Chapter

Bacterial Cell-Free Probiotics Using Effective Substances Produced by Probiotic Bacteria, for Application in the Oral Cavity

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Abstract

To avoid side effects of conventional antibiotics and disinfectants used for prevention of oral diseases such as dental caries, periodontitis, and oral candidiasis, application of probiotics has attracted attention recently. However, difficulties arise when applying those probiotics in the oral cavity, because exogenous probiotic bacteria do not colonize easily in the established oral microbiota. Even, if we are able to overcome the restriction of colonization by probiotic bacteria in the oral cavity, it comes with the risk of dental caries due to the potential acidic environment generated by probiotic bacteria. To solve these problems, "biogenics," bacterial cell-free probiotics using only the effective substances metabolically produced by probiotic bacteria, is recommended for application in the oral cavity. The concept and frontline of biogenic research will be introduced and discussed.

Keywords: biogenics, probiotics, oral diseases, dental caries, periodontitis, candidiasis

1. Introduction

More than 700 bacterial species live in the oral cavity [1, 2]. These bacteria form their own indigenous flora in their habitats, such as teeth, gingival sulcus, and tongue dorsum, making the oral environment considerably complicated. Oral indigenous bacteria coexist with humans and are vital for preventing colonization by foreign pathogenic microorganisms in the oral cavity. Such oral indigenous bacteria proliferate with time, and together with the extracellular polymeric substance (EPS) that they produce, form a biofilm visible even to the naked eye known as dental plaque [3–5].

It has recently been clarified that the formation of biofilms is controlled by quorum-sensing (QS) signals in a communication system between microorganisms that sense each other's abundance [6–8]. It has further been shown that microorganisms constituting a biofilm activate the expression of pathogenic factors when QS signaling molecules, so-called "autoinducers (AI)," act as transcription factors [8].

2. The potential pathogenicity of dental plaque as oral biofilm

Oral plaque contains also dental caries and periodontal disease causing organisms [9–12], and when these exert their potential pathogenicity, they are considered to accelerate biofilm formation even more. Generally it can be said that with increasing thickness of the biofilm, the bacterial metabolites build up at the bottom of the biofilm, and the caries and periodontitis occurrence proliferate.

Caries and periodontal diseases are called the two major dental diseases, and both of them occur as oral infectious diseases which are caused by specific bacteria known as cariogenic bacteria (such as *Streptococcus mutans*) and periodontal pathogens (such as *Porphyromonas gingivalis*) growing in plaque. This status is interpreted as dysbiosis of the oral flora. In addition, a small number of fungal genus *Candida* is also present in the indigenous oral resident microflora, and some factors also cause its growth in the plaque, resulting in dysbiosis, which causes a major oral mucosal disease, oral candidiasis. However, there is currently no technique to selectively eliminate only those causative microorganisms from the flora.

3. Oral application of probiotics and problems

The method of preventing caries and periodontal disease is basically the mechanical removal of the entire plaque by brushing, etc. However in the case of onset of the disease, antimicrobial drugs are administered for the treatment of the acute phase of periodontal disease, and antifungal administration is the first choice for treatment of oral candidiasis. However, the use of antimicrobial agents has shown problems regarding adverse effects such as drug-resistant bacteria and allergies, indicating the limitation of chemotherapy [13]. Therefore, attention has recently focused on probiotic bacteria such as bifidobacteria and lactobacilli recognizing the usefulness for improving dysbiosis [14]. Although probiotics were originally intended to improve dysbiosis of the intestinal flora [15], their usefulness is also assumed in the dental field. Attempts have been made for direct use in the oral cavity to prevent diseases such as caries and periodontal disease, and several results have been reported [16–18]. Ishikawa *et al*. reported that 4 weeks of oral administration of *Lactobacillus salivarius* TI 2711 (LS1) significantly reduced the major periodontal pathogens of *P. gingivalis*, *Prevotella intermedia*, and *Prevotella nigrescens* [19].

However, in these reports, there are few basic facts on the effects of probiotics on the oral flora and the antibacterial substances produced by them, so progress and establishment of practical applications based on the underlying mechanism has not been accomplished. In addition, genera *Lactobacillus* and *Bifidobacterium*, which are representative probiotic bacteria, exist though in minority in the oral microbiota, but because they metabolize sugar and produce large amounts of organic acid, the general understanding is that they work cooperatively with cariogenic bacteria or induce hypersensitivity.

As another fundamental issue, previous studies have highlighted the limitation of colonization and fixation of nonnatural probiotic bacteria in the intestinal tract [20, 21]. This phenomenon of transiency, but not permanency in colonization, is also relevant for probiotic applications in the oral cavity [16, 22, 23]. Even if we are able to address the restriction of colonization of probiotic bacteria in the oral cavity, it comes with the risk of dental caries due to the potential acidic environment generated by probiotic bacteria.

