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Co-fermentation of onion and whey: A promising synbiotic combination

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ABSTRACT

Juice from three different onion varieties was mixed with sweet whey and used as growth substrate for four lactic acid bacteria strains, isolated from agri-food by-products, to evaluate the possibility to exploit such substrates, known to be reach in bioactive molecules, as fermented drinks for human consumption. Results show good growth performance for *Lactobacillus fabifermentans*, *L. plantarum* and *Streptococcus macedonicus*. On the contrary *S. thermophilus* did not grow in the mixture while *S. macedonicus* did not develop in pure onion juice. After 48 h the overall sugar content decreased significantly. In particular, glucose was not utilized while inulin was completely preserved. Moreover, MS/MS analysis revealed the presence of the rare trisaccharide lactosucrose.

In the light of these considerations, the formulation obtained may be considered a potential synbiotic product with pleasant taste and beneficial effects for consumers and also an eco-friendly solution to convert an agro-food by-product into value added products.

1. Introduction

Currently, the research of new functional foods is focused on synbiotic obtained by combining probiotics and prebiotics in order to further improve human health benefits (Krumbeck, Maldonado-Gomez, Ramer-Tait, & Hutkins, 2016; Pandey, Naik, & Vakil, 2015).

Lactic acid bacteria (LAB) are the most widely used microorganisms for production of fermented foods (Kumar, Vijayendra, & Reddy, 2015) for their capability to enhance shelf-life, safety, organoleptic and nutritional properties (Lante, Nardi, Zocca, Giacomini, & Corich, 2011). They are widely recognized for their beneficial effects towards gastrointestinal diseases, human immune system, lactose intolerance, allergies and fungal infections (Masood, Qadir, Shirazi, & Khan, 2011) as a consequence of their metabolic activities, immunomodulation, and interaction with the intestinal microbiota (De Almada, De Almada, Martinez, & De Souza Sant'Ana, 2015; Parvez, Malik, Ah Kang, & Kim, 2006). The key role of LAB in vegetable fermentation has been definitely established (Tamang, Watanabe, & Holzapfel, 2016). During growth, microbial metabolism transforms plant material leading to the production of bioactive compounds and/or improve the final pleasantness of the foods (Di Cagno, Coda, De Angelis, & Gobbetti, 2013). Spontaneously fermented foods (Lucena-Padrós & Ruiz-Barba, 2016; Paramithiotis, Doulgeraki, Karahasani, & Drosinos, 2014; Paramithiotis, Kouretas, & Drosinos, 2014; Pogačić et al., 2010) or even by-products from food production (Bovo et al., 2012; Favaro, Corich, Giacomini, Basaglia, & Casella, 2013) contain autochthonous microbial communities that constitute the source for the isolation of new potentially interesting bacteria and yeasts.

Onion (*Allium cepa* L.), which is taxonomically included in the *Liliaceae* family, is a vegetable whose cultivation is widespread worldwide and accounts for more than 88 million t produced in 2014 (FAO, 2017). Compounds from onion have been reported to have a range of health benefits including anticarcinogenic, antiplatelet, antithrombotic, antiasthmatic and antibiotic activities (Griffiths, Trueman, Crowther, Thomas, & Smith, 2002). Onions contain also prebiotic substances such as fructooligosaccharides (FOS) and inulin (Sangeetha, Ramesh, & Prapulla, 2005) which are indigestible to humans enzymes but promote growth and activity of bacterial strains and simultaneously enhance health benefits in the large intestine (Al-Sheraji et al., 2013). Moreover, lactic acid fermentation can reduce the typical pungency flavor of raw onions leading to a more favourable product for the consumers (Roberts & Kidd, 2005).

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Sweet whey is the main by-product of cheese production, a highly polluting material produced in high amounts, since only about 10% of the milk is transformed in cheese (Ryan & Walsh, 2016). It is rich in fermentable sugars (ca. 5% lactose) and contains numerous bioactive compounds (Madadlou & Abbaspourrad, 2016).

The present work is aimed at developing a new functional food enriched in prebiotics and lactic acid bacteria by co-fermenting onion juices and sweet milk whey with *Lactobacillus* and *Streptococcus* strains. After evaluating microbial growth performance in juices of different onion varieties, the characteristics of the fermented products have been investigated in order to formulate a potential synbiotic product that, besides being beneficial for consumers, can convert an agro-food by-product into value added products.

2. Materials and methods

2.1. Strain selection and culture conditions

The lactic acid bacteria (LAB) used in this study were from our laboratory collection, namely *Lactobacillus fabifermentans* T30PCM01 (DSM 28391) (Treu, Vendramin, Bovo, Giacomini, & Corich, 2014) and *L. plantarum* T30PCM38 (DSM 28393) (Campanaro et al., 2014) isolated from grape marcs (Maragkoudakis et al., 2013), *Streptococcus macedonicus* 33MO (Vendramin et al., 2014) and *S. thermophilus* TH1436 (Treu et al., 2014) isolated from dairy products.

Lactobacilli were grown in de Man, Rogosa and Sharpe (MRS) broth (Oxoid) at 30 °C while streptococci were cultivated in M17 medium (Oxoid) supplemented with 0.5% lactose at 37 °C. Growth in liquid media was measured by optical density at 600 nm (OD_{600}).

