Potential prebiotic activity of *Pholiota nameko* water-based extracts. By D. Rodrigues[1*], A.C. Freitas[1,2], S. Sousa[3], T.A.P. Rocha-Santos[1,2], A.C. Duarte[1] and A.M. P. Gomes[3], [1]*CESAM - Centre for Environmental and Marine Studies & Department of Chemistry, University of Aveiro, Campus Universitário de Santiago, 3810-193 Aveiro, Portugal*, [2]*ISEIT/Viseu, Instituto Piaget, Estrada do Alto do Gaio, Galifonge, 3515-776 Lordosa, Viseu, Portugal* and [3]*CBQF - Centro de Biotecnologia e Química Fina – Laboratório Associado, Escola Superior de Biotecnologia, Universidade Católica Portuguesa/Porto, Rua Dr. António Bernardino Almeida, 4200-072 Porto, Portugal*
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Abstract
Mushrooms are an excellent source of bioactive compounds with a wide range of biological activities. In this study, prebiotic activity of water-based extracts from *Pholiota nameko* was assessed. Sustainable and food compatible extraction methods such as hot-water extraction (HWE) as well as enzyme-assisted extraction (EAE) and ultrasound-assisted extraction (UAE) were performed to tentatively identify new sources of prebiotic compounds. All water-based extracts, especially the HWE and UAE extracts and the EAE with Viscozyme and cellulase extracts, promoted the growth and viability of tested probiotic bacteria. Increases of 1.4 to 2 log cycles on *Lactobacillus acidophilus* La5 and *Bifidobacterium animalis* BB-12 were observed after 48h of incubation, thus revealing potential prebiotic activity for these water-based extracts from *Pholiota nameko*.

Introduction

Edible mushrooms have been recognized as a valuable source of nutrients and of bioactive compounds being increasingly appreciated for their sensory characteristics such as flavor and texture (1). Their nutritional value is due to high content of protein, fiber, vitamins and minerals associated to a low fat content (1,2). In fact, mushrooms have been considered an important natural food source but also a nutritional supplement or medicinal resource (3). Mushroom polysaccharides are potential sources of prebiotics given their composition in chitin, hemicelluloses, α & β-glucans, mannans and/or xylans; among these, the carbohydrates include ribose, xylose, fructose, mannose, glucose and trehalose (4). A prebiotic compound is defined as "a selectively fermented ingredient that allows specific changes, both in the composition and/or activity in the gastrointestinal microflora that confers benefits upon host wellbeing and health" (5). Foods that contain carbohydrates and, in particular, some types of oligosaccharides may present a potential prebiotic effect (6).

Pholiota nameko, a wood-rotting fungus, is a mushroom widely cultivated in China and Japan (7) but much less in Europe. It is known by its pleasant flavor being very popular in many Japanese dishes. Several researchers have studied its biological properties especially among constituent polysaccharides in terms of anti-inflammatory (7, 8), hipolipidemic (9), antioxidant (10) and immunomodulator activities (11).

Due to this excellent array of bioactive compounds with a wide range of biological properties the use of sustainable and food compatible extraction methods such as enzyme-assisted extraction (EAE) and ultrasound-assisted extraction (UAE) is important and these methods were therefore scrutinized in this study to search for new sources of prebiotic compounds. For EAE carbohydrate and protein degrading enzymes are normally applied. Indeed, carbohydrate degrading enzymes and proteases are enzymes that help to convert water insoluble plant material in water-soluble materials (12), improving both quantitative and qualitative extraction efficiency. The main objective of this study was to apply water-based extraction through combined EAE and UAE extraction technologies to *Ph. nameko* evaluating the prebiotic potential of each different extract obtained.

Material and Methods

Different water-based extracts *Ph. nameko* were prepared. Hot water extraction, EAE and UAE were performed according to Wang et al. (13) and Huang et al. (14) with modifications. Schematic representation of extraction procedure is displayed in Figure 1. Dried specimens of edible and cultivated *Ph. nameko* were supplied by Bioinvitro, Biotecnologia, Lda. **(**Gandra, Portugal), and were subsequently milled to less than 1.0 mm particle size.

