***Sargassum muticum* and *Osmundea pinnatifida* seaweeds from Buarcos Bay, Portugal: Potential prebiotic activity of water-based extracts.** By D. Rodrigues[1*], S. Sousa[2], A.G. Silva[2], L. Pereira[3], T.A.P. Rocha-Santos[1,4], A.M. P. Gomes[2], A.C. Duarte[1] and A. C. Freitas[1,4], [1]*CESAM - Centre for Environmental and Marine Studies & Department of Chemistry, University of Aveiro, Campus Universitário de Santiago, 3810-193 Aveiro, Portugal*, [2]*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,*[3]*IMAR-CMA (Institute of Marine Research)/MARE, Department of Life Sciences, Faculty of Sciences and Technology, University of Coimbra, P-3000-456 Coimbra, Portugal* and [4]*ISEIT/Viseu, Instituto Piaget, Estrada do Alto do Gaio, Galifonge, 3515-776 Lordosa, Viseu, Portugal*
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Abstract
Seaweeds are an excellent source of bioactive compounds with a wide range of biological activities. In this study, prebiotic activity of water-based extracts from *Sargassum muticum* and *Osmundea pinnatifida* were scrutinized. Food compatible extraction methods were performed such as hot-water extraction (HWE), enzyme-assisted extraction (EAE) and ultrasound-assisted extraction (UAE). Seaweed species, extraction mode and incubation time were significant factors for *L. acidophilus* La-5 and *B. animalis* BB-12 growth and viability over 48h of incubation at 37 ºC. *Sargassum muticum* extracts possessed higher prebiotic potential in particular for *L. acidophilus* La-5.

Introduction

Seaweeds are still an untapped source of diverse chemical compounds of interest including nutrients such as polysaccharides, proteins, minerals, vitamins and dietary fibbers among other compounds (1,2). Seaweeds such as *Sargassum* species (Phaeophyceae*,* brown algae), have been found to be good sources of dietary fiber and carotenoids which play important roles in the prevention of intestinal and neurodegenerative diseases, respectively (3,4,5). The biological importance of seaweeds can be found in recent reviews (6,7,8). Prebiotics have been defined as non-digestible compounds mainly of carbohydrate nature that selectively enhance the activity and viability of beneficial bacteria in the intestine, by providing these with fermentable substrates that lead to the production of short chain fatty acids which play an important role in gut health (9). Several health benefits have been associated to prebiotics in the large intestine such as modulation of beneficial gut bacteria, hypocholesterolemic effect, reduction of cancer risk, increase of the bioavailability and uptake of minerals (Ca and Mg) as well as reduction of the risk of obesity (9,10). Recently, marine resources have been envisaged given their rich profile in polysaccharides present in the cell walls (11).

Extraction and isolation of bioactive compounds of interest from seaweeds, able to be ingested or used for food purposes, need to rely upon compatible methods. Seaweeds are known to have a chemical and structural heterogeneous rigid wall which poses limitations to efficient extraction of the intracellular and wall compounds (12). Therefore the main objective of this study was to obtain water-based extracts using alternative approaches such as EAE and UAE from representative species of Central West Portuguese Coast (*Sargassum muticum* and on *Osmundea pinnatifida)* and to evaluate the potential prebiotic activity of each different extracts. Two carbohydrate enzymes (Viscozyme® L, Cellulase) and two proteases (Alcalase, Flavourzyme) were tested for EAE. According to Heo et al. (13), carbohydrate degrading enzymes and proteases can convert water insoluble seaweeds in water-soluble materials, improving both quantitative and qualitative extraction efficiency.

Material and Methods

Specimens of red algae (Rhodophyta, Florideophyceae) *Osmundea pinnatifida* and brown algae (Heterokontophyta, Phaeophyceae) *Sargassum muticum* were harvested in April 2012 from Buarcos bay, Portugal. The classification of seaweeds was based on *AlgaeBase* (14) and Pereira (15). The seaweeds were first washed with tap running water and then with deionised water to eliminate residues from the thalli surface and then dried in an oven at 60 ºC. The dried samples of seaweeds were milled to less than 1.0 mm particle size.

