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Effect of Sucrose Ester of Fatty Acids on Fruit Body Formation of *Pleurotus ostreatus*

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ABSTRACT: Sucrose ester of fatty acids (SE) is one of a group of food additive surfactants. Three kinds of SE (SE-1,2 and 3) containing the same fatty acids but with different hydrophilic -lipophilic balance values (HLB; 1, 7, and 15, respectively) were tested for their ability to stimulate fruit body differentiation of *Pleurotus ostreatus*. Experiments were performed initially with SE applied on paper disks placed on *P. ostreatus* colonies grown on malt extract agar. Among the three SE examined, the addition of SE-2 caused a stimulation of complete fruit body differentiation. SE-1, which has a smaller HLB value stimulated primordia differentiation earlier than SE-2, but the subsequent development into fruit bodies took much longer time than SE-2. SE-3 was not effective in inducing fruit bodies although stimulation of primordia was observed. Experiments of SE-2 using a sawdust medium revealed its inhibitory effect on linear growth of mycelium into the medium, but stimulation of earlier production of larger fruit bodies.

1 INTRODUCTION

When mycelium has reached a point of full growth, appropriate environmental stimuli such as light (Eger 1976), lower temperature (Eger 1968), humidity etc. are applied to induce fruit body differentiation in commercially grown *P. ostreatus*. Additionally, the procedure of soaking cultures in water after removing overgrown mycelium above the substrate is empirically known to increase numbers of primordia and subsequent yield of fruit bodies of such mushrooms as *P. ostreatus*, *Flammulina velutipes* and *Lentinula edodes* (Matsumoto *et al.* 1987). In addition to these physi-

cal stimuli, some chemical substances are reported to stimulate fruit body differentiation; Tween and Triton stimulated sporophore production with *L. edodes* (Song *et al.* 1989) and with *Schizophyllum commune*, surfactants such as digitonin and SDS stimulated fruit body formation (Oita *et al.* 1993). These works suggest that a surfactant might have a potential of stimulating fruit body development of mushrooms.

However, those surfactants reported in the past are not allowed for use as food additives. Currently, five surfactants, or emulsifiers, are available for food additives, in Japan i.e., sucrose fatty acid ester, glycerol fatty acid ester, stearoyl lactylates, propylenglycol fatty acid esters, and sorbitan fatty acid esters. Among these, sucrose fatty acid ester has the broadest range of HLB values. HLB designates the degree of hydrophilicity and lipophilicity i.e. greater the value, more soluble to water. HLB of sucrose fatty acid ester can be varied by changing numbers of fatty acid molecules (8 at highest) bound to one molecule of sucrose.

The purpose of the present study was to evaluate the stimulating effect of sucrose fatty acid ester (SE), on fruit body production of *P. ostreatus*. Among the three sucrose esters of palmitic and stearic acids examined, one with the HLB value of 7 was found to be very effective.

2 MATERIALS AND METHODS

2.1 *Organism and media*

Dikaryotic strain, *Pleurotus ostreatus* KO1 was obtained from Kawamura Edible Mushroom Laboratory. This strain is able to differentiate basidiocarps at temperature at 17 to 18°C and is compatible with *P. ostreatus* ATCC 60691 (Magae *et al.* 1990). Malt extract (2%), Agar (1.8%) (MA; 20ml) contained in petri dish as (90 mm diameter) were initially used for the assay of fruiting body differentiation.

2.2 *Condition for fungal growth*

Pleurotus ostreatus KO1 was grown on MA medium at 24°C for 4 days. Then a piece of grown colony was cut out by a cork borer (7 mm diameter) and transferred onto the center of a new MA plate. After growth for 17 days at 24 °C, in darkness, it was used for the assay of fruiting body differentiation by the following method. Six paper disks to which 50μl of 2%(w/v) SE was applied in successive x1/2 dilution were placed on about 1 cm inside the periphery of the colony grown on MA plate. Those plates were then cultured at 17 °C, 100 lux light to stimulate fruit body formation. Assays were performed in duplicate. If fruiting bodies were induced around the paper disks, stimulating effect was examined again by growing *P. ostreatus* on MA containing SE for 17 days at 24 °C and shifting the temperature to 17 °C. Assays were performed in five replicates. SE's were dissolved in a mixture of water and methanol. Filter sterilized SE's were added to the autoclaved MA. Malt extract without SE was used as a control medium.

