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Induction of fruiting in oyster mushroom (*Pleurotus ostreatus*) by polymeric 3alkylpyridinium salts

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Running title: Fruiting induction in P. ostreatus

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#### Summary

Polymeric 3-alkylpyridinium salts (poly-APS), surface-active compounds from the marine sponge *Reniera sarai*, have been shown to stimulate the fruit body formation in *Pleurotus ostreatus* mycelium. In nutrient media supplemented with poly-APS ( $\geq 0.01$  g ml<sup>-1</sup>), the formation of primordia and development of fruit bodies were detected approximately 10 d earlier than in the absence of poly-APS, and also led to a considerably larger quantity of young mushrooms. This effect appears to be specific, as other surface-active compounds, lysophospholipids and fatty acids, showed no induction of fruiting.

#### Introduction

Identification of environmental and biochemical factors that stimulate fruit body formation of mushrooms is extremely important for biotechnological and commercial production. In some parts of the world, mushrooms constitute a very important and highly appreciated source of food, and are also widely consumed for their medicinal properties. In this regard, the oyster mushroom (*Pleurotus ostreatus*) is one of the most important, being the second most important in terms of worldwide mushroom production after *Agaricus bisporus* (Kües & Liu 2000).

In recent years, several fungal or plant secondary metabolites, proteins and different synthetic compounds have been tested for their ability to stimulate the fruiting of *P. ostreatus* and other commercially important mushrooms (Upadhyay & Hofrichter 1993; Magae 1999; Sugimoto *et al.* 2001; Domondon *et al.* 2004; Berne *et al.* 2007). Among such metabolites, much attention has been devoted to surface-active compounds. In an extensive study carried out by Magae and co-workers, several natural, commercially available, and synthetic surfactants were tested for their ability to induce the fruiting process in *P. ostreatus*. The results suggested that the presence of a sugar moiety in its structure was a prerequisite for the surface-active compound to trigger the fruiting process (Magae 1999; Magae *et al.* 2005; Magae & Ohara 2006). 3-*O*-octyl- and 3-*O*-decyl-D-glucose have proved to be particularly effective in enhancing the hyphal aggregation, formation of primordia, and subsequent fruiting of the mushroom (Magae *et al.* 2005).

Polymeric alkylpyridinium salts (poly-APS, Fig 1) are water-soluble, surfactant-like compounds that can be purified in large amounts from the marine sponge *Reniera sarai* (Pulitzer-Finali). At concentrations above 230 g ml<sup>-1</sup> in water, they form large non-covalently bound supramolecular aggregates with a mean hydrodynamic radius of 23±2 nm (Sepčić *et al.* 1997a). Poly-APS display a broad spectrum of biological activities including cytotoxic (Sepčić *et al.* 1997b), haemolytic (Malovrh *et al.* 1999), anti-cholinesterase (Sepčić *et al.* 1998), antimicrobial (Chelossi *et al.* 2006), and antifouling effects (Faimali *et al.* 2003), and all of these activities are probably due to their detergent-like properties. However, they are

not effective against fungal cells (Eleršek *et al.* 2008). Because of all these characteristics, and the possibility of obtaining poly-APS-like oligomers by chemical synthesis (Mancini *et al.* 2004), these molecules could constitute good candidates as inducers of fungal fruiting.

To test this hypothesis, poly-APS were tested for the ability to induce the fruiting process in *P. ostreatus*, and their activity was compared with those of the selected commercially available surfactants, lysophospholipids and fatty acids.

