EPS of *T. elegans* production (Table 1) ranged from 1.2 to 8.4 g. dr. w/L and 2.6 to 7.2 g. dr. w/L respectively. Maximum production of the biomass produced with the soybean oil (0.5%) used as oil source was 7.2 and 8.4 g. dr. w/L after 7 and 14 days of incubation, respectively. The maximum production of EPS was 6.9 and 7.2 g. dr. w/L with the same soybean oil (0.5%) after 7 and 14 days of incubation. In *T. gibbosa*, the biomass production ranged from 1.8 to 7.1 g. dr. w/L and EPS production ranged from 1.5 to 7.7 g. dr. w/L were demonstrated. Biomass production was the highest when soybean oil (0.5%) was used as oil source with 6.8 and 7.1 g. dr. w/L

after 7 and 14 days of incubation, respectively. EPS production showed the maximum with soybean oil (0.5%) which were 7.1 and 7.7 g. dr. w/L after 7 and 14 days of incubation, respectively. The addition of soybean oil gave the best stimulatory effect on the polysaccharide and biomass production after treated *Trametes* spp. with 0.5% of soybean oil that showed maximum production over 1%. It was also nobserved with the other two oils but the maximum production was observed in soybean oil. Comparing with the sample without oil addition, increased cell concentration was found in soybean addition when compared with the other two plant oils. The lower pH in oil addition might cause by the uptake of plant oils as the carbon sources supplies the cell growth continuously. This result was in consistent with a previous study that various carbon sources were suitable for the cell growth of *Cordyceps sinensis* and the cell could grow better with lower final pH of fermentation broth (Hsieh *et al.*, 2005).The result of the enhancement of EPS production with oil addition was also shown with agreements of the previous studies (Yang *et al*., 2000; Park *et al*., 2002). According to some studies, the low pH of broth resulted from oil addition might favor the EPS to produce continuously (Hsieh *et al*., 2006) A possible reason is that high oxygen concentration might inhibit the formation of cell wall and result in leaking polysaccharides to broth.

Table 1. Effect of plant oils on biomass and EPS production of *Trametes elegans and Trametes gibbosa*.

Oils	Days	Conc (%)	*Trametes elegans*			*Trametes gibbosa*		
			pH	B	EPS	pH	B	EPS
Castor Oil	7	0.5	3.23	3.8	5.2	3.91	4.1	5.4
		1	3.19	5.1	2.8	3.26	4.8	2.6
	14	0.5	3.42	6.2	4.2	3.62	6.2	4.7
		1	4.11	6.4	4.0	3.74	6.1	4.2
Soybean Oil	7	0.5	3.32	7.2	6.9	3.42	6.8	7.1
		1	3.71	6.4	6.5	3.84	6.2	6.3
	14	0.5	3.42	8.4	7.2	3.62	7.1	7.7
		1	3.65	6.9	6.8	3.72	6.8	6.6
Pepermint Oil	7	0.5	5.04	2.2	3.5	5.12	2.4	2.4
		1	5.02	2.4	3.7	5.24	2.2	2.2
	14	0.5	5.14	1.7	4.4	5.16	2.1	1.9
		1	5.08	1.2	2.6	5.05	1.8	1.5
Control	7	-	2.70	1.0	6.8	1.77	1.6	2.8
	14	-	2.85	1.8	4.6	2.12	6.0	7.4

B: Biomass, EPS: Exopolysaccharides

When 2% glucose was used as a carbon source along with the plant oils, stimulated the mycelial growth of *G. frondosa* and slightly increased the EPS