2.3 *Test tube and bottle assay*

Rice bran (10%), sawdust (3.7%) and water (53%) with various concentrations up to 2%(w/w) of SE-2 were mixed together. The mixture was used for each test tube test and 500g for 800 ml polypropylene bottle assays. *P. ostreatus* was inoculated from MA plate and grown at 22 °C for 17 days for test tube, 27 days for 800 ml bottles. Then they were moved to temperature of 17°C under 100 lux light.

2.4 *Chemicals*

Sucrose esters were the product of Mitsubishi-kagaku Foods Co. Constituent fatty acids of SE were stearic acid (70%) and palmitic acid (30%). SE-1 has the HLB value of 1 and is 1% mono esters and 99% poly esters. SE-2 has the HLB of 7 and consists of 40% mono esters. SE-3 has the HLB of 15 and consists of 70% mono esters. Mono ester is more hydrophilic than polyesters. Both SE-2 and SE-3 are soluble in water but SE-1 does not dissolve in water.

3 RESULTS

3.1 *Paper disk assays*

SE-3 was most effective in promoting primordium formation around the paper disks (data not shown). The weakest effect was shown by SE-1. Fruit bodies appeared in the highest frequency around the paper disks with SE-2. Neither sucrose nor fatty acid alone on paper disks promoted fruit body differentiation on MA plate.

3.2 *Medium assay*

When SE was added directly to malt extract agar, apparent mycelial growth was not different from the control except for SE-2, which colony showed a whiter and more knobby appearance. The earliest primordia for-

ɘ 1. Production of primordia and/or fruitbodies on a medium containing sucrose
ɾ of fatty acids (SE).

Conc(%)		Time of incubation at 17°C (days)				
		8	12	18	25	36
-1	1	pm 5				pm 2, fb 3
	2	pm 2				pm 3, fb 2
E-2	1		pm 3, fb 1	pm 3, fb 2		
	2		fb 1	fb 5		
SE-3	1		pm 3	pm 4	pm 4, fb 1	pm 4, fb 1
	2		pm 3	pm 5	pm 5	pm 5

Assays were performed in five replicates. Numerals represent the number of plates in which primordia (pm) or fruit bodies (fb) were formed.

mation occurred with SE-1. But the subsequent growth into a mature fruit bodies was not observed (Table 1). Also, the number of primordia induced by SE-3 in each plate was greater than that induced by SE-2, but only one of them grew into mature fruit bodies. However, with 2%(w/v) SE-2, formation of complete fruit body was obtained in every plate assayed (Fig. 1). When sucrose or fatty acid was added alone to the medium, no such effect was obtained. Sucrose promoted mycelial growth but depressed fruiting. With only MA, fruit bodies developed rarely, if any, after 30-days of the incubation at 17 °C . Therefore, it was concluded that SE-2 stimulated differentiation of the fruiting bodies of P. *ostreatus* and was further examined for its effect in sawdust rice bran medium.

3.3 *Test tube and bottle assays*

With 2%(w/w) of SE-2, the number and size of fruit bodies were greater than those without SE-2 on 11th day of culture in test tubes (Fig.2-a). But SE-2 was inhibitory to linear growth of the mycelium into the medium. At the commercial scale, effect of SE-2 was apparent at 13th day of 17 °C incubation (Fig. 2-b). The size of fruit bodies were larger compared with the control. As consistent with the plate assay, SE-2 contributed to full mycelial growth, rather than increased number of fruit bodies.

4 DISCUSSION

In this study, one type of food additive surfactant was shown to be markedly stimulating not to the growth of mycelium but to the differentia-

