Probiotics Used for Respiratory Diseases

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Continuous increase of bacterial resistance to antibiotics causes many problems such as the advent of resistance to pathogenic bacteria, difficulty of microbial disease treatments, environmental pollution and others. It is inevitable to find potential substitutes for antibiotics in order to solve the above mentioned problems. Recently many literatures have shown that probiotics could be applied to the treatment or amelioration of respiratory diseases in addition to intensively studied gut related diseases. Target diseases for collecting data and analysis of the efficacies were chosen because viral respiratory infections are the most common diseases in humans. They were mainly viral diseases like common colds, pneumonia in addition to allergies and asthma. Papers on clinical efficacies, safety risks and mechanisms of microbial action of respiratory diseases were secured through known information sites and analyzed for their exact evaluations. The present analysis of research results on probiotics efficacies for respiratory diseases showed discrepancies in efficacies. On the whole, half to one third of papers reviewed only showed certain level of efficacies against respiratory viral diseases. It is very difficult to compare the results directly because the studies varied highly in study design, outcome measures, probiotics, dose, and matrices used. However, the results obtained so far show the potential applications of probiotics to the prevention or amelioration of the diseases. Conclusively, further well organized studies using randomized, double-blind, placebo-controlled clinical trials are needed to elucidate the realities of probiotics on respiratory related diseases and to obtain more definite efficacy results.

Key Words: Probiotics, Respiratory diseases, Allergies, Efficacy

INTRODUCTION

It is true historically that continuous fight against a number of pathogens and resultant saves of many lives became possible by the discovery of antibiotics by Fleming in 1920s. However, recent misuses of antibiotics by human in various fields including animal husbandry made increase a lot of antibiotic resistant microbes. Appearances of resistant bacterial strains caused many problems in the field of human (1) and veterinary medicine (2), agriculture and environment. For examples, antibiotic resistances made treatment of infectious diseases difficult by the super-bacteria appearance in addition to raising environmental contaminations through livestock and human activities.

Therefore, substitutes for antibiotic uses are required for the solution of antibiotic problems in all related fields. One of possible substitute candidates for antibiotics could be
probiotics recently known for their efficacies and extensively studied for their application possibilities. Probiotics have been reported to play many roles for health improvement by the regulation of microbiota and many other beneficial activities (3−6) including decreasing the antibiotic resistances and increasing nutrient utilizations. Many recent researches have revealed that probiotics could be used for prophylaxis or disease therapy by prevention or killing of pathogenic bacteria (7, 8).

Recently it is known that microorganisms in human body account for approximately 1 to 3% of the human body mass. Therefore, constituents of microorganisms including probiotics within human body tracts play great roles in homeostasis of human body. On the other hand, probiotics have many advantages over antibiotics in their specificity of action and pressures of selection on bacteria. The basic concept of probiotics is traditionally based on the theory of Metchnikoff more than one hundred years ago. His hypothesis on life extension by probiotic use was that the ingestion of many quantities of lactic acid bacteria would increase life expectancy by protecting human from many diseases. However, recent extensive studies on probiotics have improved the old concept on lactic acid bacteria in the light of recent microbiological insights.

The definition of probiotics has been changed in time to adapt to the broadening of organ target and modes of administration. World Health Organization's definition (WHO) on probiotics is as follows, namely "live micro-organisms which, when administered in adequate amounts, confer a health benefit on the host" (9, 10). They consist mostly of bacteria including lactic acid bacteria, bifidobacteria and yeasts including Saccharomyces that may be present either in food supplements or drugs.

So far, most application and studies of probiotics were limited to gastrointestinal areas because of their importance and traditional target of uses. However, recently studies on other human body including respiratory tracts are increasing because of possible prevention or therapies of infections, allergy and atopic dermatitis, and broad ranges of applications.

Therefore, the present articles reviewed related reference published on clinical trials, and described the mechanisms of action and safety of probiotics used for the respiratory tract diseases. Probiotics are known to be different in efficacies and other properties depending on strains developed for products even though within same species. This specific properties of probiotics require caution in reading of related study results.

1. Probiotics and respiratory tract

Respiratory tract has specific properties of microbiota other than those of intestinal tract. Therefore, in order to understand probiotics acting on respiratory tract diseases, composition of microbiota in respiratory tract and related functions should be investigated. Research papers on the microbiota in respiratory tract and potential use of probiotics for the related diseases were reviewed and analyzed in the followings.

