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A potential of brown rice polish as a substrate for the lactic acid and bioactive compounds production by the lactic acid bacteria newly isolated from cereal-based fermented products

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ABSTRACT

This work aims to evaluate the fermentability of brown rice polish for the growth of eleven *Lactobacillus* spp. and *Pediococcus* spp. strains in a single step process at a solid-state fermentation (SSF) conditions. In addition, technological and functional properties of the tested lactic acid bacteria (LAB) were assessed. The brown rice polish was a suitable substrate for the cultivation of newly isolated LAB without supplements. The acidification rate, lactic acid (LA) production ability, endo-xylanase and protease activities significantly ($p < 0.001$) depended on the bacterial strain and fermentation time. Some of the tested lactobacilli were the best producers of LA (up to 116.51–160.97 g/L) with higher contents of L(+)-LA (L/D ratio 1.05–4.70). In many cases, rice polish medium as a nutrient source was comparable to MRS medium for the production of metabolite with antifungal activity against *Fusarium graminearum*, *F. avenaceum* and *F. nivale*. The *L. brevis* LUHS173, *L. uvarum* LUHS245 and *P. pentosaceus* LUHS100 strains show the potential for the technological applications due to their antifungal, xylanolytic and protein degrading capacity.

1. Introduction

The rational recycling of natural by-products from food production is a very promising area. Using alternative biotechnology tools for the processing of such by-products it is possible to obtain biologically valuable natural components useful for high-value food production (Lemes et al., 2016). The “green” processing and fermentable capability of many of the agro-industrial by-products make them attractive candidates in fermentation biotechnology to produce a valuable product with multiple applications.

The fermentation with lactic acid bacteria (LAB) is an effective biotechnological process to keep and improve the safety, nutritional and functional value of products (Yonekura, Sun, Soukoulis, & Fisk, 2014). The main criteria used to select microbial cultures are desirable technological and nutritional properties such as the growth of bacterial biomass and acidification rate, and synthesis of antimicrobial and antifungal compounds (Rather et al., 2013). The ability of bacteria to grow in cereal processing by-product substrates without supplements and viability during storage is important for the development of technological and physiological properties of food. According to the literature, cereals, such as barley, wheat, and pseudocereals are potential substrates for the cultivation of microorganisms (Pelikanova, Liptakova, Valík, & Stančeková, 2011). While the by-products of rice processing consist of the protein, dietary fiber, phospholipids, essential fatty acids, vitamins, minerals, and antioxidants (Bhosale & Vijayalakshmi, 2015), it could be processed into high-value products by using fermentation technologies.

The fermented substrate is a source of microorganisms producing different enzymes useful in biotechnological applications. Enzymes, such as xylanases and proteases are examples of the technological and nutritional potential of the microbial biomass of fermented products. Xylanolytic enzymes and proteases are used in foods to eliminate antinutritional factors and increase availability of functional compounds such as soluble xylans and arabinoxylans, free amino acids, bioactive peptides are desirable (Beg, Kapoor, Mahajan, & Hoondal, 2001; Katina et al., 2012).

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Moreover, *Fusarium* fungi could cause contamination with mycotoxins in cereal grains including brown rice and their milling by-products (Desjardins et al., 2000; Lee et al., 2011). Fermentation with selected microorganisms could be one of the easiest and cheapest means of food preservation additionally imparting nutritional and organoleptic benefits to those fermented foods (Juodeikiene, Basinskiene, Bartkiene, & Matusevicius, 2012).

This work aims to evaluate the ability of brown rice polish to support the growth of newly isolated lactic acid bacteria at solid-state fermentation (SSF) conditions without the addition of hydrolytic enzymes or nutrients. In addition, technological and functional properties of tested lactic acid bacteria (LAB) were evaluated.

2. Materials and methods

2.1. Rice polishing by-products

Rice polish powder (moisture 11.38 g/100 g, protein 8.89 g/100 g, fat 1.27 g/100 g, sugars 205 mg/100 g, dietary fiber 7.46 g/100 g) obtained after polishing of brown rice was from the mill SC Ustukiu malūnas (Pasvalys, Lithuania). A sterilization (dry heating) was used for the rice polish treatment to inactivate the residual enzymes and microorganisms. Each sample was placed to open thermostable glass vessel and spread uniformly in thin layer (0.5 cm). Then, the vessels were transferred into the autoclave and heated at 121 °C for 15 min. The total average microorganisms count after polish sterilization was $< 10^{-1}$ CFU/g.

2.2. Lactic acid bacteria

Eleven lactic acid bacteria (LAB) (Lactobacillus paracasei (NBRC 15889), Lactobacillus uvarum (strain 8), Lactobacillus farraginis (NRIC 0676), Lactobacillus brevis (ATCC 367; n = 2), Lactobacillus plantarum (WCFS1), Lactobacillus plantarum (JCM 1149), Pediococcus acidilactici (DSM, 20284; n = 2), Pediococcus pentosaceus (ATCC 25745; n = 2) previously isolated from a spontaneously fermented wheat flour media and identified by phenotypic and molecular techniques (Bartkiene et al., 2017) were used for the fermentability assay. Prior to each of the experiments, all bacteria were propagated twice in MRS broth (CM 0359, Oxoid Ltd., Hampshire, UK) at 30 °C for 48 h.