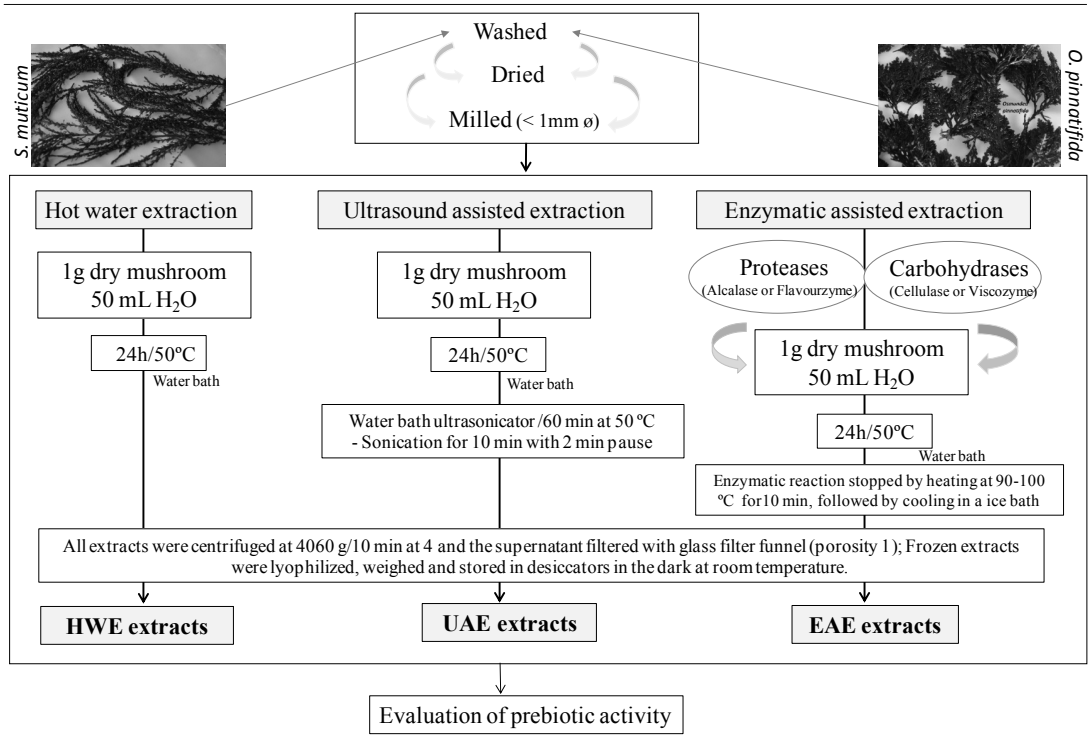


Fig. 1. Water-based extraction procedures applied to *S. muticum* and *O. pinnatifida*.

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 carbon source and supplemented with the extracts (6%) throughout 48h at 37 ºC. Growth of both probiotic bacteria were also evaluated in MRS broth with glucose (6%), with fructooligosaccharides (FOS) (6%) as well as without glucose. All media contained 0.5 g/L L- cysteine-HCl were inoculated with a 24-h probiotic culture (2%). Two replicas of inoculated media were incubated in 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 platted with 20 µL aliquots, in duplicate, on MRS agar with 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 La-5, and under anaerobic conditions for 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.

Results and discussion

The potential prebiotic effect of seaweed water-based extracts was evaluated by using pure cultures of representative beneficial bacteria including strains of lactobacilli and bifidobacteria in parallel with well-established prebiotics, such as FOS (17). In figure 2 and 3 it can be observed the viable cell numbers of *L. acidophilus* La-5 and of *B. animalis* BB-12 throughout 48h of incubation at 37 ºC in the presence of the two seaweeds species extracts. For comparison purposes, both probiotic strains were also grown under the same conditions but in the absence of sugar (negative control) as well as in the presence of glucose or FOS at similar concentrations (positive controls). Seaweed species, extraction mode and incubation time were revealed to be significant factors for both probiotic strains viability ($p<0.05$). The viable cell numbers of *B. animalis* BB-12 and *L. acidophilus* La-5 in the presence of the different *S. muticum* extracts were higher than those obtained with Glucose or FOS after 24 and 48h of incubation (Fig.2). Enzymatic extracts were able to maintain the number of viable cells over time in particular the EAE extracts with Cellulase. Regarding *L. acidophilus* La-5 significantly higher values ($p<0.05$) of viable cell numbers were observed for the majority of culture media enriched with seaweed water-based extracts after 24h of incubation in comparison to growth in media with glucose or FOS.