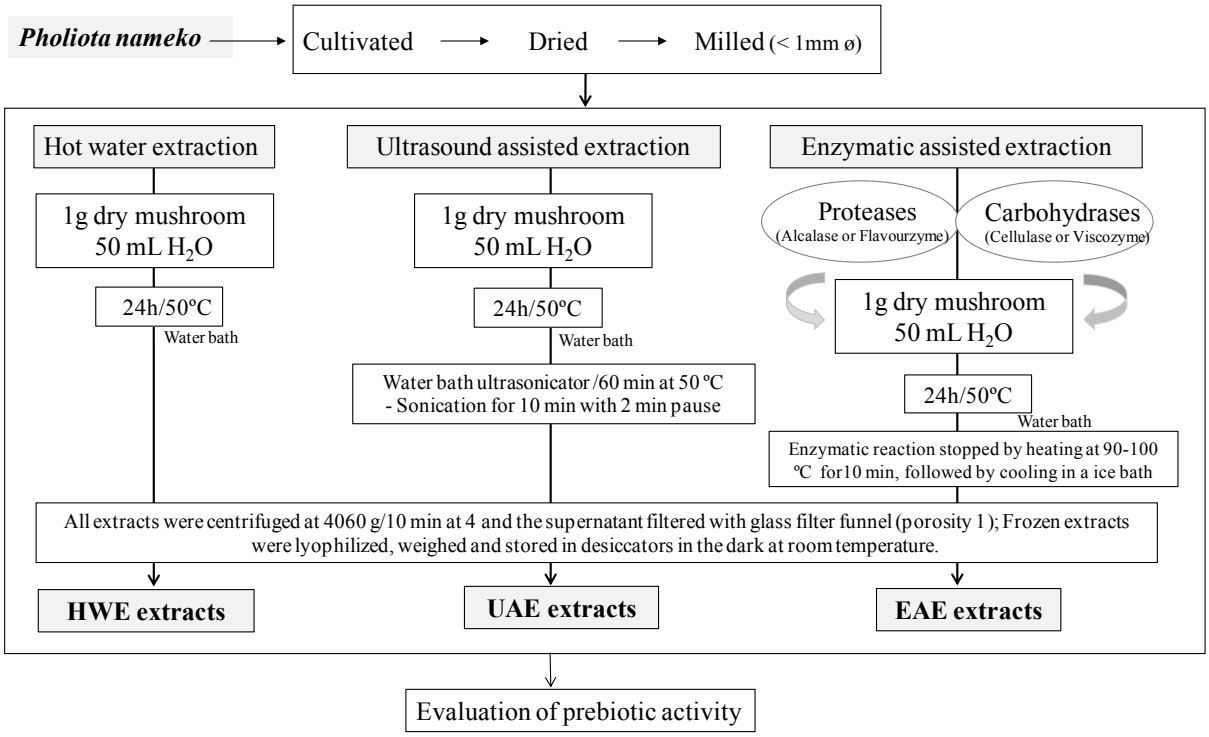

Fig 1. Water-based extraction procedures applied to *Pholiota nameko*.

The evaluation of potential prebiotic activity of mushroom extracts was performed by enumeration of viable cells of two probiotic strains namely *Lactobacillus acidophilus* La-5® and *Bifidobacterium animalis* BB-12® (CHR-Hansen, Denmark) in MRS broth without conventional carbon source and supplemented with the mushroom extracts (6%) throughout 48h at 37 ºC. Growth of both probiotic bacteria was also evaluated in MRS broth with glucose (6%), with fructooligosaccharides (FOS) (6%) as well as without glucose (negative control). All media contained 0.5 g/L L- cysteine-HCl and were inoculated with a 24-h probiotic culture (2%). Two replicas of inoculated media were incubated at 37 °C under agitation and sampled at 0, 4, 8, 12, 24 and 48 h. At each sampling time, decimal dilutions using sterile 0.1 % (w/v) peptone water were plated with 20 µL aliquots, in duplicate, on MRS agar containing 0.5 g/L L- cysteine-HCl according to Miles and Misra (15). Plates were incubated at 37 °C for 48 h under aerobic conditions for *L. acidophilus* La-5, and under anaerobic conditions for *B. animalis* BB-12 with GENbox.

Statistical analysis

A two-way analysis of variance (ANOVA) was carried out with SigmaStat™ (Systat Software, Chicago, IL, USA), to assess whether the extract and incubation time at 37 ºC were significant sources of variation at a significance level of P=0.05. Since significant differences were observed for each factor (extract and incubation time) as well as significant interactions,

one-way ANOVAs were carried out to observe if the source of carbon (glucose, FOS or extract) were statistically significant for the number of viable cells of *L. acidophilus* La-5 or *B. animalis* BB12, after 24 or 48 h of incubation, respectively. One-way ANOVAS were also performed to evaluate statistical significance of the viable cell numbers after 24 and 48h, in comparison to values at 0h.

Results and discussion

In the last years the search for bioactive polysaccharides among natural terrestrial sources has risen including among plant materials, such as cereals, vegetables, fruits, mushrooms, or in their by-products, as new prebiotic alternatives (16).

The effect of the different *Ph. nameko* extracts on the number of viable cells of *L. acidophilus* La-5 and of *B. animalis* BB-12, throughout 48h of incubation at 37 ºC, is reported in Figures 2 and 3, respectively. For comparison purposes both probiotic bacteria were also grown in the same conditions but in the absence of sugar (negative control) as well as in the presence of glucose or FOS (positive controls).