4. The concept of biogenics

To overcome the above problems, "biogenics" as a new idea has been introduced. Biogenics is a kind of functional food, using only the ingredients, which have a positive effect on the host with regard to immunostimulating or immunosuppressing mutagenesis, tumorigenesis, peroxidation, hypercholesterolemia, or intestinal putrefaction [24]. Achieving a probiotic effect by the intake of nonviable probiotic bacteria has been proposed in previous reports. For example, the life span of mice increased, when they were fed with pasteurized fermented milk [25, 26]. A significant reduction of the Ehrlich ascites tumor growth in mice was also reported [26]. In addition, it was shown that heat-inactivated *Enterococcus faecalis* [27] or *L. gasseri* [28] showed a beneficial regulatory effect in the gut. Moreover, Nakamura *et al*. [29] found an angiotensin-I-converting enzyme (ACE) inhibitor in a Japanese sterilized milk beverage fermented by *L. helveticus* and *Saccharomyces cerevisiae*. The active substance in this fermented beverage was identified as lactotripeptide metabolically generated in the fermentation pathway. Follow-up studies were able to determine the bioactive metabolites of probiotic bacteria in addition to the antimicrobial substances, such as bacteriocin [30, 31], and other beneficial active substances, such as conjugated linoleic acid (CLA) [30–32], proteins or peptides [33, 34], and polyphenols [35, 36]. Taking all these observations into account, biogenics, which makes use of the bioactive metabolites as foods or medicine, was recently advocated as a new concept [24, 37]. The biogenic effect is independent of the colonization and viability of probiotic bacteria. Hence, biogenics is the direct delivery of an isolated and purified active ingredient of probiotics to the local environment. This strategy may also be useful for oral disease prevention. It may be possible to purify the effective ingredients against oral pathogenic activity of probiotic bacteria for use in the biogenics process. However, this idea requires further study prior to clinical use.

5. Antibacterial substances produced by lactic acid bacteria

Research of probiotics for intestinal health has revealed several antibacterial substances produced by lactic acid bacteria in addition to organic acids such as lactic and acetic acids [38]. These are (1) hydrogen peroxide [39], (2) bacteriocins [40], and (3) low-molecular-weight antimicrobial substances.

5.1 Hydrogen peroxide (H_2O_2)

Hydrogen peroxide is produced by most *lactobacilli* in the presence of oxygen. *lactobacilli* possess oxidases that reduce oxygen to hydrogen peroxide, oxidizing substrates such as pyruvate or NADH [41]. Since they do not produce catalases, H_2O_2 does not suffer auto-degradation. H_2O_2 has a broad-spectrum planktonic bacteria, but the effect decreases dramatically on biofilm. It appears that *Lactobacilli* do not produce effective concentrations of H_2O_2 against fungi [42], unlike other bacteria [39].

5.2 Bacteriocins

Lactic acid bacteria produce bacteriocins, proteinaceous antimicrobial substances with molecular weights of several thousand daltons or more. Bacteriocins can be divided into five classes according to their primary structure, molecular composition, and physical and functional properties [43, 44]. However, bacteriocins produced by lactic acid bacteria against *S. mutans* and *P. gingivalis* are not yet known. Bacteriocin L23 produced by *Lactobacillus fermentum* L23 [44], plantaricin produced by *L. plantarum* [45], and pentocin TV35b produced by *L. pentosus* [46] appear to be effective against the yeast form of *Candida*. Bacteriocins effective for the hyphal forms of *Candida* have not yet been identified [47, 48].

5.3 Low-molecular-weight antimicrobial substances

Reuterin, an antibacterial substance (also known as 3-hydroxypropionaldehyde; molecular weight, 74 Da; composition formula, $C_3H_6O_2$), is a product of glycerol fermentation, which has been seen in several probiotic bacteria. These probiotic bacteria include not only *L. reuteri* [49] but also *L. brevis*, *L. buchneri* [50], and *L. collinoides* [51]. Under anaerobic conditions *L. coryniformis* [52] also produces a low-molecular-weight antimicrobial substance that does not contain amino acids [53]. Reuterin was found to exert its antibacterial effects by causing oxidative stress within the bacterial cell [54]. In addition to reuterin, the low-molecular-weight substances of lactobacilli, reutericyclin [55] and diacetyl [56] also showed effectiveness against the yeast forms of *Candida* [57].

As the smallest peptides, diketopiperazines (DKPs, cyclic dipeptides) are known to possess several physiological activities, including an antimicrobial effect.

diketopiperazines are a group of cyclic organic compounds where two amino acids are connected by a peptide bond, forming a lactam, and it is the first peptide whose three-dimensional structure has been completely solved by Robert Corey in the 1930s [58]. Corey determined the structure of the cyclic anhydride of the dipeptide glycylglycine. Diketopiperazines are also biosynthesized from amino acids in diverse organisms including mammals and are considered to be secondary metabolites [59]. Although some protease enzymes, such as dipeptidyl peptidase, produce a dipeptide by cleavage from the protein terminus, it is known that the