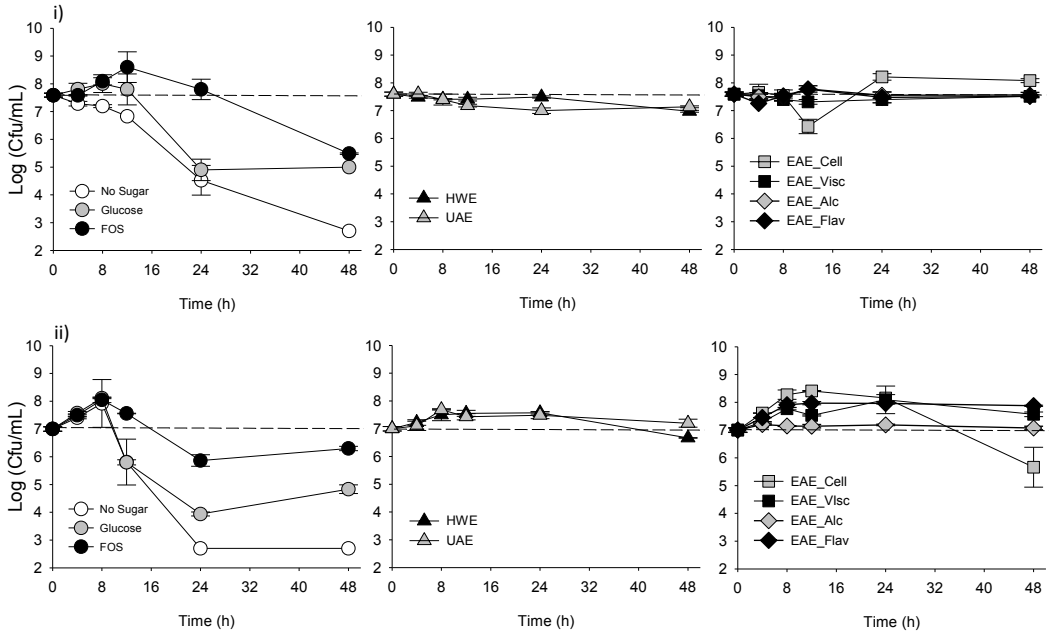
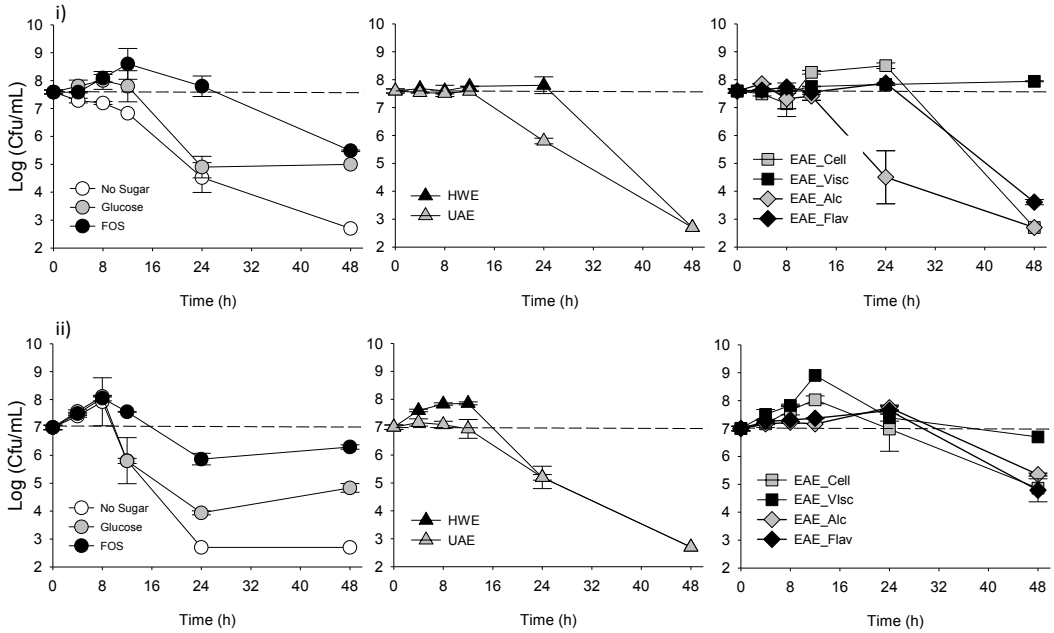


Fig. 2. Mean and standard deviation of cell counts of *B. animalis* BB-12® (i) and *L. acidophilus* La-5® (ii) throughout 48h of incubation at 37 ºC in the presence or absence of sugars or *S. muticum* extracts.

Lower potential of prebiotic activity was observed for *O. pinnatifida* extracts (Fig. 3), in particular for *B. animalis* BB-12. Despite the enhanced growth by 24 h incubation, after 48h a decrease of viable cells occurred for all extracts except for EAE with Viscozyme; end-product formation, substrate depletion or the largest amount of sulphated sugars observed in these extracts could in part justify this decrease in viable cell numbers.

According to the main results it was found that, in general, *S. muticum* extracts seemed to have a higher prebiotic potential. The viable cell numbers of *B. animalis* BB-12 and *L. acidophilus* La-5 in all *S. muticum* extracts after 48h of incubation were significantly higher than those obtained with prebiotic FOS and glucose ($p<0.05$).

Although of preliminary nature, the results show that the majority of the extracts obtained from both seaweeds possess carbon sources that can be metabolized by *L. acidophilus* La-5 and *B. animalis* BB-12. A more promising prebiotic potential was however observed for *S. muticum* extracts especially for those obtained by enzymatic action. Given the promising reported results obtained in terms of growth promotion of *L. acidophilus* La-5 and *B. animalis* BB-12 and considering the prebiotic potential of polysaccharides and seaweed extracts reported in literature concerning other seaweed species, further *in vitro* (non-digestibility and selective fermentation capacity) and *in vivo* studies must be performed with the enzymatic extracts assisted by Cellulase of *S. muticum.* The identification of polysaccharides (and associated molecular weight) and oligosaccharide fractions in the extracts derived from the enzymatic action will also be determinant to understand and correlate with the prebiotic activity.



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Acknowledgments

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