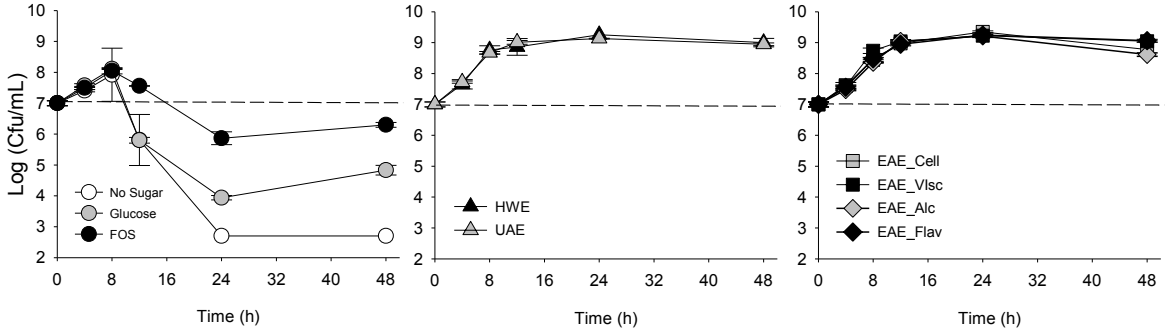

Fig. 2. Mean and standard deviation of viable cell numbers of *L. acidophilus* La-5® throughout 48h of incubation at 37 ºC in the presence or absence of sugars or mushroom extracts.

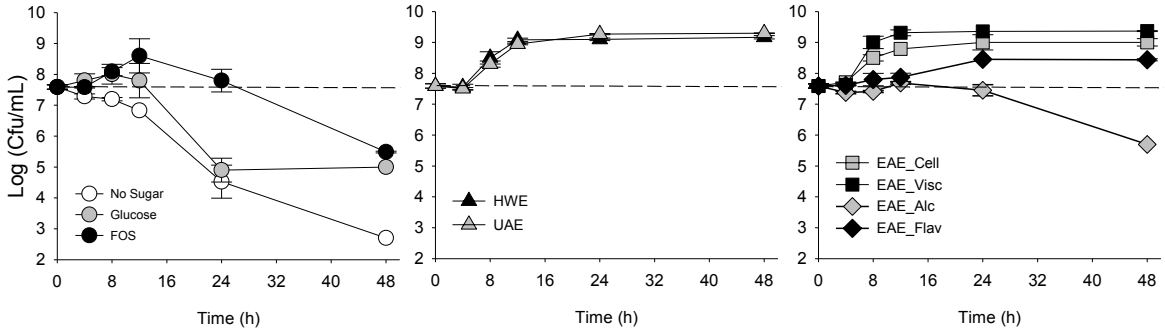

Fig. 3. Mean and standard deviation of viable cell numbers of *B. animalis* BB-12® throughout 48h of incubation at 37 ºC in the presence or absence of sugars or mushroom extracts.

For *L. acidophilus* La-5 significantly higher values ($p<0.05$) of viable cell numbers were observed for all the extracts after 24 and 48h of incubation in comparison to growth with glucose or FOS (Fig. 2). An increase of 2 log cycles was observed with HWE, UAE as well as with enzymatic extracts assisted by Viscozyme and Flavourzyme resulting in ratios of 1.3 after 48h. Slightly lower values of *L. acidophilus* La-5 viable cell numbers were observed in assisted enzymatic extracts with alcalase and cellulase especially after 48h of incubation resulting in lower ratios at this stage.

Potential prebiotic effect by the *Ph. nameko extracts* was also observed for *B. animalis* BB-12. Its number of viable cells in all extracts, except for enzymatic extract with alcalase, after 24 and 48h of incubation, were significantly higher (Fig. 3) than those obtained with

prebiotic FOS and glucose ($p<0.05$). Increments of 1.4 to 1.8 log cycles were observed with HWE, UAE as well as with enzymatic extracts assisted by Viscozyme and cellulase resulting in positive ratios after 48h.

Content and type of sugars present in the extracts are probably determinant factors for the growth and viability of probiotic bacteria because cellulose, chitin, β-glucans, α-glucans and glycoproteins have been described in the outer or inner layer of fungal cell wall being more or less extractable depending on the extraction method; water-soluble polysaccharides from mushrooms have been extracted with hot water whereas water-insoluble polysaccharides by alkali solutions (17). To our best of knowledge no studies on prebiotic potential from *Ph. nameko* exist and therefore no comparison to other research works is possible.

Despite the preliminary nature of the present study, the results show that the majority of the extracts obtained from the *Ph. nameko* possess carbon sources that can be metabolized by *L. acidophilus* La-5 and *B. animalis* BB-12. A more promising prebiotic potential was observed for HWE, UAE, and EAE with Viscozyme and cellulase. Given the reported results obtained in terms of growth promotion of *L. acidophilus* La-5 and *B. animalis* BB-12 and considering that prebiotic potential of polysaccharides and extracts from other mushroom species have been reported by literature (16, 18, 19), further *in vitro* studies such as non-digestibility and selective fermentation capacity as well as *in vivo* studies must be performed with the HWE, UAE and enzymatic extracts with Viscosyme and cellulase. The identification of polysaccharides (and associated molecular weight) and oligosaccharides in the extracts will also be determinant to understand and correlate with the prebiotic activity.

Acknowledgments

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