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Note

Effect of protein hydrolizates on growth kinetics and aminopeptidase activities of some *Bifidobacterium* species

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ABSTRACT

This study evaluated the effect of a new hydrolizate from poultry feathers on growth kinetics and aminopeptidase activities of eight bifidobacteria compared with common peptones. The growth kinetics results suggest that the experimental hydrolizate could be a cheaper medium ingredient without affecting the modulation of common aminopeptidase activities.

Bifidobacteria are probiotics microorganism widely used in food industries [1]. They are a nutritionally heterogeneous group, whose growth is often slow or limited on synthetic media lacking of a growth-promoting factor [2]. Although Bifidobacteria are able to use ammonium salts as their only source of nitrogen [3], the supplementation of peptides and amino acids is considered requirement for the economical production of these strains [4]. Lazzi and colleagues [5] demonstrated that also poultry feathers enzymatic hydrolizate could be a suitable nitrogen source for the production of microbial biomass with improved viability. Chemical composition of poultry feathers hydrolizate has revealed that it is poor in free amino acids and is mainly composed by peptides [5]. To deepen this aspect, in this work, the growth performances of poultry feathers hydrolizates on eight *Bifidobacterium* strains belonging to different species were compared with two commercial hydrolizates, i.e. Tryptone and Peptone. The maximum cell density (OD_{max}) and the maximum specific growth rate (μ_{max}) were used to determine the nitrogen source preferred by each species tested. Moreover, in order to investigate the effect of the growth condition on the modulation of aminopeptidase pattern, the aminopeptidase activities (AA) of each strain in each condition were studied. Bifidobacterial proteolytic system is poorly investigated in contrast to the huge research works about lactic acid bacteria proteolytic system. Instead this is an important topic because more and more often bifidobacteria are added to several kinds of foods rich in proteins such as acid milk, cheese, soy based food etc [6–8]. The knowledge of aminopeptidase activities induced by different growth condition is useful because it could affect the enzymatic activities in the food matrix [4]. Cheng and Nagasawa [9] found that in cell extract of *Bifidobacterium breve* there were aminopeptidase and iminopeptidase, El-soda and colleagues [10] discovered that *Bifidobacterium infantis*, *Bifidobacterium longum*, and *Bifidobacterium adolescentis* show amino-, di-, tri-, and carboxy-peptidase activities. By sequencing the genome of *B. longum* NCC2705 more than 20 predicted peptidases were found that could provide amino acids from proteinaceous substrates in the gastrointestinal tract and vagina, where carbohydrates are less abundant [11].

Eight strains of *Bifidobacterium* genus were used in this work. *Bifidobacterium pseudolongum* B340, *B. longum* B350, *Bifidobacterium angulatum* B397, *Bifidobacterium pseudocatenalatum* B328, *Bifidobacterium bifidum* B382 belonging to the collection of Department of Food Science (University of Parma) [12]. Reference strains, *Bifidobacterium animalis* subsp. *animalis* ATCC25527,

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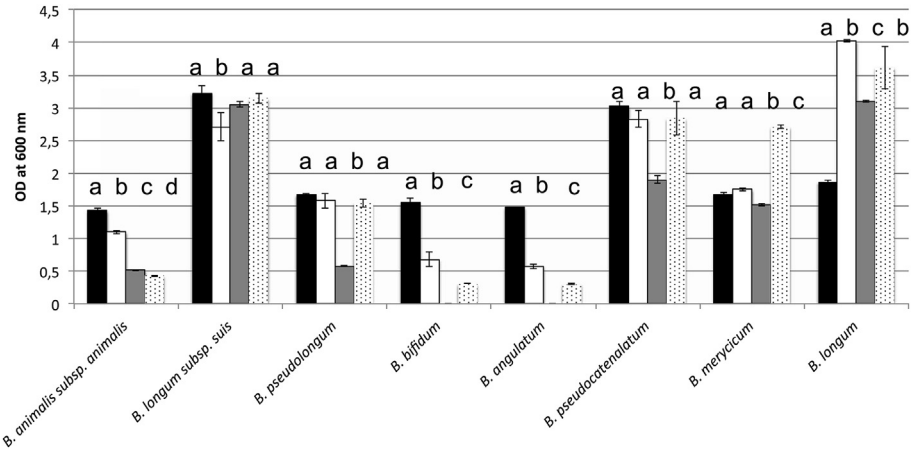

Fig. 1. OD_{max}. The maximum cell density (OD_{max}) of eight strains considered in this work. MRS (Black bars), MRSN-6L (white bars), MRSN-PEP (light gray bars) and MRSN-TRY (dotted bars). The bars bearing different letters are significantly different by Tukey's test ($p < 0.05$). All the data represent the means ± standard deviation.

