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Lactobacillus gasseri requires peptides, not proteins or free amino acids, for growth in milk

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ABSTRACT

Lactobacillus gasseri is a widespread commensal lactic acid bacterium inhabiting human mucosal niches and has many beneficial effects as a probiotic. However, *L. gasseri* is difficult to grow in milk, which hurts usability for the food industry. It had been previously reported that supplementation with yeast extract or proteose peptone, including peptides, enables *L. gasseri* to grow well in milk. In this study, our objective was to confirm peptide requirement of *L. gasseri* and evaluate efficacy of peptide release by enzymatic proteolysis on growth of *L. gassei* in milk. Three strains of *L. gasseri* did not grow well in modified DeMan, Rogosa, Sharpe broth without any nitrogen sources (MRS-N), but addition of a casein-derived peptide mixture, tryptone, promoted growth. In contrast, little effect was observed after adding casein or a casein-derived amino acid mixture, casamino acids. These results indicate that *L. gasseri* requires peptides, not proteins or free amino acids, among milk-derived nitrogen sources for growth. *Lactobacillus gasseri* JCM 1131^{T} hardly had growth capacity in 6 kinds of milk-based media: bovine milk, human milk, skim milk, cheese whey, modified MRS-N (MRSL-N) supplemented with acid whey, and MRSL-N supplemented with casein. Moreover, treatment with digestive proteases, particularly pepsin, to release peptides made it grow well in each milk-based medium. The pepsin treatment was the most effective for growth of strain JCM 1131^{T} in skim milk among the tested food-grade proteases such as trypsin, α-chymotrypsin, calf rennet, ficin, bromelain, and papain. As well as strain JCM 1131^{T}, pepsinolysis of milk improved growth of other *L. gasseri* strains and some strains of enteric lactobacilli such as *Lactobacillus crispatus*, *Lactobacillus gallinarum*, *Lactobacillus johnsonii*, and *Lactobacillus reuteri*. These results suggest that some relatives of *L. gasseri* also use peptides as desirable nitrogen sources, and that milk may be a good supplier of nutritious peptides to enteric lactobacilli including *L. gasseri* after peptic digestion in the gastrointestinal tract. This is the first report showing peptide requirement of *L. gasseri* and efficacy of pepsinolysis on the growth of *L. gasseri* and its relatives in milk. This study would contribute to increasing usability of *L. gasseri* and its relatives as probiotics in dairy foods.
Key words: *Lactobacillus gasseri*, peptide requirement, proteolysis, pepsin

INTRODUCTION

Lactobacilli are generally recognized as important beneficial members of the gastrointestinal (**GI**) microbiota of human and animals, along with bifidobacteria. Among them, *Lactobacillus gasseri* has been frequently detected in the GI tract and feces of humans (Selle and Klaenhammer, 2013), and were once reported as a predominant species of lactic acid bacteria (**LAB**) in the human small bowel (Reuter, 2001). Colonization of the human GI tract by *L. gasseri* has been often observed to be established at an early stage of life and to last throughout adulthood (Wall et al., 2007). Because *L. gasseri* is one of the predominant LAB species in the oral and the vaginal cavities and on the mammary areola of humans, it is presumed to be partly transferred to the GI tract through the oral cavity of neonates from the vaginal tract and the mammary areola of their mothers during delivery and lactation (Matsumiya et al., 2002; Martín et al., 2003; Dal Bello and Hertel, 2006; Lamont et al., 2011).

As for the beneficial effects of oral administration of *L. gasseri* upon the host, many researchers have reported maintenance of gut homeostasis, regulation of immune system, reduction of allergic symptoms, prevention of bacterial and viral infections, and alleviation of infectious disease symptoms (Selle and Klaenhammer, 2013). Recently, it has been also revealed that *L. gasseri* SBT2055 has a clear antiobesity effect for humans (Kadooka et al., 2010). The greater part of these benefits should be derived by survival and transient colonization of the commensal LAB in the GI tract. The viability and the colonization ability of many *L. gasseri* strains have been already confirmed on in vitro

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and in vivo assays, and are regarded as depending on acid tolerance, bile resistance, adhesion capacity to the GI epithelial and mucus layers, metabolic capacity and several host-dependent factors such as core temperature, nutritional environment, and immune system (Azcarate-Peril et al., 2008; Selle and Klaenhammer, 2013). In anticipation of such beneficial effects, several fermented food products and dietary supplements using *L. gasseri* as a probiotic are widely developed and distributed by food companies.