2.3. Evaluation of acid and temperature tolerance and gas production ability

The tested LAB strains were screened for their growth at different temperatures and ability to survive at low pH conditions. The temperature tolerance assay was monitored at 10 °C, 30 °C, 37 °C, and 45 °C for 24 h in MRS broth. The turbidity of the bacterial suspensions was measured as the absorbance at 420–580 nm using the wideband filter in the Bioscreen C instrument, an automated turbidity reader (Labsystems Oy, Helsinki, Finland). Each strain was tested in three replicates using 100-well Honeycomb microplates. The ability of the strains to survive at low pH was evaluated by measuring survival at 37 °C for 3 h in MRS broth acidified with 1 M HCl to final pH 2.5. Aliquots of 0.1 mL were then removed at constant intervals (0, 1, 2, 3 h) for determination of total viable count. After appropriate dilutions (up to 10^{-4}–10^{-8}) cells were plated in duplicate on MRS agar to be incubated at 30 °C for 48 h. The count of viable cells was expressed as a log of colony forming units per milliliter (CFU/mL). Additionally, a Durham tube method for testing of gas production by LAB in MRS broth (for 24 h at 30 °C) was used.

2.4. Evaluation of rice polish fermentability

For the fermentation assay, the sample was prepared by mixing sterilized rice polish powder with distilled water at a ratio 1:3, wherein 2% (w/v) of the pure culture of each LAB strain was inoculated. After incubation for 60 h at 30 °C, the sample (10 g) was mixed with 90 mL of saline (9 g/L NaCl), and the serially diluted bacterial suspension was further used for the estimation of the number of viable cells. Sterile MRS agar (CM0361, Oxoid) was used for the evaluation of bacterial growth in Petri plates under anaerobic conditions for 72 h at 30 °C. The total titratable acidity (TTA) was determined using a 10 g sample homogenized with 90 mL of distilled water, and the value is expressed as the volume (mL) of 0.1 M NaOH required to titrate 100 g of sample to pH 8.3 using phenolphthalein as an indicator. The pH values were measured using the pH-meter and a pH electrode (Sartorius, Goettingen, Germany). The acidity characteristics and microbial counts were evaluated after each 12 h of fermentation.

2.5. Evaluation of D-/L-lactic acid production

The concentration of LA was determined using the D-/L-lactic acid Megazyme kit (Megazyme Int. Ireland Ltd., Wicklow, Ireland). For the analysis, fermented sample (10 g) was centrifuged (4500 rpm, 20 min), and 1 mL of the supernatant was diluted to 100 mL with distilled water. Each sample was analysed with the kit according to the manufacturer's instructions.

2.6. Determination of amino acid profile

Amino acids (AA) content in control and fermented rice polish samples was analysed by ultrafast liquid chromatography (UFLC) with automated o-phthalaldehyde (OPA)/9-fluorenylmethyl chloroformate (FMOC). Standard solutions of the amino acids alanine (ALA), aspartic acid (ASP), arginine (ARG), cystine (CYS), glycine (GLY), valine (VAL), leucine (LEU), isoleucine (ILE), threonine (THR), serine (SER), proline (PRO), methionine (MET), glutamic acid (GLU), phenylalanine (PHE), lysine (LYS), histidine (HIS), tyrosine (TYR), and tryptophan (TRP) were analysed (A9781 Sigma-Aldrich, Germany). For the analysis, the sample (1 g) was hydrolysed with 7.5 mL of 6 M HCl for 24 h at 37 °C. After centrifugation (5000 rpm, 20 min) 2.5 mL of the solution was mixed with 7.5 mL of deionized water. Prior to injection, all samples were filtered through 0.45-μm filters. The AA were separated with UHPLC column YMC-Triart C18 (1.9 pm, YMC co. ltd.) using a UFLC instrument (Shimadzu, Japan) equipped with the fluorescence detector RF-20Axs and pre-treatment function equipped automatic injector SIL-30AC (Shimadzu, Japan). Analytical conditions were as follows: mobile phase: solvent A (20 mmol/L potassium phosphate buffer (pH 6.5), solvent B (45/40/15 acetonitrile/methanol/water); flow rate: 0.8 mL/min, column temperature 35 °C; detection: RF-20Axs Ex. at 350 nm, Em. at 450 nm to Ex. at 266 nm, Em. at 305 nm (9.0 min). A five-level calibration set was used, covering a concentration range of 0.006–0.20 μmol/mL, except for alanine and cysteine, each covering a concentration range of 0.06–1.00 μmol/mL.

2.7. Determination of xylanase and protease activities

The xylanase and protease activities of LAB were evaluated during 60 h of fermentation. The sample (10 g) was mixed with 20 mL of distilled water or 50 mM potassium phosphate buffer (pH 7.5), respectively, and centrifuged (4500 rpm, 20 min). The obtained supernatants as enzyme solutions were used for activity assay. The xylanase activity was determined by the reducing sugars assay (Miller, 1959). One unit of xylanase activity was defined as the amount of enzyme required to release 1 μmole of xylose reducing sugar equivalents per minute from the birchwood xylan (0.5%) under the assay conditions (pH 4.5, 37 °C). The mode of action of LAB protease was determined by Sigma-Aldrich non-specific protease assay using casein (0.65%) as substrate. One unit of enzyme activity was defined as the amount of enzyme that liberates of equivalent of 1 μg of tyrosine per minute from the substrate (casein) under the conditions of the assay (37 °C and pH 7.5) and was reported