production. This result was not consistent with the previous reports (Wei *et al.,* 2003). Adding plant oil to the mycelial culture of the fungus showed a positive effect. However, the effect was showed a negative when the supplement concentration was too high. The feasibility of using plant oils for the mycelial growth and polysaccharides production of *S. commune.* The plant oils such as olive, castor and peppermint oil could be favorable used as an additive for the EPS production of *S. commune* (Krishna *et al*., 2008). The biomass and EPS of *T. elegans* production (Table 2) ranged from 2.3 to 5.9 g. dr. w/L and 3.2 to 4.7 g. dr. w/L respectively. Maximum production of the biomass produced with the triton x 100 (0.5%) used as oil surfactant producing 5.1 and 5.9 g. dr. w/L after 7 and 14 days of incubation respectively. Maximum production of EPS is 4.7 and 4.5 g. dr. w/L with the surfactant tween 20 (0.5%) after 7 and 14 days of incubation respectively. In *T. gibbosa*, the biomass production ranged from 2.5 to 5.1 g. dr. w/L and EPS production ranged from 3.1 to 4.8 g. dr. w/L. Biomass production showed the highest when tween 80 (0.5%) and triton x 100 was used as surfactant with 4.9 and 5.1 g. dr. w/L after 7 and 14 days of incubation respectively. EPS production showed the maximum with tween 20 (0.5%) and triton x 100 showing 4.8 and 4.7 g. dr. w/L after 7 and 14 days of incubation. EPS production was found to decrease with all concentrations of Tween 80 addition. Tween 80 having an 18 C side chain, had been reported that they could be hydrolyzed by microbial enzymes, such as lipase, to release oleic acid (Breuil *et al*., 1978). That is, the activity of lipase was induced by the presence of Tween 80; oleic acids releasing from the hydrolyzates of Tween 80 were able to enhance the cell growth. However, the increase of cell growth from the media with Tween 80 was less than from the media with plant oil. The finding showed that the less polysaccharides production was similar to that from plant oil addition in 2% glucose media and it might concern in Tween 80 that would increase cell growth instead of polysaccharides production.

With 12 C side chain, surfactants of Tween 20 (polyoxyethylene sorbitan monolaurate) addition were significantly showed the inhibition on the cell growth of *G. frondosa*. The less carbon chain of surfactants exhibited higher diffusion rate in cell wall of *G. frondosa*. The high concentration of surfactant might damage the cell membrane or interact with other bio-compounds in cell and then resulted in low cell growth. However, Tween 80, with longer side chain of surfactants, might also be able to diffuse through the cell wall with less concentration of surfactants. To accommodate the surfactants media the composition of fatty acid in cell was altered. The presence of Tween 80, which containing fatty acids a decrease in the amount of *n*-palmitate and an increase of octadecenoate were observed (Umesaki *et al.*, 1977) . These results were consistent with those in previous studies which showed the effect of Tween 80

on stimulatory enzyme secretion by *Candida lipolytica* in submerged cultures (Nascimento *et al.*, 2000). The effect of organic acids on biomass and EPS of *Trametes* spp after 7 and14 days of the incubation as seen in Table 3. Three organic acids were tested for the biomass and EPS production. Lactic acid was found to be effective compared to the other organic acids.

Table 2. Effect of surfactants on biomass and EPS production of *Trametes. elegans and Trametes gibbosa.*

Surfactants	Days	Conc (%)	*Trametes elegans*			*Trametes gibbosa*		
			pH	B	EPS	pH	B	EPS
Tween 20	7	0.5	4.22	3.0	4.7	4.12	3.2	4.8
		1	4.01	2.3	3.9	4.29	2.5	4.2
	14	0.5	3.92	3.4	4.5	4.21	3.4	4.3
		1	3.71	2.7	3.7	3.84	2.9	3.9
Tween 80	7	0.5	3.52	5.1	4.5	3.92	4.9	4.4
		1	3.66	3.1	3.6	3.79	4.2	3.9
	14	0.5	3.89	5.9	4.3	3.62	3.9	4.4
		1	3.77	3.2	3.4	3.54	3.6	3.6
Triton X 100	7	0.5	4.72	4.3	4.2	4.65	4.4	4.2
		1	4.55	4.1	3.2	4.44	4.2	3.1
	14	0.5	4.62	5.0	3.9	4.32	5.1	4.7
		1	4.32	4.2	3.6	4.41	3.9	4.5
Control	7	-	2.70	1.0	6.8	1.77	1.6	2.8
	14	-	2.85	1.8	4.6	2.12	6.0	7.4

B: Biomass, EPS: Exopolysaccharides

The biomass and EPS of *T. elegans* production (Table 3) ranged from 4.1 to 5.8 g. dr. w/L and 4.5 to 6.4 g. dr. w/L respectively. Maximum production of the biomass produced with the lactic acid (0.5%) which were 5.6 and 5.8 g. dr. w/L after 7 and 14 days of incubation, respectively. Maximum production of EPS was 6.2 and 6.4 g. dr. w/L with the propionic acid (0.5%) after 7 and 14 days of incubation. In *T. gibbosa*, the biomass production ranged from 4.2 to 5.7 g. dr. w/L and EPS production ranged from 4.7 to 6.7 g. dr. w/L. Biomass production was the highest with lactic acid (1%) and valeric acid (0.5%) which used with 5.4 and 5.2 g. dr. w/L after 7 and 14 days of incubation, respectively. EPS production showed the maximum with lactic acid (0.5%) which were 6.5 and 6.7 g. dr. w/L after 7 and 14 days of incubation, respectively. Succinic acid might responsible for both cell growth inhibition and EPS stimulation. The contribution of exhausted succinic acid for cell growth was limited and the major carbon source for cell growth and product formation was glucose. Similar observations that citric acid was not the main energy source for cell growth were reported elsewhere in the citric acid supplemented cultures (Souw

and Demain, 1980; Jana and Ghost, 1998; Shu *et al*., 2002). The observations are not supporting the previous results in the case of biomass inhibition.

