Carbohydrate Polymers 79 (2010) 1040–1045

Contents lists available at ScienceDirect

Carbohydrate Polymers

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

Effects of carbohydrate source on physicochemical properties of the exopolysaccharide produced by *Lactobacillus fermentum* TDS030603 in a chemically defined medium

Kenji Fukuda [a,*], Tala Shi [a], Kentaro Nagami [b], Fiame Leo [a], Tadashi Nakamura [b], Kumi Yasuda [b], Akitsugu Senda [a], Hidemasa Motoshima [c], Tadasu Urashima [a]

[a] Department of Animal and Food Hygiene, Obihiro University of Agriculture and Veterinary Medicine, 2-11 Inada-cho, Obihiro, Hokkaido 080-8555, Japan
[b] Department of Food Science, Obihiro University of Agriculture and Veterinary Medicine, 2-11 Inada-cho, Obihiro, Hokkaido 080-8555, Japan
[c] Research Center, Yotsuba Milk Products Co. Ltd., 465-1 Wako, Kitahiroshima, Hokkaido 061-1264, Japan

ARTICLE INFO

Article history:
Received 27 April 2009
Received in revised form 25 September 2009
Accepted 15 October 2009
Available online 24 October 2009

Keywords:
Chemical structure
Chemically defined medium
Exopolysaccharide
Lactic acid bacteria
Viscosity

ABSTRACT

A thermophilic lactic acid bacterium, *Lactobacillus fermentum* TDS030603, produced about 100 mg/L of EPS in purified form when grew in de Man–Rogosa–Sharpe (MRS) broth. The 1% (w/v) solution of the purified EPS was highly viscous, exhibiting an apparent viscosity (η_{app}) of 0.88 Pa s at a shear rate of 10/s. To investigate the impact of carbohydrate source on the production yield and chemical structure of EPS and the viscosity of EPS solution, a chemically defined medium (CDM) has been developed. Results of TLC, HPLC, and ^{1}H NMR spectroscopy indicated that the chemical structures of EPS released in MRS and in the CDM supplemented with glucose, galactose, lactose or sucrose were very similar. All the 1% solutions of EPSs released in CDMs were highly viscous similar to the EPS released in MRS, but their viscosities appeared to differ, presumably because of the differences in their molecular mass distributions.

1. Introduction

An exopolysaccharide (EPS) is a sugar polymer that is produced mainly by bacteria and microalgae, either in a form bound to the cell-wall, a so-called capsular polysaccharide (CPS), or in a free form liberated into the culture medium, known as a slime EPS (Sutherland, 1972). It is believed that the physiological function of EPS is as the first line of biological defense against phagocytosis, phage attack, antibiotics, toxic metal ions and physical stresses such as desiccation and osmotic stress (Looijesteijn, Trapet, de Vries, Abee, & Hugenholtz, 2001; Roberts, 1996; Weiner, Langille, & Quintero, 1995; Whitfield, 1988). EPS produced by lactic acid bacteria (LAB) is very useful in the food industry, because it provides consistency to the resulting fermented milk products, such as Scandinavian ropy milks, viili and långfil (Duboc & Mollet, 2001). Furthermore, some EPSs have been claimed to show bioactivities beneficial to health, including prebiotic or anti-inflammatory effects (Salazar, Gueimonde, Hernández-Barranco, Ruas-Madiedo, & de los Reyes-Gavilán, 2008; Vinderola, Perdigón, Duarte, Farnworth, & Matar, 2007).