In order to use the same amount of bacteria for each experiment, Cryobeads, porous beads intended as carriers to support the viability of microorganisms during storage (Thermo Fisher Scientific, Rodano, IT) were prepared according to manufacturer's instruction using stationary phase cultures and stored at −80 °C. The number of bacterial cells adherent to each Cryobead was verified cells by plate count to be 10^7. One bead was used as inoculum for each experiment by transferring it into the growth substrate.

2.2. Preparation of onion juices

Onions from red, white and yellow varieties (RO, WO and YO, respectively) were purchased in a local market and stored at 4 °C in the dark until required. Onions were peeled of their dry outer skin, destemmed and finally processed in a centrifugal juicer (Moulinex JU655, Groupe SEB, Ecully, France) to obtain juice (Tinello & Lante, 2017). After pH measurement, juices were centrifuged at 3864*g* for 15 min at 4 °C, filtered through a Millipore 0.45 µm filter membrane (Merck Millipore, Billerica, MA, USA) and stored at −20 °C in the dark.

2.3. Fermentation of onion juice

Microbial cultures were grown in 15-ml sterile falcon tubes without agitation. Each strain was inoculated into 10 mL of raw onion juice at the final concentration of 10^6 cells/mL by adding one Cryobead to each tube. Samples of raw onion juice without inoculum were used as control.

Tubes were incubated at 30 °C for lactobacilli and 37 °C for streptococci. Each experiment was repeated three times. After 96 h samples were collected for microbiological and chemical analyses.

2.4. Co-fermentation of onion and whey

Milk whey powder (Lactalis, Laval, France) was dissolved into deionized water to 20% w/v in 250-mL flasks and pasteurized for 15 min at 72 °C (Maragkoudakis et al., 2016).

Fermentation media were prepared by mixing sweet whey with onion juice at 1:1 ratio. Each strain was inoculated into the mixture at a concentration of 10^6 cells/mL and incubated for 48 h at 30 °C or 37 °C, depending on the species. One sample of medium without inoculum was used as negative control. Each experiment was repeated three times.

2.5. Microbiological analyses

Viable cell counts were determined by the spread plate method. Samples were diluted in sterile saline solution (0.9% NaCl w/v) and spread on plates. Plate Count Agar (Oxoid) was used for total bacterial counts, incubated at 30 °C for 24 h.

2.6. High performance liquid chromatography (HPLC) analysis of sugars

Sugars (fructose, galactose, glucose, inulin, and sucrose) contents were determined by HPLC analysis using a SpectraSYSTEM™ UV6000LP HPLC system (Thermo Finnigan, San Jose, CA, USA) with diode-array detection and an Aminex HPX-87C anion exchange column (Bio-Rad, Hercules, CA) in accordance with the method proposed by Kagkli, Corich, Bovo, Lante, and Giacomini (2016). Before injection into the column, samples were filtered through Millipore 0.22 µm filter membranes (Merck Millipore, Billerica, MA, USA). The mobile phase was deionized water, the temperature of analysis was 85 °C and the flow rate was 0.6 mL/min. Sugars were identified by comparing their retention times with those of commercial standards.

2.7. Tandem mass spectrometry (MS/MS) analysis

Further analysis of the peaks collected from the HPLC elution of the fermented mixtures of sweet whey and yellow onion juice (YOW) with *L. fabifermentans, L. plantarum* and *S. macedonicus* (retention time of 9.02 min) was made by MS/MS analysis using a Thermo Scientific™ LTQ Orbitrap XL™ Hybrid Ion Trap-Orbitrap Mass Spectrometer system (Thermo Fisher Scientific, Waltham, MA, USA) with Fourier Transform (FT) detector. Prior to analysis the sample was dissolved in methanol/0.1% v/v formic acid (1:1 v/v). The flow rate of the constant infusion was 3 µL/min. The capillary voltage and temperature were 4 kV and 200 °C, respectively. Identification of the compounds composing the peak was performed according to the mass-to-charge ratio (m/z) of its corresponding fragment ions.

2.8. Statistical analysis

Data, which were presented as means ± standard deviation (SD) of three replicates, were subjected to one-way analysis of variance (ANOVA), after verifying the normal distribution and homogeneity of variance, using the PROC GLM of SAS (Statistical Analysis System, 2013). The model included the onion cultivars and LAB strains as fixed effects. Differences among means with $P \leq .05$ were accepted as representing statistically significant differences in accordance with the Tukey's multiple range test.

3. Results and discussion

3.1. Evaluation of onion juices

Firstly, WO, RO and YO juices were assessed for their suitability as substrate for LAB growth, since it is well known that these microbes have complex nutritional requirements (Özcelik et al., 2016).

Four strains were chosen: *L. fabifermentans* T30PCM01 and *L. plantarum* T30PCM38 isolated from grape marcs; *S. macedonicus* 33MO and *S. thermophilus* TH1436 coming from dairy products. LAB belonging to the genera *Lactobacillus* and *Streptococcus* are widely recognized for their health benefits as probiotics (Masood et al., 2011; Parvez et al., 2006).