Apart from that, some strains of *L. gasseri* have been reported to produce antibacterial peptides, namely bacteriocins. *Lactobacillus gasseri* LA39 (JCM 11657) produces a circular bacteriocin, gassericin A, linked at the N- and C-terminal ends (Kawai et al., 1998; Arakawa et al., 2010). *Lactobacillus gasseri* SBT2055 and LA158 (JCM 11046) produce the same 2-component bacteriocin, gassericin T, that is highly similar to acidocin LF221 and gassericin K7 from *L. gasseri* LF221 and K7, respectively (Kawai et al., 2000; Majhenic et al., 2004; Treven et al., 2013; Yasuta et al., 2014). These bacteriocins are heat-stable and pH-tolerant and have broad-spectral antibacterial activity against various strains of LAB and food-spoilage and pathogenic bacteria including *Bacillus cereus*, *Listeria monocytogenes*, and *Staphylococcus aureus*; and therefore, they and their producers are expected to be used for safe food preservation (Toba et al., 1991; Itoh et al., 1995; Kawai et al., 1997; Bogovic-Matijasić et al., 1998; Bogovic-Matijasić and Rogelj, 2000). Indeed, the gassericin A-containing culture supernatant of *L. gasseri* LA39 and its concentrate have been effectively used as biopreservatives on custard cream manufacturing (Arakawa et al., 2009; Nakamura et al., 2013).

Thus, *L. gasseri* strains are considered to be live materials useful as probiotics or biopreservatives in the food industry. However, it is empirically demonstrated that *L. gasseri* cannot grow well in milk without any supplement, despite the good growth in semisynthetic media such as de Man, Rogosa and Sharpe (**MRS**) broth and *Lactobacillus* Selection broth. The poor growth in milk has also been observed with some strains of the other relative lactobacilli: *Lactobacillus acidophilus*, *Lactobacillus gallinarum*, and *Lactobacillus johnsonii* (Elli et al., 1999; Masuda et al., 2003a,b; Avonts et al., 2004). We previously showed that *L. gasseri* successfully grew in milk-based media by supplementation with yeast extract or proteose peptone that are components of MRS broth, not free amino acids, nucleotides, glucose, minerals, or vitamins (Arakawa et al., 2008). Avonts et al. (2004) had also reported that addition of yeast extract into milk-based media is effective on growth of *L. acidophilus* and *L. johnsonii* strains as well as *L. gasseri*. These findings suggest that *L. gasseri* and the relatives would require peptides as nitrogen sources in milk-based media. In this study, we confirmed peptide requirements of *L. gasseri* and evaluated efficacy of proteolysis, particularly using pepsin, of milk proteins on growth of *L. gasseri* in milk-based media. In addition, the growth-promoting effect of pepsinolysis of milk was examined in strains of the *L. acidophilus* complex members including *L. acidophilus*, *Lactobacillus crispatus*, *Lactobacillus amylovorus*, *L. gallinarum*, *L. gasseri*, and *L. johnsonii* (Johnson et al., 1980; Fujisawa et al., 1992), and other lactobacilli.

MATERIALS AND METHODS

Bacterial Strains

Lactobacillus strains used in this study were listed in Table 1. All strains were precultured 3 times with a 1% (vol/vol) inoculum at 37°C for 24 h in lactobacilli MRS broth (Oxoid, Basingstoke, UK). The MRS broth was autoclaved at 121°C for 15 min.

Determination of Nitrogen Sources Required by L. gasseri

Lactobacillus gasseri JCM 1131^{T}, JCM 11046, and JCM 11657 were cultivated with a 1% (vol/vol) inoculum at 37°C for 48 or 72 h in modified MRS broth (**MRS-N**) without peptone, beef extract (Lab-Lemco), and yeast extract, and 8.3% (wt/vol) reconstituted skim milk (protein content 3.0%, **RSM**; Megmilk Snow Brand, Hokkaido, Japan) supplemented with casein (Nacalai Tesque, Kyoto, Japan), tryptone (casein enzymatic hydrolysates, peptide mixture; Becton Dickinson, Franklin Lakes, NJ), or casamino acids (casein acid hydrolysates, free amino acid mixture; Becton Dickinson). Casein, tryptone, and casamino acids were added at 3.0% (wt/vol) each to MRS-N and 1.0 or 3.0% (wt/vol) each to RSM. The MRS-N-based media were autoclaved at 121°C for 15 min. The RSM-based media were autoclaved at 110°C for 20 min. Cell growth was monitored with pH and optical density (**OD**) at 620 nm (in the case of the MRS-N-based media) or titratable acidity (in the case of the RSM-based media) of culture solutions. Results given are mean values of at least 2 independent determinations.

Effect of Digestive Proteases on Growth of L. gasseri in Milk-Based Media

Lactobacillus gasseri JCM 1131^{T} was cultivated with a 1% (vol/vol) inoculum at 37°C for 72 h in whole bovine milk (**WBM**), human breast milk (**HBM**), RSM, 21.5% (wt/vol) reconstituted cheese whey (pro-