1-1) Microbial constituents and diseases in respiratory tract

There are possibilities that microbiota which is distinct in constitution in human body tract interacts each other and its host regardless of not enough evidence in vivo (11, 12). Even though most researches on microbiota in human body had been performed from human intestinal tracts, those on respiratory tract microbiota are being elucidated and advanced owing to modern biotechnologies (13). Even today, it is difficult to secure evidence that respiratory microbiota constituents and functions are similar in developing and maintaining immune activities of the lung to intestinal microbiota. Nonetheless, there is increasing evidences that relationships between the respiratory microbiota and many lung diseases are shown and microbial changes in respiratory microbiota could cause diseases in human (Fig. 1). Furthermore, studies on the possible existence of "core respiratory tract microbiota" are suggested. It is known that microbiota of respiratory tract are mainly composed of Pseudomonas, Streptococcus, and many other bacteria. The "core respiratory microbiota" might be distinct depending on factors such as ethnic groups, environments like diets and many others. The presence of core respiratory microbiota and changes in its constituents dependent on individual health conditions
suggest the possibility that respiratory microbiota could have roles in causing chronic respiratory diseases. Much fewer numbers of Bacteroidetes and Firmicutes in respiratory tract are confirmed to be present in persons with diseases other than healthy persons.

It was reported that respiratory tracts including alveoli and bronchioles of healthy persons demonstrated the presence of bacteria by using more sensitive molecular methods including PCR (14). Microbial constituents of the above tracts were the same as those found in the upper respiratory tracts, whereas the bacterial concentration in lung is in much fewer levels. It is suggested that bacteria in alveolar could come from the upper respiratory tracts when considering similar bacterial constituents in alveolar and lung (15). It was also reported that the pulmonary microbiota in patients showing pneumonia (16) and bronchitis (17) was complex in microbial compositions. Owing to understanding of predominant bacteria in human respiratory tracts in healthy persons and patients, it was possible to understand the changes induced in a particular microbiota when using probiotics.

Experimental evidences on association between the respiratory tract microbiota and many chronic lung diseases have been accumulated recently. Furthermore, there is possibility that changes in the composition of the respiratory tract microbiota among subjects cause different disease status depending on persons with different ethnical backgrounds and factors such as environmental factors.

1-2) Clinical trials on persons with respiratory diseases using probiotics

For the demonstrations of clinical efficacies on respiratory tract diseases by probiotics, randomized and well organized clinical trials are needed. Presently many clinical results have been reported using various kinds of probiotics. Higher proportions of probiotics developed for health promotion so far have been used for the digestive tract diseases including diarrhea and inflammatory diseases (18–20). On the contrary, probiotic products for respiratory tract and urogenital infections have been developed relatively recently (21). It is theoretically presumed that probiotics effective for digestive infections might have effects on respiratory and urogenital infections owing to the interactions between the probiotic and the digestive immune system.

Many clinical reports on beneficial effect by probiotics for respiratory infectious diseases have been obtained recently (22–25). Fourteen clinical studies on "probiotics and prevention of upper respiratory tract infections" were reviewed (22). More children and less geriatric patients were included as research target populations of upper respiratory diseases including common colds. Combined probiotics consisted
of Lactobacillus and Bifidobacterium species were used alone or combined against the respiratory tract infections mentioned above. The patients in groups treated by one or several probiotic(s) had presented at least one acute episode less than those in the placebo even though no significant difference between the two groups was shown in the mean duration per infection. The test groups treated with probiotics showed lower number of antibiotic prescriptions and adverse effects of probiotics were fewer. Therefore, the authors concluded that the probiotic use groups showed better results than control groups in the number of upper respiratory tract infections and less antibiotic administrations. Nonetheless, it should be cautious for exact interpretations of clinical trial results because objective opinions should be involved.

Another clinical trial results were also reported on 224 infants vaccinated against pneumococcus, aged 7 to 13 months, and presented risks for acute otitis media (26). A combinations of prescribed probiotics were administrated daily for 12 months. Although no significant differences in the important criteria were observed between groups, it was suggested that some studies showed the potential of probiotics to prevent upper respiratory tract infections. In addition, large scale clinical trial with 465 persons administered by 2 different subspecies of Bifidobacterium and one subspecies of Lactobacillus were performed (27). Bifidobacterium animalis subsp. lactis BI-04 administered group showed lower illness episode, compared with control group, while there was no significant difference in illness risk between other groups. It was concluded that Bifido BI-04 group was effective because there was approximately 20 days meantime delay to an illness episode in BI-04 and NCFM & Bi-07 used groups, compared to concerned control group.