Cyclic dipeptide	Origin	Biological function	References
Cyclo(Leu-Pro)	*Lactobacillus casei* AST18	Antifungal activity	[64]
Cyclo(Phe-Pro) Cyclo(Phe-4-OH-Pro)	*L. plantarum* MiLAB393	Antifungal activity	[65]
Cyclo(Gly-Leu)	*L. plantarum* VTT E-78076	Antimicrobial activity	[66]
Cyclo(Phe-Pro) Cyclo(Tyr-Pro)	*L. reuteri* RC-14	Antimicrobial activity	[67]
Cyclo(Pro-Pro) Cyclo(Leu-Pro) Cyclo(Met-Pro) Cyclo(His-Pro)	*L. amylovorus* DSM 19280	Antifungal activity	[68]
Cyclo(Leu-Leu)	*L. plantarum* AF1	Antifungal activity	[69]
Cyclo(4-OH-Pro-Leu)	*L. fermentum* ALAL020	Antimicrobial activity	[70]

Table 1.
Diketopiperazines (cyclic dipeptide) produced by probiotic bacteria.

resulting dipeptide cyclizes spontaneously to form a diketopiperazine. In addition, diketopiperazines are attractive scaffolds for drug design due to their structural properties such as a rigid structure, optical activity, and various side-chain structures [59]. Both natural diketopiperazines and synthetic diketopiperazines have been reported to possess various physiological activities including antitumor activity [60], antiviral activity [61], antibacterial activity [62], and antimicrobial activity [63]. However, there are only few reports on DKP produced by probiotic bacteria (**Table 1**). In addition, the antimicrobial mechanism is also poorly understood.

6. Anti-inflammatory substances produced by lactic acid bacteria

Periodontitis and candidiasis are both inflammatory diseases; therefore, inflammation symptoms are desired to be cured by biogenics, but there are few candidates for that.

CLA is a general term for regioisomers and structural isomers of linoleic acid having a conjugated diene structure.

Diene structure means there are two double bonds with a single bond in between. Rumenic acid, for example, is one of the 28 isomers of CLAs and exists in the fat and dairy products of ruminants [71]. It is a trans fat; however, CLAs can also appear as cis-fats. CLAs are known to reduce the production level of IgE and a chemical mediator leukotriene in a rat inflammatory model [72]. However, the opposite effect of increasing serum C-reactive protein value and reducing serum adiponectin level in human by CLA supplementation was observed recently [73].

7. Understanding the property of biofilm

Most bacteria and fungi have the potential to grow in a biofilm, in an environment with liquid flow and solid surfaces. Biofilm formation, which has been experimentally observed in single bacteria, is now known not only to cross species but also to cross the kingdom of microbes. In human bodies, such situations particularly exist in the resident microbiota. Microorganisms including oral pathogens have the potential to express pathogenic properties in biofilms, contrary to the planktonic type. In other words, the so-called biofilm phenotypes upregulate the production of EPS that block the stimuli or stress from outside the biofilm, such as antibiotics and disinfectants. The EPS also provides sticky intercellular binding material and extracellular energy storage compounds [74, 75] to promote interaction among contacting microbial cells [76], resulting in complex and dynamic interplay.

8. Disruption of the quorum-sensing signals

Recently, a QS inhibitor (QSI) and QS signal quencher (QQ) molecule attracted attention in regard to understanding biofilm infections. Biofilm formation is triggered and controlled by a cell-to-cell communication process in harmony with the bacterial population density known as quorum-sensing system, which is based on small molecules termed autoinducers [77]. Some reports revealed that bacteriocins produced by probiotic lactobacilli such as *L. acidophilus*, *L. plantarum*, and *L. reuteri* functioned as QSI or QQ molecules [78]. It may be possible to purify the effective ingredients of probiotic bacteria against oral pathogenic activity in biofilms for use in the biogenics process. Recently, some instance of QC disruption by cyclic dipeptides has been reported. *L. reuteri*, a human vaginal isolate, was capable of producing the cyclic dipeptides cyclo(L-Phe-L-Pro) and cyclo(L-Tyr-L-Pro), inhibiting the staphylococcal quorum-sensing system driven by the AI named agr, to suppress the expression of toxic shock syndrome toxin-1 in *S. aureus* [79]. The report is useful for a better understanding of interspecies cell-to-cell communication between *Lactobacillus* and *Staphylococcus* and provides a hint to attenuate virulence factor production by bacterial pathogens. However, this idea requires further study before clinical application.

9. Conclusion

Biogenics is based on the concept of using the active ingredients which were revealed by the mechanism of oral probiotics. Biogenics is expected to be a prevention method for oral diseases that can be implemented without the problems associated with the use of probiotic bacteria, namely the involvement of acids harmful to teeth. The emergence of resistant bacteria against naturally occurring substances of biogenic candidates is not yet known. Furthermore, it is possible to combine substances which contribute to the health of the oral cavity, with those contributing to systemic health, such as control substances for blood sugar level, blood pressure, neutral fat, antioxidants, anti-stress, immune enhancement, anti-inflammation, antianxiety, and antidepressants. Therefore, the progress of practical application is expected. However, the elucidation of the mechanism of action is still in the beginning, and further study is needed.

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Conflict of interest

There are no conflicts of interest.

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