Biotechnology Reports 10 (2016) 105–110

Contents lists available at ScienceDirect

Biotechnology Reports

journal homepage: www.elsevier.com/locate/btre

The γ-aminobutyric acid-producing ability under low pH conditions of lactic acid bacteria isolated from traditional fermented foods of Ishikawa Prefecture, Japan, with a strong ability to produce ACE-inhibitory peptides

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ARTICLE INFO

Article history:
Received 3 February 2016
Received in revised form 6 April 2016
Accepted 7 April 2016
Available online 9 April 2016

Keywords:
γ-Aminobutyric acid
ACE inhibitory activity
Lactic acid bacteria
Fermented food

ABSTRACT

Many traditional fermented products are onsumed in Ishikawa Prefecture, Japan, such as *kaburazushi*, *narezushi*, *konkazuke*, and *ishiru*. Various kinds of lactic acid bacteria (LAB) are associated with their fermentation, however, characterization of LAB has not yet been elucidated in detail. In this study, we evaluated 53 isolates of LAB from various traditional fermented foods by taxonomic classification at the species level by analyzing the 16S ribosomal RNA gene (rDNA) sequences and carbohydrate assimilation abilities. We screened isolates that exhibited high angiotensin-converting enzyme (ACE) inhibitory activities in skim milk or soy protein media and produced high γ-aminobutyric acid (GABA) concentrations in culture supernatants when grown in de Man Rogosa Sharpe broth in the presence of 1% (w/v) glutamic acid. The results revealed that 10 isolates, i.e., *Lactobacillus buchneri* (2 isolates), *Lactobacillus brevis* (6 isolates), and *Weissella hellenica* (2 isolates) had a high GABA-producing ability of >500 mg/100 ml after 72 h of incubation at 35 °C. The ACE inhibitory activity of the whey cultured with milk protein by using L. *brevis* (3 isolates), *L. buchneri* (2 isolates), and *W. hellenica* (2 isolates) was stronger than that of all whey cultured with soy protein media, and these IC_{50} were < 1 mg protein/ml. Three of 10 isolates had high GABA-producing activities at pH 3, suggesting that they could be powerful candidates for use in the fermentation of food materials having low pH.

1. Introduction

Ishikawa Prefecture in the Hokuriku region, which is located in the northern part of central Japan, is one of the major areas in which people produce various fermented fish products, such as fermented fish with cooked rice (*narezushi*), fermented fish and vegetable with malted rice (*kaburazushi*), fish fermented with rice bran mash (*konkazuke*), and fish sauce (*ishiru*). These products are known to be lactic acid fermented foods, in which various lactic acid bacteria (LAB) are involved in the production process [1–5].

Till date, many studies have demonstrated that several LAB species produce a ubiquitous four-carbon amino acid, γ-aminobutyric acid (GABA), which is synthesized from glutamic acid via a reaction catalyzed by glutamate decarboxylase [EC 4.1.1.15], a pyridoxal 5′-phosphate-dependent enzyme [6]. In mammals, GABA has various physiological functions, such as neurotransmission and the induction of hypotensive effects [7]. It is well known that it is involved in the regulation of cardiovascular functions such as blood pressure and heart rate, and that it plays an important role in the sensations of pain and anxiety [8]. In addition, GABA is found

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http://dx.doi.org/10.1016/j.btre.2016.04.002

to have diuretic and tranquilizing effects [9]. Wong et al. [10] reported that GABA intake can help treat various neurological disorders such as Parkinson's disease, stiff-man syndrome, and schizophrenia. Although GABA is present in many fruits and vegetables, the amount of GABA present is very low, ranging from 0.03–2.00 μmol/g fresh weight [11]. Therefore, the development of new pharmaceutical and functional foods containing a considerable amount of GABA is required. Many studies have reported the mass production of GABA using *Lactobacillus brevis* isolated from alcohol distillery lees [12] and kimchi [13], *Lactobacillus paracasei* from fermented fish [14], and *Lactococcus lactis* from cheese starters [15].