Bifidobacterium longum subsp. *suis* LMG21814, *Bifidobacterium merycicum* LMG11341 were purchased from: the "American Type Culture Collection" (ATCC, Manassas, U.S.A., http://www.atcc.org/) and "Belgian co-ordinated collection of microorganism" (LMG, Gent, Belgium, http://bccm.belspo.be/). All the strains were maintained as culture stocks in 15% glycerol (w/v) at −80 °C, and routinely grown for 48 h in anaerobic conditions at 37 °C in MRS (Oxoid Italia, Milano, Italy) with cysteine hydrocloride at 0.05% (Sigma–Aldrich Corporation, St. Louis, Missouri, USA) (MRS).

Four different growth media were used in this study: MRS (Oxoid), three media based on MRS without the major nitrogen sources [5] (MRSN) supplemented with 22 g/L of FFP 6L (6L) from poultry feathers, Tryptone (TRY) (Oxoid) or Peptone (PEP) (Oxoid). MRSN was composed by (g/l): glucose, 20.0, dipotassium hydrogen phosphate, 2.0, sodium acetate trihydrate, 5.0, triammonium citrate, 2.0, magnesium sulfate eptahydrate, 0.2, manganese sulfate tetrahydrate, 0.05, complex nitrogen source, 22.0 g/l (referred to as peptone, yeast extract, "Lab Lemco" powder) and TritonX100, 1 ml/l.

Their growth was monitored during 66 h by reading turbidity at 600 nm (OD_{600}) to obtain growth curves for each strain in each cultured medium. The maximum cell density, OD_{max}, was determined as the maximal OD value reached at the stationary phase. The maximum specific growth rate, μ_{max}, was determined by calculating the slope of the exponential growth phase ($\mu_{max} = \Delta \ln(OD600)/\Delta t$, where t is time and expressed as h^{-1}). Each growth curve was carried out in duplicate.

From the culture of each strain in MRS, MRSN-6L, MRSN-TRY, MRSN-PEP cell extract was obtained. After three-repeated subculture in each media, cells extracts (CE) were obtained following the methods of Chen and Steele [13]. Protein concentrations were determined as using the Bradford commercial kit (Bio-Rad Laboratories, Hercules, Calif.) with bovine serum albumin (Sigma–Aldrich) as a standard.

Aminopeptidase activity (AA) was investigated against five different chromogenic substrates: β-naphthylamide (β-NA) derivates of L-anomers of leucine (Leu), lysine (Lys), proline (Pro), glycine–proline (Gly–Pro) and phenilalanine–proline (Phe–Pro). Each CE was incubated with 0.650 mmol/L solutions of β-naphthylamide derivates (Bachem Feinchemikalien AG, Switzerland) and phosphate buffer at 37 °C for proper time to maintain linear range of the reaction: for Gly–Pro and Leu 30 min and for Phe–Pro, Lys and Pro 3 h. The reaction was stopped adding 250 μL of 2.0 mol/L HCl. The degree of hydrolysis was determined by measuring the colored product of an azocopulation reaction by reading spectrophotometrically at 580 nm [14].

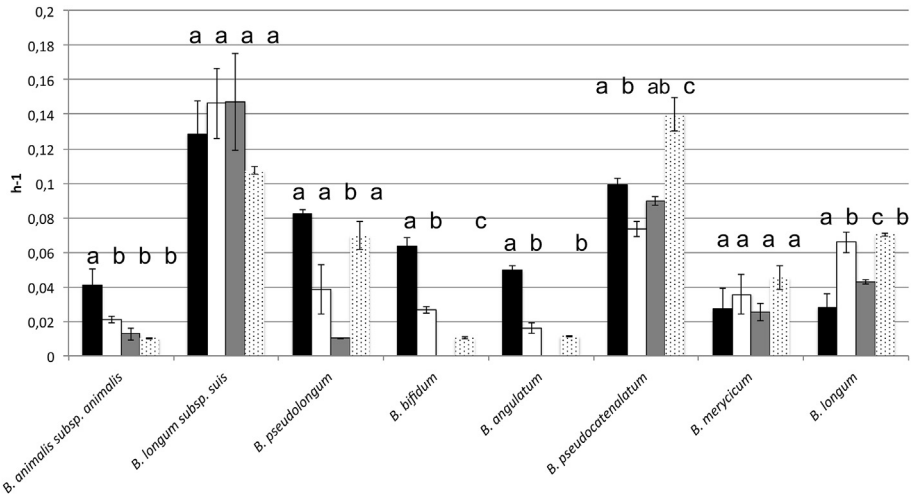

Fig. 2. μ_{max}. The maximum specific growth rate (μ_{max}, h^{-1}) of eight strains considered in this work. MRS (Black bars), MRSN-6L (white bars), MRSN-PEP (light gray bars) and MRSN-TRY (dotted bars). The bars bearing different letters are significantly different by Tukey's test ($p < 0.05$). All the data represent the means ± standard deviation.

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