Table 3. Effect of organic acids on biomass and EPS production of *Trametes elegans and Trametes gibbosa*.

Organic acids	Days	Conc (%)	*Trametes elegans*			*Trametes gibbosa*		
			pH	B	EPS	pH	B	EPS
Propionic acid	7	0.5	4.32	4.2	5.3	4.42	4.4	5.4
		1	4.12	4.1	5.1	4.24	4.2	5.2
	14	0.5	4.64	4.8	5.6	472	4.9	5.9
		1	4.19	4.3	5.1	4.53	4.7	4.7
Lactic acid	7	0.5	5.32	5.6	6.2	5.54	5.3	6.5
		1	5.24	5.2	4.8	5.26	5.4	4.9
	14	0.5	5.65	5.8	6.4	5.43	5.7	6.7
		1	5.18	5.4	4.9	5.21	5.1	4.7
Valeric acid	7	0.5	4.42	5.1	5.2	4.45	4.9	5.5
		1	4.41	4.9	4.6	4.32	5.2	4.7
	14	0.5	4.72	5.3	5.1	4.81	4.8	5.5
		1	4.53	5.1	4.5	4.24	5.2	5.8
Control	7	-	2.70	1.0	6.8	1.77	1.6	2.8
	14	-	2.85	1.8	4.6	2.12	6.0	7.4

B: Biomass, EPS: Exopolysaccharides

An interesting observation was made on the formation of an insoluble gel when the culture filtrate was frozen prior to polysaccharide precipitation. In the Table 1, these strains are marked. This characteristic could help polymer separation, since there is no need of an organic solvent such as isopropanol, ethanol or acetone for the precipitation of the polymer, thus increasing the process viability. Moreover, it is important to observe that the product obtained by solvent precipitation can not consider as pure polysaccharide because proteins and salts present in the medium coprecipitate. The data obtained from this screening were just indicative for selecting strains for further investigations on EPS production. Some of the studied strains were submitted to perform a lignin degradation activity test (Capelari and Zadrazil, 1997.) All strains produced more EPS showed good lignin degradation activity. Okino (1996) studied some of these strains for laccase and peroxidase production and all of them showed enzyme activity. The conditions of submerged culture could be considered as an adequate for biomass production. Data were also presented in literatures of Manachini (1979); Compere *et al*., (1980); Masaphy and Levanon, (1992) and Burns *et al*. (1994) that showed lower production for *Pleurotus* sp with other culture parameters. During estimation of polymer and biomass

produced, it is important to consider that EPS adherent to the hyphae that are also entrapped into the pellets formed during the submerged culture. It means that the dry weight of biopolymer which precipitated from the culture filtrate does not correspond to the total EPS and that the biomass can be overestimated. To minimize this problem, biomass was washed twice with distilled water. During the screening, it was observed that the submerged cultures showed different characteristics according to the fungal species. The pellets formed can be regular or irregular in form and size. The form varies from spherical to cylindrical and the size from 1 to 20 mm. In some cases the formation of pellets was not observed, but rather a mycelial agglomeration without a defined form (Maziero, 1996). The pellets were smooth, hairy (with looser outer zones) or with fringes of aggregated hyphae that give the pellet a star form. The color and consistency were also different, as well as the flavour. Sometimes the culture filtrate was shown very clear, other times was turbid and very viscous. In most of the cultures the presence of crystals with different forms was observed, which could indicate, in some cases, the presence of excreted metabolites. When there is a depletion of glucose in the medium it was observed that pellets begin to become darker and break up. The dead hyphae are decomposed and the resulting substances are reabsorbed by the mycelium. Results showed that most of the Basidiomycetes strains screened are potential EPS producers. The possibility of using these biopolymers for medical application promises a large opportunity to improve the study of such group of fungi. Besides the South Indian basidiomycetes has been scarcely investigated although it has a great potentiality.

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(Received 8 March 2010; accepted 30 May 2011)