A large number of EPSs produced by LAB have been described (Cerning, 1990; De Vuyst & Degeest, 1999; Jolly, Vincent, Duboc, & Neeser, 2002; Laws, Gu, & Marshall, 2001; Welman & Maddox, 2003), but little is known about the effects of medium components on the chemical structure of the EPS and its rheological properties (Vaningelgem et al., 2004). Since the complexity of media composition can lead to an incorrect structural analysis of EPS (De Vuyst & Degeest, 1999), a chemically defined medium (CDM) is of great advantage when assessing the effects of medium components on the chemical structure and the physicochemical properties of EPS (Grobben et al., 1998). It has been shown, using CDM, that adenine or orotic acid stimulates both the cell growth and the yield of EPS in Lactobacilli (Petry, Furlan, Crepeau, Cerning, & Desmazeaud, 2000; Torino, Hébert, Mozzi, & de Valdez, 2005). Composition of carbohydrate source also exhibited significant effects on the EPS yield, but the preferences for sugar for the maximum EPS production were strain-dependent (Cerning et al., 1994; Tallon, Bressollier, & Urdaci, 2003; Torino et al., 2005). On the other hand, the effects of carbohydrate source on the monosaccharide composition of EPS are still unclear: the constitutive monosaccharides were found to be the same in *Lactobacillus helveticus* following alterations in the carbohydrate source (Torino et al., 2005), but the relative proportions of the individual monosaccharides varied in *L. delbrueckii* subsp. *bulgaricus* (Petry et al., 2000). The rheological

* Corresponding author. Tel.: +81 155 49 5564; fax: +81 155 49 5577.
E-mail address: fuku@obihiro.ac.jp (K. Fukuda).

0144-8617/$ - see front matter © 2009 Elsevier Ltd. All rights reserved.
doi:10.1016/j.carbpol.2009.10.037

properties of the EPS produced by LAB are attributed to its molecular mass, molecular mass distribution, constituent sugar residues, linkages between the sugar monomers and the presence of side groups (Shene, Canquil, Bravo, & Rubilar, 2008). However, effects of altered medium composition on the rheological properties of EPS have been scarcely reported.

In the present paper we aimed to evaluate the effects of carbohydrate source on the yield, chemical structure and viscosity of a neutral hetero-EPS produced by *L. fermentum* TDS030603. In this context, we have developed a CDM for this strain, having modified the previously reported media (Morishita, Deguchi, Yajima, Sakurai, & Yura, 1981). The chemical structure and viscosity of EPS released into CDM were assessed using the EPS released into MRS as the reference. Possible determinants leading to rheological variations in the EPS will be discussed.

2. Materials and methods

2.1. Bacterial strain and chemicals

Lactobacillus fermentum TDS030603 was obtained from the bacterial collection of our own laboratory (Leo et al., 2007). MRS was from Oxoid (Cambridge, UK). DEAE-Sephadex A-50 and Toyopearl HW-55F was from GE Healthcare (Uppsala, Sweden) and Tosoh (Tokyo, Japan), respectively. D_2O (99.99% atom % D) was from Sigma–Aldrich (St. Louis, USA). All the chemicals used were analytical grade.

2.2. Development of CDM and culture condition

Following the previous report (Morishita et al., 1981), we firstly tested a prototype CDM consisting of 48 constituents, of which six were non-essential amino acids (see below) and the other 42 are listed in Table 1. Essential or important chemical compounds for cell growth were determined by checking the cell density of culture medium from which one of the above constituents had been omitted. After static culture in MRS for 24 h at 30 °C under aerobic conditions, the cells were harvested, washed thoroughly with sterilized phosphate buffered saline, and inoculated into 1 L of either MRS or CDM to yield an optical density (OD) of 0.2 at 600 nm. Cell growth ($OD_{600\ nm}$) and pH of the static culture were monitored. Simultaneously, cultivable cell numbers were counted on MRS-agar plates; after a given time period, a 1-ml aliquot of culture medium was collected, diluted with MRS and spread on a MRS-agar plate, which was incubated at 30 °C for 24 h under anaerobic condition. Colonies appearing on the plate were counted as cultivable cells. To measure the amount of EPS produced, a 100-ml aliquot of the culture medium was collected, and the EPS was purified following the procedure described below.