Studies on effects of probiotics on respiratory diseases of various animals in vivo and in vitro showed that there might be high potential of benefit roles by administered probiotics (28). The mechanisms of the probiotics on diseases of animals are not definite even though there could be explained by several respects on differences in clinical efficacy data. Therefore, using probiotics for animal respiratory diseases should be only one of many choices because of discrepancies between in vivo and in vitro tests in addition to differences of results among studies performed. Nonetheless, it is very difficult directly to compare the different studies because of different probiotic strains and many other factors applied. Other factors include various endpoint, populations employed, genetics and environments.

1-3) Efficacy of probiotics against viral infections

Many researchers performed studies on the efficacies of probiotics against viral diseases (28–30). One study on rhinovirus infection using reported results on 94 infants who were treated at a hospital allocated to receive oral probiotics or probiotics on days of 3 and 60 (29). The main parameters of the study were the incidence of virus-associated respiratory tract infection episodes and the severity and duration of viral respiratory infections. A significantly lower incidences of respiratory infections were detected in infants receiving probiotics, contrary to control groups. Furthermore, rhinovirus-induced incidences in the probiotic administered groups were lower, compared with the controls. No differences in rhinovirus RNA load during infections or occurrence of rhinovirus RNA in asymptomatic infants were shown in the study groups. The study concluded that microbiota modification with specific prebiotics and probiotics might be a means for reducing rhinovirus infections (30). Antibiotics are mostly used for the treatments of common viral respiratory diseases which are usually caused by common cold and influenza (31). For these reasons, future main targets of probiotics uses could be for respiratory diseases following gastrointestinal diseases (32).

Furthermore, the respiratory tract is a suitable target for probiotic immune stimulation owing to mucosal surfaces which are functionally related with other mucosal surfaces of the mucosal-associated lymphoid tissues (33). There are several reports that consumption of probiotics by infants may be protective against respiratory tract infections (34–36). Influenza vaccinations have increased recently in people aged 65 or higher by the high prevalence of the disease. Probiotics have been shown to stimulate antibody response to viral vaccination in many clinical studies conducted so far (37–40). Aubin et al. reported the study that a probiotic fermented dairy product improved immune response to
influenza vaccination in the elderly group (37). Boge et al. (38) also performed the double blind clinical trials to investigate the impact of probiotics consumptions on target groups. Based on study results, they concluded that daily consumption of probiotics increased specific antibody responses to influenza vaccination in the age group of over 70 years (38). Davidson et al. used Lactobacillus GG as an immune adjuvants of influenza vaccine in adults. They found the enhancements of immune responses owing to probiotics adjuvants in double blind clinical tests (39). Namba et al. also had similar results which showed enhancement of antibody titers and cell mediated immunity when Bifidobacterium longum BB 536 strain was administered to the elderly (40).

One study group tried to investigate efficacies of probiotics administered persons using Lactobacillus gasseri and Bifidobacterium longum (41). They evaluated clinical data, cellular immune parameters and symptom score of 479 persons participated in the research. Patients having received at least 3 months of probiotics supplements had shorter cold episodes by 2 days and experienced less severe symptoms (41). When administered orally at a dose of $2 \times 10^9$ CFU/day for 30 days, Bacillus coagulans increased cytokine production when exposed to an influenza A strain (41). Twelve week consumption of a probiotic product with $10^9$ CFU Lactobacillus plantarum and Lactobacillus paracasei was shown to reduce the risk of developing common cold infections (41). Nonetheless, it is evaluated that administrations of these probiotics for amelioration or preventions of the designated respiratory diseases are too early to be accepted because of no assurances of the efficacies against viral infections. On the contrary, combination therapies with antibiotics or other drugs in necessary situations are recommended in the present time.