Moreover, people have become increasingly interested in functional foods, because of a growing awareness among consumers of the link between diet and health. Angiotensin I-converting enzyme (ACE) [EC 3.4.15.1], a dipeptidyl carboxypeptidase, plays an important role in the regulation of blood pressure, and the enzyme cleaves the dipeptide portion from the C-terminal end of angiotensin I to produce the strong vasopressor angiotensin II. Hence, great interest has been shown in ACE-inhibitory peptides that have the ability to lower the blood pressure of hypertensive patients. Various ACE-inhibitory peptides have been isolated from different sources that are released during or after fermentation [16–19] or by hydrolytic processes [20].

The aim of this study was to screen various LAB exhibiting a strong ability to produce GABA and ACE inhibitors, which are expected to enhance the development of functional fermented foods. We tested both these abilities in LAB isolated from traditional fermented foods of Ishikawa Prefecture that were cultured in the presence of skim milk or soy protein. Based on this study, we discussed the possibility of these isolates being used as starters in the production of new types of fermented foods.

2. Materials and methods

2.1. Media and chemicals

De Man Rogosa Sharpe (MRS) medium was purchased from Oxoid (Basingstoke, Hampshire, UK). An API 50CH test kit was purchased from bioMérieux, Marcy l'Etoile, France. GABase (a mixture of γ-aminobutyrate glutamate aminotransferase and succinic semialdehyde dehyrogenase from *Pseudomonas fluorescens*) was purchased from Sigma-Aldrich (St Louis, MO, USA). NADP + was purchased from Oriental Yeast Co. (Tokyo, Japan). GABA, dithiotheriol, α-ketoglutarate, tris (hydroxymethyl) aminomethane (Tris), and sodium sulfate were obtained from Nakalai Tesque Inc. (Kyoto, Japan). Glutamic acid was purchased from Kanto Chemical Co. (Tokyo, Japan).

Table 1
LAB isolated from the fermented foods of Ishikawa Prefecture, Japan.

Fermented foods	Origin	Raw materials	Number of fermented food samples	LAB species identified (number of isolates)[a]
Narezushi	Purchased from market	Fish (horse mackerel, mackerel, or amberjack), rice, Japanese pepper, and red pepper	9	*Lactobacillus buchneri/parabuchneri* (4), *Lactobacillus brevis* (5), *Lactobacillus alimentarius* (4), *Lactobacillus casei/paracasei* (2), *Lactobacillus plantarum/paraplantarum* (7), *Pediococcus ethanolidurans* (1), *Weissella hellenica* (1)
Narezushi (our previous work [21])	Made by manufacturer	Fish (horse mackerel), rice, Japanese pepper, and red pepper	1	*Lactobacillus buchneri/parabuchneri* (1), *Lactobacillus brevis* (2), *Lactobacillus plantarum/paraplantarum* (1), *Lactobacillus casei/paracasei* (1),
Ika-koujizuke	Purchased from market	Squid and malted rice	1	*Carnobacterium divergens* (1), *Enterococcus faecalis* (1), *Enterococcus gilvus* (1), *W. hellenica* (1)
Ika-kurozukuri	Purchased from market	Squid and squid ink	1	*W. hellenica* (1)
Kaburazushi	Made by manufacturer	Yellow tail, turnip, and malted rice	1	*Leuconostoc mesenteroides* (1), *Leuconostoc citreum* (3), *Lactobacillus sakei* (3), *Lactobacillus curvatus* (1)
Daikonzushi	Made by manufacturer	Herring, Japanese radish, and malted rice	1	*Weissella cibaria* (1), *W. hellenica* (1), *L. citreum* (1), *L. mesenteroides* (2), *L. curvatus* (1), *L. sakei* (1), *Pediococcus pentosaceus* (1)
Yamahai-syubo	Made by manufacturer	Malted rice and cooked rice	1	*L. citreum* (1), *L. sakei* (2)

[a] Identified by 16S rDNA sequence analyses.