2.3. Production and isolation of EPS

Glucose, galactose, lactose, or sucrose was used in the CDM as carbohydrate source at the final concentration of 1% (w/v). After cultivation as described above, the cells were removed by centrifugation (17,000*g*, 1 h, 4 °C). Crude EPS released in CDM was precipitated by addition of an equal volume of ice-cold ethanol to the supernatant. The ethanol precipitate was collected by centrifugation (17,000*g*, 30 min, 4 °C), dissolved in 30 ml of water, dialyzed overnight with water at 4 °C, and lyophilized. The lyophilized crude EPS (from 100 ml of the culture medium) was dissolved in 10 ml of 50 mM Tris–HCl (pH 8.7), and purified by a batch method using a 20-ml slurry of DEAE-Sephadex A-50 equilibrated with the same buffer. The non-adsorbed fraction was collected, thoroughly dialyzed against water, and lyophilized. Crude EPS released in MRS was subjected to the same procedure described above, however, it required further purification using a Toyopearl HW-55F column (2.6 × 100 cm, 15 ml/h) equilibrated with water. The polymer dry mass of purified EPS was determined by measuring the weight. The lyophilized EPS was stored in a desiccator until used.

Table 1
Chemical composition of the CDM for *L. fermentum* TDS030603.

Components	Concentration (g/L)
D-Glucose	10.0[a]
DL-Alanine	0.2[b]
L-Arginine	0.1[a]
L-Aspartic acid	0.1[b]
L-Glutamic acid	0.2[a]
L-Histidine	0.1[a]
L-Isoleucine	0.1[a]
L-Leucine	0.1[a]
L-Methionine	0.1[a]
L-Phenylalanine	0.1[a]
L-Serine	0.1[b]
L-Tryptophan	0.1[a]
L-Tyrosine	0.1[a]
L-Valine	0.1[a]
p-Aminobenzoic acid	0.002[b]
Biotin	0.00001[a]
Folic acid	0.0001[b]
Nicotinamide	0.001[b]
Nicotinic acid	0.001[b]
Pantotheic acid	0.002[a]
Pyridoxal	0.002[a]
Pyridoxol	0.001[b]
Riboflavin	0.0002[b]
Adenine	0.01[a]
Guanine	0.01[b]
Thymine	0.005[a]
Uracil	0.01[a]
Xanthine	0.01[b]
Adenylic acid	0.02[a]
Cytidylic acid	0.05[a]
2′-Deoxyguanosine	0.01[a]
Ammonium citrate	1.0[a]
Sodium acetate	6.0[b]
Sodium citrate	0.5[b]
Sodium thioglycolate	0.5[b]
$FeSO_4 \cdot 7H_2O$	0.02[b]
K_2HPO_4	3.0[a]
KH_2PO_4	3.0[a]
$MgSO_4 \cdot 7H_2O$	0.5[b]
$MnSO_4 \cdot 5H_2O$	0.2[a]
Spermidine phosphate	0.005[b]
Tween 80	1.0[a]

[a] Essential.
[b] Important but not essential.

2.4. Estimation of EPS molecular mass

The molecular mass distribution of EPS was estimated using high performance liquid chromatography (HPLC). The purified EPS was dissolved in water (1 mg/ml), and 100 μl of this solution was loaded onto a TSKgel G6000PWXL column (7.8 × 300 mm, Tosoh). Elution was done with water at 40 °C at a flow rate of 1 ml/min. The EPS was detected by measuring the refractive index of the eluate using a refractive index monitor RI-8020 (Tosoh). Shodex Standard P-82 (Showa Denko, Tokyo, Japan), a series of pullulans with known molecular masses ranging from 0.59×10^4 to 7.88×10^5 Da, was used as the standard.

2.5. Monosaccharide composition of EPS

The purified EPS (2 mg) was hydrolyzed in 250 μl of 2 M trifluoroacetic acid (TFA) at 100 °C for 5 h. Excess TFA was removed by