1-4) Probiotics for pneumonia

Pneumonia is an inflammatory condition of the lung affecting mainly air sacs called alveoli and mostly caused by infection with microorganisms including viruses and bacteria. Recently one approach for pneumonia is the introduction of probiotics at the oro-pharyngeal level or by nasal oro-gastric intake. Two hypotheses on the possible mechanism of action by probiotics on pneumonia are suggested. One is that probiotics administered could decrease the concentration of pathogens owing to competitions at the oropharyngeal and gastric organs of pneumonia patients (42). The other hypothesis is that probiotics could modulate the immune response which is common to mucosa-related lymphatic tissue in respiratory and digestive mucosa. Seven studies were performed on the efficacies of probiotics on the patients with ventilator associated pneumonia (VAP) (42~48). The probiotics employed for the studies were used alone or combined. Probiotics used include L. plantarum, L. rhamnosus, Pediococcus pentosaceus, Leuconostoc mesenteroides, Lactobacillus paracasei subsp. paracasei, and Lactobacillus plantarum. The probiotics included in the studies were administrated through oropharyngeal, nasal or oro-gastric intake. The administration methods of probiotics including doses and frequency of daily administration varied according to studies performed. There were no reported adverse effects including bacteremia especially.

In the study on the efficacy of probiotics on pneumonia, the primary endpoint was the delay before isolation of P. aeruginosa in gastric and respiratory samples, whether causing colonization or infection (43). The delay before acquisition of this bacterium was significantly different between the two groups; the delay in the control group was 11 days on average while it was 50 days in the probiotic administered group. The two groups did not show any differences in the incidence of P. aeruginosa pneumonia (43). Klarin et al. performed the clinical tests in order to evaluate the potential use of a probiotic with L. plantarum 299 strain in the oropharynx of incubated patients and to determine its safety (44). Knight group surveyed tried to assess the effect of symbiotic therapy on critically ill patients with ventilator associated pneumonia (45). Kotzampassi et al. performed the research on the benefits of a symbiotic formula. The primary outcome was the rate of systemic infection resulting in 63% in the probiotic group versus 90% in the control group (46). The primary endpoint of the study was microbiologically caused pneumonia. No adverse effect was observed after the probiotic administrations. Spindler-Vesel group performed a clinical research on the
patients in early enteral nutrition in order to evaluate the efficacies of synbiotics, prebiotics and other peptides (47). The other study was performed on 167 critically ill patients for 48 hours, receiving either probiotics diluted in water and administrated daily through a gastric tube with *L. rhamnosus* GG, *L. casei*, *L. acidophilus* and *Bifidobacterium bifidum*. Double blind, randomized placebo control group in trauma patients was included for the efficacy comparison (48). There were no differences in mortality at 28 days and rate of nosocomial pneumonia acquired under ventilation between administered group and control group. Nonetheless, there was a significant decrease of mortality in patients with a probiotic treatments when a subgroup of 101 patients presenting sepsis was analyzed. However, the mortality was higher in patients with the probiotics in the subgroup of patients not presenting severe sepsis. As based on research results above, it was concluded that the administration of probiotics was associated with a decreased rate of virus caused pneumonia and a decreased length of stay in emergency centers. The incidences and mortalities in the above clinical studies showing split results were summarized in Table 1.

### 1-5) Probiotics on allergy

Researches on patients with allergy efficacies by probiotics are in early stages. So far, several epidemiological studies have shown a clear link between early exposure to microorganisms and the incidence of asthma. However, there are discrepancies in efficacies by probiotics on allergy, depending on authors, strains of probiotics and many other factors.

Few studies have analyzed their efficacy in early allergic diseases like food allergy and atopic dermatitis. Wheeler *et al.* surveyed immunological and clinical impact of probiotics *Lactobacillus acidophilus* on asthma (49). They demonstrated on clinical improvement in 15 young adults with moderate asthma, compared with control group when treated with probiotic during treatment period. The authors concluded that they could not detect any changes in clinical and immunological parameters in asthma patients.

Helin *et al.* reported that there was no effect of probiotics (*Lactobacillus rhamnosus*) on birch-pollen allergy but a non-significant decrease of symptoms in the test group when they was orally administered for 28 weeks (50). Giovannini *et al.* also reported the effect of long term probiotics use on preschool children with allergic asthma and/or rhinitis (51). They performed a randomized blind controlled clinical trials using fermented milk containing *Lactobacillus casei* without finding any beneficial effect after 12 months of treatment.

