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FEMS Microbiology Letters 247 (2005) 231–239

FEMS MICROBIOLOGY Letters

www.fems-microbiology.org

Phytase activity as a novel metabolic feature in *Bifidobacterium*

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Received 14 March 2005; accepted 5 May 2005

First published online 23 May 2005

Edited by B. Ward

Abstract

Phytase activity has been detected for the first time in *Bifidobacterium* spp. These bacteria were able to dephosphorylate phytic acid (myo-inositol hexaphosphate, IP_6) and generate several myo-inositol phosphate intermediates (IP_3–IP_5). *B. globosum* and *B. pseudocatenulatum* were optimally active at neutral-alkaline pH and *B. adolescentis*, *B. angulatum* and *B. longum* at acid pH. *B. pseudocatenulatum* showed the highest levels of phytase activity. This species produced maximum activity in the exponential phase of growth and when fructo-oligosaccharides were used as carbon source in the culture medium. The potential role of phytase activity from *Bifidobacterium* spp. in the reduction of the antinutritional properties of IP_6 is discussed.

Keywords: *Bifidobacterium*; Phytase; Phosphatase; Phytic acid; Antinutrient

1. Introduction

The human gut is populated by at least 500 different species, which coexist in dynamic equilibrium with the host. The beneficial microbial groups develop an array of metabolic, trophic and protective functions, which have profound repercussions on human health [1]. Among them, nutrient metabolism represents an important biochemical activity of the human body and results in salvage of energy, generation of absorbable compounds and production of vitamins and other essential nutrients [2,3]. As a consequence, dietary strategies that favour the prevalence of health-promoting intestinal bacteria have been developed. These include the use of prebiotic oligosaccharides and selected bacterium strains (probiotics) as food supplements [4]. Fructooligosaccharides (FOS) are examples of prebiotics that stimulate the growth and metabolism of bifidobacteria [5]. Bifidobacteria selectively colonize the intestinal tract of breast-fed infants and are also relevant colonic bacteria in adults [6,7]. This group is considered to be one of the major microbial stimuli for newborns and one of the most important sources of probiotics. However, the information about the metabolic activities of bifidobacteria that could serve nutritional functions is still limited and mostly focused on polysaccharide degradation [8]. The metabolism of these compounds leads to the generation of small free fatty acids (butyrate, propionate, and acetate), which are utilized as energy sources and are thought to play additional roles, for instance in the solubilization and absorption of minerals [9,10].

Phytic acid (myo-inositol hexaphosphate, IP_6) or phytate is the primary storage form of phosphorus in plant seeds and is associated with fibre in many foods,

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0378-1097/$22.00
doi:10.1016/j.femsle.2005.05.008

such as soy- and cereal-based products. It consists of an inositol, which is a hexahydroxycyclohexane in chair conformation, with six phosphate ester bonds. The phosphate groups confer on it a high negative charge and therefore a strong chelating ability, which reduce the dietary bioavailability of amino acids and minerals such as Ca^{2+}, Zn^{2+} and Fe^{2+} [11]. However, the antinutritional properties of phytates can be reduced through their enzymatic dephosphorylation into lower phosphorylated products [12]. Furthermore, the generation of these intermediate products (IP_{1-4}) and myo-inositol could also have relevant health implications, as they are involved in the regulation of vital cellular functions [13].