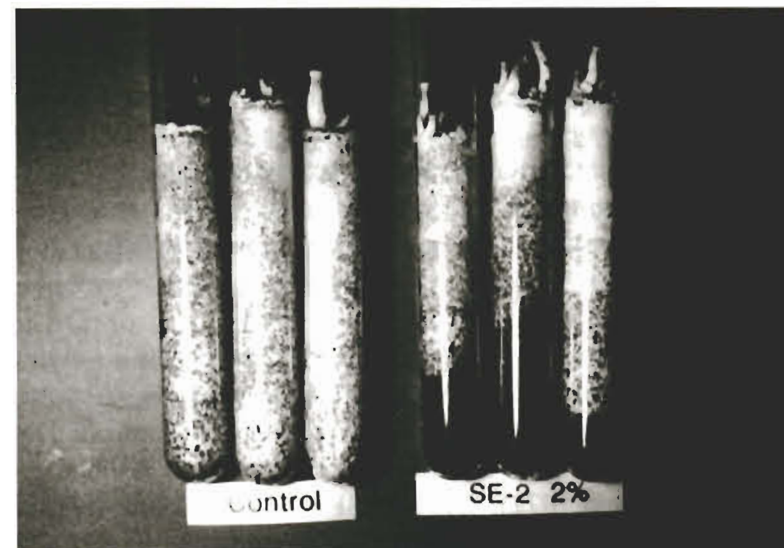

Fig. 1a. SE-2 was added to sawdust rice bran medium and *P. ostreatus* was grown as described in the Materials and Methods.

Fig. 1b. SE-2 was added to sawdust rice bran medium in 800 ml bottles and *P. ostreatus* was grown as described in the Materials and Methods. Left: control. Right: with 2%(w/v) SE-2.

tion of fruit bodies of *Pleurotus ostreatus*. Usually, high yielding substances (derived from rice, wheat, corn and soy bean) added to medium today, are rich in carbon and/or nitrogen sources to increase fruit body production as a consequence of rich vegetative growth. But the result of the present study indicated that SE did not accelerate mycelial growth. When SE-2 was present, fruit bodies differentiated even before the mycelium was fully grown to the bottom of the test tube (Fig. 1a). This means SE is not working simply as nutrient that promotes mycelial growth.

SE-3 was effective in sporphore initiation; primordia formation, but could not sustain fruit body development. In *Coprinus cinereus,* it is known that sporphore initiation and its development is controlled with independent genes since independent mutants have been isolated (Takemaru and Kamada 1971). Whether SE-1 and SE-3 affect different genes from the genes affected by SE-2 would be interesting to know.

Results of the plate assay showed that SE-2 did not increase numbers of primordia but every primordium to form perfect fruit bodies. This result is reflected again in the commercial scale bottle assay; SE-2 was more effective in promoting the growth of fruit bodies to full size rather than in increasing their numbers (Fig.1b).

The first naturally occurring substance which shows fruit body stimulation to be isolated was cerebroside (Kawai *et al.* 1985). This compound has a structure of sugar attached to lipid moiety, although the sugar itself was later proven to be not essential for activity. Sulfonated saccharides derived from lignin which are reported to activate mycelium growth and fruiting is also speculated to have surfactant activity (Inaba *et al.* 1982, Inaba *et al.* 1983). Surfactants such as SDS and Triton which do not have this sugar and lipid moiety, were also tested. But they were found not to be effective in stimulating fruiting of *P. ostreatus* (data not shown). A weak effect was observed with Tween 80 (data not shown).

Most probably, when the sucrose ester is incorporated into the mycelium, it is degraded into sugar and fatty acid by esterase. Then sucrose is taken into the cytosol to be metabolized while the fatty acid is incorporated into membranes. In *Pleurotus ostreatus*, it is reported that palmitic acid, which is a constituent of SE, is one of the most abundant fatty acids in fruit body, especially in young pilei (Kazuno *et al.* 1985) while stearic acid is the least. Therefore, it is probable that fatty acids from the sucrose ester contribute to the growth of fruit bodies.

Additionally, it is reported that in *L. edodes*, that a low temperature shift to induce fruit body development affects both glucose uptake and lipid biosynthesis (Song *et al.* 1989). In this respect, it can be said that sucrose ester of fatty acids is a substance that can affect both the phenomena at the point of fruit body differentiation. However, although there was no difference in constituent sugar and fatty acids between them, apparent differences in fruit body stimulation were observed among the three SEs. This fact indicates that the physical character of SE as surfactant played a significant role in fruit body differentiation.

From these results, it is concluded that surfactant activity is essential but not sufficient for a stimulation of fruiting. Probably, SE-2 had the right surfactant activity to injure the membrane of the mycelium and thus, provided a stress that induced fruiting. At the same time, nutrients from the injured myselium became available for fruit body growth after being metabolized as fatty acids and sucrose.

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