Stokert *et al.* reported a clinical research which showed no demonstration of definite improvements in children with asthma when they were administered with *Enterococcus*

### Table 1. Summaries of double blind randomized, controlled clinical trials for patients of intensive care units after administration of various probiotics on mechanical ventilation pneumonia and mortality

<table>
<thead>
<tr>
<th>Reference number</th>
<th>Number of patients Assessed</th>
<th>Probiotics vs. Control</th>
<th>Probiotics vs. Control</th>
<th>Probiotics vs. Control</th>
<th>Indicator disease</th>
</tr>
</thead>
<tbody>
<tr>
<td>45</td>
<td>130 vs. 129</td>
<td>9% vs. 13%</td>
<td>21.5% vs. 26.3%</td>
<td>Pneumonia</td>
<td></td>
</tr>
<tr>
<td>43</td>
<td>102 vs. 106</td>
<td>23% vs. 22%</td>
<td>NA</td>
<td>Pneumonia</td>
<td></td>
</tr>
<tr>
<td>44</td>
<td>23 vs. 21</td>
<td>4% vs. 14%</td>
<td>22% vs. 19%</td>
<td>Pneumonia</td>
<td></td>
</tr>
<tr>
<td>47</td>
<td>26 vs. 87</td>
<td>15% vs. 39%</td>
<td>8% vs. 6%</td>
<td>Pneumonia</td>
<td></td>
</tr>
<tr>
<td>46</td>
<td>35 vs. 30</td>
<td>54% vs. 80%</td>
<td>14% vs. 30%</td>
<td>Pneumonia</td>
<td></td>
</tr>
<tr>
<td>42</td>
<td>68 vs. 70</td>
<td>19% vs. 40%</td>
<td>21% vs. 17%</td>
<td>Pneumonia</td>
<td></td>
</tr>
<tr>
<td>48</td>
<td>87 vs. 80</td>
<td>18.7% vs. 26.4%</td>
<td>25.3% vs. 33.7%</td>
<td>Pneumonia</td>
<td></td>
</tr>
</tbody>
</table>

*: VAP, ventilator acquired pneumonia, and #: NA, not applicable.
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There is possibilities that school age children with asthma might not be effectively immune response to probiotics (53). So far, it is very difficult to find literatures demonstrating definite clinical effects on allergy and/or asthma by probiotics. However, there are reports that some strains of probiotics might have roles in alleviation of allergic symptoms in vitro. In the present time, most clinical researches on efficacies of asthma by probiotics were performed by taking foods containing probiotics. In these cases, survival conditions of probiotics in foods must be considered for the exact evaluations of the treatment efficacies. Furthermore, administration method, epidemiology of subjects employed and many other factors involved must be evaluated for the increase of efficacy enhancements on allergy and/or asthma. The efficacies could be different from direct administrations to respiratory airways by droplets or other methods. Conclusively, probiotics effects on allergies and/or have not been conclusive and could be different by target subjects, strains used, and authors performing the studies.

2. Mechanisms of probiotics action in respiratory tract

Exact mechanisms of probiotics action on respiratory tracts are still unclear. Furthermore, the mechanisms of probiotics seem different depending on probiotics species and specific strains. There seem to be multiple mechanisms in probiotic activity depending on probiotics characteristics and target diseases. Multiple microbe-microbe and microbe-host interactions probably account for the versatility of probiotic action. However, it seems likely that probiotics have similar effects on the whole. The same mechanisms of anti-inflammatory actions as gut system might be applied to respiratory tract very possibly.

2-1) Direct antimicrobial activity by probiotics

So far, evidence on probiotics has been mostly secured by the authors of many researches on the activity of probiotics in the digestive tract. However, most researches on the activities of probiotics on respiratory including atopic dermatitis and allergies have recently been initiated. Nonetheless, evidences of probiotics on mechanism of actions associated with the respiratory tract and allergy have accumulated recently by many researchers (54, 55). First, probiotics produce antimicrobial agents such as bacteriocins, hydrogen peroxide, and organic acids which participate in the acidification of environments. In addition, probiotics and pathogens compete for a limited number of substrates and cellular adhesion sites in addition to inhibiting the production of virulence factors. Many probiotics including Lactobacillus secret corresponding antimicrobial compounds as shown in Table 2 (56).

Furthermore, some of probiotics make antimicrobial activities directly on various pathogens. These activities are based on actions affecting microbial products like toxins, metabolic products. Mucin produced by probiotics inhibits the adherence of pathogenic microbes which can be prevented from the body. However, obtaining evidence of antimicrobial activities is very difficult to demonstrate because of many different factors involved. Early studies on antiviral activities by probiotics have been performed in patients with enteric virus infections including rotavirus and many enteroviruses. Presently many studies tries to demonstrate the antiviral activities by probiotics using related enzymes.