### Materials and Methods

Poly-APS were purified from the marine sponge Reniera sarai, as described (Sepčić deionized water. Lysophosphatidylcholine, dissolved et al. 1997a), and in lysophosphatidylinositol, and myristic, palmitic, and stearic acids (all from Sigma), were dissolved in dimethylsulphoxide (DMSO). Each of the solutions (250 I) of poly-APS, lysophospholipids, and fatty acids were mixed with malt-extract agar (MEA; Merck) just before its solidification (at 42 °C), and the mixture poured into polystyrene Petri dishes (20 ml plate<sup>-1</sup>). In control plates, solutions were replaced by 250 I deionized water, or DMSO, as appropriate. The final concentrations of the tested compounds were 0.5, 0.1, 0.01, 0.001, and 0.0001% (w/v) for the fatty acids, 0.05, 0.01, 0.001, and 0.0001% (w/v) for the lysophospholipids, and 10, 1, 0.1, and 0.01 g ml<sup>-1</sup> for poly-APS. After solidification, all the dishes were centrally inoculated with a mycelial disc (0.5 cm diam) of *Pleurotus ostreatus*, strain Plo 5, obtained from the periphery of 7-d-old potato-dextrose agar (Difco) inoculum plates from the ZIM collection of the Biotechnical Faculty, University of Ljubljana, Slovenia (Raspor et al. 1995). Each surfactant concentration was assayed five times, and the results are presented as mean ± S.E. The plates were kept in a growth chamber in the dark at 25 °C and 70 % humidity, and the growth of the mycelium was monitored regularly until the surface of the nutrient medium was homogeneously overgrown. After 7 d, plates were transferred to 8 °C in the dark for 4 d, in order to induce the formation of primordia. The subsequent fruiting proceeded in a growth chamber with a constant temperature of 17 °C, 90 % air humidity, less

than 1000 ppm CO<sub>2</sub>, and a 12:12 h photoperiod (29.4 W m<sup>-2</sup>). The plates were monitored regularly for the formation of primordia over the following 30 d.

#### **Results and discussion**

Addition of poly-APS to the growth medium inoculated with mycelium of *Pleurotus ostreatus* did not alter the rate of mycelial growth (not shown); however, it did markedly enhance the formation of primordia, which appeared 10 d earlier than in control experiments. The average size and number of fruit bodies on the 23<sup>rd</sup> d after exposure to low temperature are shown in Fig 2. All poly-APS concentrations tested markedly enhanced the yield of fruit bodies, reaching a peak at 1 g ml<sup>-1</sup>. In contrast, no formation of primordia was observed on plates treated with either the lysophospholipids or the fatty acids. At concentrations greater than 0.01 %, all lysophospholipids and fatty acids exerted a concentration-dependent inhibitory effect on the mycelial growth of *P. ostreatus* (not shown).

In conclusion, poly-APS are shown to markedly enhance the fruiting process and yield of oyster mushrooms. In keeping with previous findings showing that pure commercial surfactants like sodium dodecyl sulphate (SDS), 3-[(3-Cholamidopropyl)dimethylammonio]-1propanesulfonate (CHAPS), 3-[(3-Cholamidopropyl)dimethylammonio]-2-hydroxy-1propanesulfonate (CHAPSO), octanoyl-N-methylglucamide (MEGA 8), nonanoyl-Nmethylglucamide (MEGA 9), decanoyl-N-methylglucamide (MEGA 10), or Triton do not induce the fruiting process in P. ostreatus (Magae 1999; Magae et al. 2005; Magae & Ohara 2006), surface-active fatty acids and lysophospholipids also proved to be ineffective. Poly-APS contain large amounts of nitrogen, which could enable them to stimulate the fruiting by acting as a nutrient supplement, as previously reported for some other nitrogen-containing compounds like amino acids (Eger 1976). Thus, surface activity of a certain compound is not a sole prerequisite for triggering fruiting of *P. ostreatus*. Our results, combined with those obtained by other authors (Magae et al. 2005), indicate that the presence of an appropriate nutrient source (e.g., a sugar or a nitrogen-containing moiety), combined with a

surfactant moiety, constitutes the necessary structural basis for the induction of fruiting in *P. ostreatus*.

Controlling of the fruit body initiation is a very important step in the production of mushrooms, and all the factors that influence this process could have potential biotechnological and commercial applications. The data presented in this paper justify the efforts to chemically synthesize larger amounts of poly-APS analogues (Mancini *et al.* 2004) and other surfactant-based compounds, which could be applied as inductors of fruiting in *P. ostreatus*, and possibly in other mushrooms, in mushroom farms.

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Figure 1. Chemical structure of poly-APS.

Figure 2. Effect of exogenously applied poly-APS on the fruiting of *Pleurotus ostreatus*. The cultivation of *P. ostreatus* on poly-APS-supplemented MEA plates was carried out as described in the Materials and Methods section. (A) Representative control and poly-APS-treated plates photographed on the 23<sup>rd</sup> d after the induction. (B) The average number of fruit bodies grown on plates supplemented with various concentrations of poly-APS on the 23<sup>rd</sup> d after induction. Numbers near the data points denote the average dimensions ± standard error (in millimetres) of respective fruit bodies.