Phosphatases constitute a diverse group of enzymes that catalyze the hydrolysis of phosphomonoester bounds of a wide variety of phosphate esters. Phytases are a subgroup of phosphatases with general preference for phytate, which is hydrolysed in a stepwise manner generating phosphoric acid and myo-inositol phosphates [14]. Non-specific acid phosphatases constitute another subgroup of phosphatases, which show high hydrolysis rates with monophosphorylated compounds but low level of activity against phytate. However, this group of enzymes could also contribute to the hydrolysis of myo-inositols with lower number of phosphate groups [15–17]. Phytases are particularly important in human nutrition for their possible role in the degradation of phytate during both food processing and gastrointestinal transit [18]. Previous studies have demonstrated that the degradation of phytate in the stomach and intestine is mainly due to dietary phytases and, probably, to the metabolic activity of the colonic microflora [18,19]. So far, the only phytic acid degrading bacteria identified in human faeces are members of the genera *Bacteroides* and *Clostridium* and the Gram-negative bacteria *Escherichia coli* and *Klebsiella pneumoniae* [20,21]. This biochemical property has not been attributed to intestinal isolates of the genera *Lactobacillus* and *Bifidobacterium,* which are important integrants of the gut microflora and the preferred source of probiotics. This activity has only been screened in *Lactobacillus* strains isolated from food fermentations. These isolates rarely produce phytase activity although they normally possess non-specific acid phosphatase activity [15,17,22].

In this work, phytase and general phosphatase activities have been screened in different *Bifidobacterium* spp., representative of common intestinal isolates and probiotic species. The ability of certain species to reduce the levels of phytate and generate lower phosphorylated derivatives was detected for the first time. The optimal conditions for phytase activities as well as the study of the environmental factors that regulate their synthesis are reported, providing relevant information about their potential as novel technology or probiotic traits.

2. Materials and methods

2.1. Bacterial strains and culture conditions

The *Bifidobacterium* strains used in this work are listed in Table 1. The bacterial strains were routinely cultured in modified Garche broth [23]. For enzyme activity assays and determinations of *myo*-inositol phosphates,

Table 1
Phytase and phosphatase activities of different *Bifidobacterium* strains

Strain	Specific activities (U mg protein^{-1})				Hydrolysis (%)	Generation (%)		
	Phytase[a] at 50 °C	Phytase[b] at 37 °C	Phosphatase[c] at 50 °C	Ratio[d]	IP_6[e]	IP_5	IP_4	IP_3
B. adolescentis ATCC 15703	0.38	0.08	0.17	2.24	5.93	ND[f]	1.43	1.40
B. angulatum ATCC 27535	0.33	0.08	0.06	5.50	8.76	ND	4.42	4.14
B. animalis DSM 10140	0.54	0.20	0.40	1.35	3.70	ND	0.99	1.41
B. animalis DSM 20104	0.44	0.49	0.36	1.22	3.70	0.43	0.54	2.88
B. breve ATCC 15700	0.01	0.00	0.08	0.13	0.56	0.30	0.47	–
B. catenulatum ATCC 27539	0.10	0.03	0.08	1.25	5.96	ND	1.87	2.05
B. globosum DSMZ 20092	0.61	0.04	0.47	1.30	8.00	ND	0.51	1.42
B. longum ATCC 15707	0.26	0.18	0.06	4.33	12.81	0.41	2.02	1.08
B. pseudocatenulatum ATCC 27919	3.43	2.01	0.52	6.60	100.00	ND	0.90	5.88

[a] Phytase activity was determined using sodium phytate as substrate. One unit of phytase activity (U) was defined as the amount of enzyme that produces 1 μmol of inorganic phosphorous per hour at 50 °C. Data are the mean of two independent determinations. The standard deviations of the means were less than 0.10.

[b] Phytase activity was determined using sodium phytate as substrate. One unit of phytase activity (U) was defined as the amount of enzyme that produces 1 μmol of inorganic phosphorous per hour at 37 °C. Data are the mean of two independent determinations. The standard deviations of the means were less than 0.10.

[c] Phosphatase activity was determined using *p*-nitrophenyl phosphate as substrate. One unit of phosphatase activity (U) was defined as the amount of enzyme that produces 1 μmol of *p*-nitrophenol per hour at 50 °C. Data are the mean of two independent determinations. The standard deviations of the means were less than 0.03.

[d] Ratio specific phytase activity/specific phosphatase activity at 50 °C.

[e] IP_3 to IP_6: myo-inositol containing 3–6 phosphates per inositol residue. Data are the mean of two independent determinations. The standard deviations of the means were less than 0.35.

[f] ND, not detected.