Table 2. Antimicrobial substances produced by probiotic bacteria (56)

<table>
<thead>
<tr>
<th>Probiotics</th>
<th>Compounds produced</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lactobacillus GG</td>
<td>Wide-spectrum peptides</td>
</tr>
<tr>
<td>L. acidophilus</td>
<td>Acidolin, Acidophilin</td>
</tr>
<tr>
<td>L. delbrueckii subspecies bulgaricus</td>
<td>Bulgarian</td>
</tr>
<tr>
<td>L. plantarum</td>
<td>Lactol</td>
</tr>
<tr>
<td>L. brevis</td>
<td>Lactobacillin, Lactobrevin</td>
</tr>
<tr>
<td>L. reuteri</td>
<td>Reuterin</td>
</tr>
<tr>
<td>L. sake L145, L. sake Lb706</td>
<td>Lactocin S</td>
</tr>
<tr>
<td>L. johnsonii</td>
<td>Lactocin F</td>
</tr>
<tr>
<td>L. helveticus</td>
<td>Helveticin J</td>
</tr>
<tr>
<td>L. cremoris</td>
<td>Dipliococin</td>
</tr>
<tr>
<td>Lactococcus lactis</td>
<td>Nisin, Lactostrepsin</td>
</tr>
<tr>
<td>Pediococcus pentosaceus, P. acidilactis</td>
<td>Pediocin</td>
</tr>
<tr>
<td>S. thermophilus</td>
<td>Streptophilin</td>
</tr>
<tr>
<td>Enterococcus faecium DPC 1146</td>
<td>Enterocin 1146</td>
</tr>
</tbody>
</table>

faecalis (52).
instead of virological tests. Direct demonstration of antimicrobial effects in vivo and in vitro by enzymes of probiotics is comparatively easier. However, it should be very cautious to interpret the results obtained because of high non-specific reactions. For example, probiotics containing lactase have been shown to help lactose be digested.

Another mechanisms of probiotics action can be determined by modification of probiotics concerned to produce therapeutic substances. For example, genetically engineered Lactococcus lactis strain could deliver the anti-inflammatory cytokines or enzymes to the gastro-intestinal organs. However, the introduction of genetically modified probiotics in human require no risk to public health and technology of controlling and eliminating the modified organism if necessary.

2-2) Probiotics action on epithelium barrier function

In epithelial layer in host, there are three different kinds of modulation of barrier effects. Namely, pathogens can be inhibited by antimicrobial compounds produced by probiotics, and inhibitions of quorum-sensing and virulence factors in addition to cell adhesion competition between probiotics and pathogens within the environments (15) as shown in Fig. 2. Probiotics can also act against the pathogen by interacting with the host's epithelial membranes (57). Guglielmetti et al. studied the potential of various lactic acid bacteria to inhibit Streptococcus pyogenes, a pathogenic bacterium, at the pharyngeal level in vitro (58). They demonstrated that Lactobacillus helveticus MIMLh5 inhibited 55% of S. pyogenes, in adhesion on a line of oropharyngeal cancerous cells lines and 32% on a cell line of keratinocytes. The reinforcement of the epithelium's barrier function could
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2-3) Increase of immune function

A number of research have been performed on the influence of probiotics on immune parameters, inflammatory conditions in humans. However, most studies have been performed in gastrointestinal tract so far. Immunological effects on respiratory and/or allergy by probiotics are assumed to be lower or indirect, compared with gastrointestinal diseases. Probiotics have high possibilities of immunomodulatory effects. Namely, probiotics might be able to modulate the hosts of the innate as well as the acquired immune system. Release of soluble factors by probiotics can trigger activities of immune cells or epithelial cells which subsequently affect immune cells. Probiotics can also modulate the immune system's activity nearby or remotely from the organ target. Furthermore, particular strain of L. helveticus inhibited cytokine pathway in the presence of IL-1. Thus some probiotics and especially those belonging to the Lactobacillus genus are known to stimulate the activity of particular immune related cells.

