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Progress and Development of Exopolysaccharides of *Lactobacilli*

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Abstract

Exopolysaccharides as well as other bioactive substances of Lactic acid bacteria (LAB) attracted much attention due to its specific structure and functions, e.g. tolerance to gastrointestinal (GI) stress, adhesion on the intestinal mucosa, inhibition against pathogens, and modulation of immune system. In this mini-review, we have summarized the recent progress of exopolysaccharides of *Lactobacillus spp*. on composition, antioxidant activity, colonization and immunity effect, with the aim to highlight the development tendency in the approach of exopolysaccharides of LAB.

Keywords: *Lactobacillus*; Exopolysaccharides; Probiotic; Antioxidant activity; Colonization

Introduction

Progress on disclosing the beneficial effects of Lactic acid bacteria (LAB) (*Lactobacillus plantarum L. rhamnosus, Bifidobacterium spp*. etc.) had been achieved recently on some of metabolic substances (exopolysaccharides, bacteriocins, bioactive peptides etc.). Traditionally, those benefits or healthy effects of LAB including preserving food, improving food flavor and enhancing health [1-3] were attributed to yielding of organic acid e.g. acetic acid, lactic acid, propionic acid and butyric acid etc., and change of pH or oxidation-reduction potential as well as the antagonistic effect of LAB against pathogenic microorganisms. As one of the popular species of LAB in fermented food of dairy products, sourdough, pickles and fermented sausage, *Lactobacillus spp*. were proved to ameliorate the flora balance of human or animals, strengthen the immunity and reduce the symptom of hypertension. Recently, accumulative evidence of the health effects of *Lactobacillus spp*. was related to the substances of exopolysaccharides (EPS), bacteriocins, and bioactive peptides etc. In this mini-review, we focused on the recent progress on composition, antioxidant activity, colonization and immunity effect of exopolysaccharides of *Lactobacillus spp*., with the aim to highlight the development tendency in the approach of EPS of LAB.

Composition Difference of Eps of Lactobacillus Spp

The probiotic function of Lactobacillus spp. might be closely relevant to the composition and structure of those specific EPS. Different strain of Lactobacillus spp. maintained its unique synthesis pathway for EPS due to the survival environment of using different carbohydrates e.g. glucose, sucrose, galactose, lactose etc., specific gene clusters might be conservative on the bacterial chromosome during the long term evolution in food matrix [4,5]. Different strains even in the same species presented different composition and structure of EPSs. For instance, Wang et al. [6] reported that the EPS of *L. plantarum* YW32 was composed of mannose, fructose, galactose and glucose in an approximate molar ratio of 8.2:1:4.1:4.2, whereas that of *L. plantarum* ZDY2013 was composed of only xylose and galactose, the later accounted for 98.3% (wt/wt) [7]. Some species of Lactobacillus spp. produced two or more EPSs. For example, EPS of fraction S1 from *L. rhamnosus* KF5 consisted of glucose, arabinose, glucosamine, galactosamine and galactose in an approximate molar ratio of 2.03:1.29:1.25:0.72:0.61and the other fraction S2 contained rhamnose, glucose and galactose in a molar ratio of approximately 1.73:1.47:1.00 [8]. There are some disputes about the forming of EPSs under different environments. Generally, it was accepted that culture conditions or some other factors (pH, temperature, incubation time and medium composition etc.) significantly influenced the yield and composition of EPS of *Lactobacillus spp*., however, few publications mentioned that the yield of EPS of *Lactobacillus spp*. is rarely affected by those factors [9,10].

Antioxidant Activity of Eps of Lactobacillus Spp

In vitro antioxidant activity of EPS of Lactobacillus spp. was mainly evaluated by 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging, hydroxyl radical scavenging, and super-oxide radical scavenging abilities of EPS. Constitution of EPS decided on the difference of antioxidant activity. For example, the EPS of *L. plantarum* YW32 at a dose of 5mg/ml showed strong scavenging abilities toward hydroxyl (77.5%) and superoxide radicals (66.5%), but low DPPH radical scavenging rate (30%) [6]. In a parallel study, the EPS from *L. plantarum* ZDY2013 at a dose of 2mg/mL showed lower rate of DPPH (less than 5.06%) and superoxide radicals scavenging (47.95%) [11]. However, the sulfonation modified EPS increased its antioxidant activity by almost four fold. Sulfonating of EPS enhanced the antioxidant activity not only *In vitro* but also in the Caco-2 cell model [12]. It was reported that carboxymethylation modification could also enhance the potential of polysaccharide as oxidation inhibitor *In vitro* [13]. Update, data on the appropriate modification of EPS components to enhance antioxidant activity *In vivo* had not been documented.

Colonization and Immunity of Eps of Lactobacillus Spp

Apart from the antioxidant ability, EPS of LAB might play a role in improving the cells survival by challenging the adverse digestion in gastrointestinal tract. Russo et al. [14] demonstrated that glucans from Pediococcus parvulus improved the stress tolerance and colonization of *L. plantarum* WCFS1 in human intestinal epithelial cells; on the other hand, Dertli et al. [15] proposed that EPS of *Lactobacillus spp*. might hinder the specific adhesion factors on the bacterial cell surface to reduce the adhesion on intestinal epithelial cells. Few publications disclosed that EPS as prebiotic substances help the colonization of Lactobacillus spp. in GI as native flora. For the effect of EPS on host immunity, there is a cross-talking between probiotics and host receptors via recognition between host pattern recognition receptors and microorganisms associated molecular patterns. Although there is no direct evidence or even little information about the EPS involved into the recognition receptors directly, some studies found that EPS had immunomodulatory properties in vitro and in vivo. In Caco-2 cells model, four immune-related genes that include interleukin-1α (IL-1α), chemokine C-C motif 2 (CCL2), tumor necrosis factor α (TNF-α), and pentraxin 3 (PTX3) were stimulated by EPS (*L. acidophilus* NCFM) preparations [16]. Remarkably, EPS of *L. rhamnosus* GG (LGG) as a shielding for the cells was proved to prevent the attacking initiated by host defense factors e.g. LL-37 [17].

Conclusion and Future Trends

Gene cluster guiding the synthesis of EPS together with environmental factors decided on the structure and composition of EPS of LAB, which is critical for the antioxidant activity. EPS might help LAB or other probiotics colonizing in gastrointestinal tract by protecting the cells from digestion, or using as substrates for other beneficial microorganisms, and thereafter affect the immunity of host. Although there were quite a few *in vitro* or *in vivo* studies on the aspect of composition, structure and antioxidant activity, colonization and immune regulation of EPS from different species of LAB, however, lack of comparative study of EPS lead to the contradict conclusion of the functions (e.g. adhesion) of EPS. Future perspective research guideline for EPS should be included: 1. Investigation of the change of function of EPS by using gene-knockout technique, namely, comparing the structure, antioxidant activities, colonization ability of the wild strain and EPS-gene knockout strain of LAB; 2. Systematical evaluation of the adhesion ability and immunity response of EPS yielding strain of LAB in germ-free mice or other animal model; 3. The heredity of EPS gene cluster of LAB should be evaluated for its stability under different environments by using genomic analysis and bio-information. To obtain objective conclusions, joint research work on describing the role of EPS of LAB should be encouraged in the future.

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