Charlson et al. administered L. rhamnosus GG to the mouse nasally for three days for stimulating the cytotoxic activity of pulmonary NK cells and then infected influenza virus H1N1 [14]. They found that the secretion of IL-1 and TNF-α was increased. These effects were interpreted to be related to a better survival of mice at 15 days, with 60% of survivors in treated mice compared with 20% in control mice.

Another example of research demonstrated that the action of Lactobacillus casei CRL431 in a murine model of Streptococcus pneumoniae pulmonary infection allowed confirming immunological responses by probiotics. In the study, L. casei CRL431 was ingested 3 times and then instilled S. pneumonia into mouse nasally. The ingestion of L. casei CRL431 during two days was effective with a stimulation of innate and adaptive immune responses. In addition to immunological responses, it induced a faster clearance of S. pneumonia with a lower pneumococcal inoculum in the lungs and a shorter bacteremic period than in the control group. Furthermore, fifteen days after the infection, the anti-pneumococccic IgG levels in BAL and in blood were superior to those observed in control mice in addition to superior TNF levels in the lungs of treated mice compared to those of controls after 8 hours. It is interpreted that stimulation by anti-inflammatory agent produced could prevent tissue damage caused by a strong inflammatory response [15]. In order to demonstrate the protective effects by probiotics on respiratory tract, the development of well organized and randomized double blind clinical trials is considered mandatory.

As another example of clinical study, West et al. (27) examined cell-mediated immune mechanisms following daily supplementation with Bifidobacterium animalis subsp. lactis BI-04 and a combined Lactobacillus acidophilus NCFM & B. animalis subsp. lactis BI-07. A cohort of 144 individuals were allocated to daily supplementation consumed as a beverage with BI-04. They concluded that probiotic supplementation had little effect on parameters of the innate immune system and certain probiotics is not effective on immune response.

3. Safety of probiotics

Probiotics has been used as a constituent of food or drinks for a long time without particular problems of risk. However, safety, specifically in the case of immune compromised persons should be evaluated critically in the preparation of functional food or drugs. For the persons with immune deficient or aged persons, special criteria should be applied for the persons with opportunistic infections theoretically.

Probiotics may be responsible for bacteremia or even endocarditis. So far, reported cases of bacteremia and endocarditis due to Lactobacillus are very difficult to find. Husni et al. reported the review study which found 45 cases of bacteremia for 15 years, showing 0.085% of positive cultures from blood specimens. Immune compromised persons, diabetic patients and/or patients with recent surgery were surveyed especially in the reports (59, 60). However, the origin of bacteremia could be caused by endogenous bacteria from organs like intestine and genitalia. In addition, there is
potential positive results caused by other factors like false positive. Husni et al. (59) concluded that the chances of bacteremia after the ingestion of probiotics is very low with only a few cases reported so far. The exact identification of strain origins of bacteremia in patients included in the study should be elucidated because of potential recurrences of endogenous strains in the patient body.

The other potential risk of probiotics is the gene transfer from commensal bacteria to probiotics or vice-versa, including antibiotic resistance. Tannock et al. demonstrated that antibiotic resistance plasmids were transferred from Lactobacillus sp. to Enterococcus faecalis in vitro and in vivo (61). Furthermore, Haug et al. also demonstrated that gene was transferred from Enterococcus faecium to Listeria monocytogenes in vitro model of colic fermentation (62). Klein et al. reported that lactobacilli carry an intrinsic resistance to vancomycin but this resistance was proved to have no relationship with \textit{vanA}, \textit{vanB}, or \textit{vanC} genes (63). It is interpreted that continuous repetition on the acquisitions of resistance genes was originated from exogenous probiotics because of potential acquirements of new genes.

The other potential adverse effect of probiotics could be a decreased immune modulating effect for the administered individuals, especially in pregnant women and neonates. Nevertheless, researchers even majoring on probiotics have not reported any such adverse effects (22). However, it should be kept in mind that even probiotics is a living organism and opportunistic infection could occur theoretically, even though probiotics including the \textit{Lactobacillus} genus are tested to be some of the safest micro-organisms. Therefore, safety of all the probiotics strains should be demonstrated completely before use since each specifically interacts with the host. In addition, all the properties of bacteria used as probiotics at the infra-specific level should be identified completely for making sure they are safe for the target patients.

4. Requirements for the development as probiotics

In order to be used as probiotics for human, several criteria should be satisfied with properties necessary for safety and function mainly. First of all, the safety of probiotic strains should be secured for human use in addition to several basic properties including human origin of strains, production of antimicrobial substances, antagonism against carcinogenic and pathogenic bacteria and clinically validated health effects. Main guidelines for the safety assessment and other properties can be found and discussed in several articles (64, 65). Several approaches are possible in the assessment of the probiotic safety and functions including studies on the intrinsic properties (66) and pharmacokinetics studied \textit{in vivo} using related biopsy techniques (67). In relationship with properties of probiotic product, Marteau et al. reported potential detrimental properties of bile salts or mucus of probiotics in certain circumstances (68).

Korpela et al. performed the study on the potential risk of platelet aggregation by \textit{Lactobacillus rhamnosus} GG which is known as one of the safest probiotics (69). In their studies, confirmed that the bacterial strain did not induce spontaneous aggregation as shown in other previous studies using other bacterial strains.

Charteris et al. (70) performed the study on the antibiotic susceptibility of potential probiotic \textit{Lactobacillus} species. Antibiotic resistance of probiotics could be intrinsic or acquired. They found that lactobacilli display a wide range of antibiotic resistances naturally, but in most cases antibiotic resistance is not of the transmissible type (70). \textit{Lactobacillus} strains with non transmissible resistances usually do not form a safety problem. Another criterion to consider in the use of probiotics for respiratory diseases is strain origin, possibly from human. Human origin of the probiotics has many advantages over the other animal species. Other points to consider for probiotic action are adhesion of probiotic strains to tract surfaces and subsequent colonization, immunity, and stabilities at target organ of the body. Thus, adherent probiotics have been thought effectively to induce immune effects and to stabilize the intestinal barrier (70). Adhesion may also provide competitive exclusion of pathogens from the intestinal epithelium. However, exact mechanism of probiotics and role of adhesion in safety still not elucidated detailedy.

Being ideal probiotics for respiratory diseases requires good functions working in human body. These requirements
are good stability of strains and secretion of beneficial materials and persistence in tracts in addition to good immune stimulant. Furthermore, competitive and ideal probiotics should have good sensory properties, phase resistance, stability during processing and storage, and heat resistance. Other requirements to be considered for competitive probiotics are anti-mutagenic and anti-carcinogenic properties, antagonistic activity against pathogens and satisfaction of various criteria in concerned regulations with corresponding authority.

CONCLUDING REMARKS

In this review, many literatures on the clinical trials using probiotics were collected and analyzed, depending on health benefit and infection control. Many research results obtained so far showed that there are some potential benefits to the respiratory conditions including infectious diseases in vitro and in animal tests. However, well controlled clinical trials showed split results on the whole. Some data from clinical trials did not show convincing results. The reasons for the results are supposedly caused by various ways of clinical trials themselves. There were differences in uses of administered probiotics, study populations and designs together with various clinical endpoints. For the applications of probiotics to human diseases, additional studies on various targeted populations are required. The present evidences obtained so far are not enough for a therapy or prevention. Therefore, some additional ways and administrations should be prepared. More numbers of clinical trials on the efficacy owing to probiotics are required. Combined administrations of probiotics and known drugs should be considered for the decrease of antibiotic resistance.

On the whole, continuous in vitro and animal studies on probiotics actions made mechanisms of clinical trials better understood recently. Probiotics can also be effective against infections agents by various ways including inhibition and competition with other infectious agents. Supposed mechanisms of pathogen inhibitions include reinforcement of the epithelium barrier functions and modifications of the immune system response.

It is concluded that probiotics have many advantages in preservations of commensal bacteria and inhibitions of resistant bacteria. In addition, combined uses of various probiotic bacteria have higher level of clinical benefits. However, for the uses of the airway microbiota in respiratory therapy, the more detailed mechanisms of microbiota functions should be elucidated. Presently it is interpreted that differences in strains, hosts and study performances might cause split results of efficacies. However, considering all these results obtained so far, it is considered that probiotics might have the potentials of preventions for respiratory diseases. In addition, for the human uses of probiotics, safety problems should be considered with special emphasis on compromised persons. Conclusively there are possibilities that probiotics could be used in the areas of allergies, asthma or wheezing in addition to various respiratory diseases when many technical problems concerned are solved.

REFERENCES

29) Luoto R, Ruuskanen O, Waris M, Kalliomäki M, Salminen S, Isolauri E. Prebiotic and probiotic supple-


