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REVIEW ARTICLE

Beneficial effects of probiotics in upper respiratory tract infections and their mechanical actions to antagonize pathogens

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Summary

Probiotics are live micro-organisms with beneficial effects on human health, which have the ability to counteract infections at different locations of the body. Clinical trials have shown that probiotics can be used as preventive and therapeutic agents in upper respiratory tract infections (URTIs) and otitis. Their mechanical properties allow them to aggregate and to compete with pathogens for nutrients, space and attachment to host cells. Consequently, they can directly antagonize pathogens and thus exert beneficial effects without directly affecting the metabolism of the host. An overview of the probiotics with such traits, tested up to date in clinical trials for the prevention or treatment of URTIs and otitis, is presented in this review. Their mechanical properties in the respiratory tract as well as at other locations are also cited. Species with interesting *in vitro* properties towards pharyngeal cells or against common respiratory pathogens have also been included. The potential safety risks of the cited species are then discussed. This review could be of help in the screening of probiotic strains with specific mechanical properties susceptible to have positive effects in clinical trials against URTIs.

Introduction

When they were discovered in the middle of the twentieth century, antibiotics offered the promise of efficient and cheap treatment of bacterial infections and even the possibility to eliminate infectious diseases. Pathogens have, however, found a way to survive by developing resistance to a range of antibiotics. This not only makes treatment of disease more difficult but also presents a serious threat to immunodeprived populations. As alternative antimicrobial approaches are being developed, probiotics have gained special interest in the last years.

According to the latest definition by the World Health Organization, probiotics are live micro-organisms that when administered in adequate amounts confer a health benefit to the host (FAO/WHO 2001). Probiotic treatment aims to direct the composition of the microbiota from potentially harming to a microbiota that would be beneficial to the host. Probiotics can be used as means of prevention by reducing the risk for overgrowth of potential pathogenic bacteria, thus suppressing colonization by the latter (Ouwehand *et al.* 2002). They also aim to restore lost bacteria or metabolic activities in colonized organs or to stimulate the immune response (Kalliomaki and Isolauri 2003). Probiotics have been shown to be beneficial at different sites of the human body – oral cavity, respiratory tract, gastrointestinal tract and urogenital tract. They have well established positive effects in the treatment of diarrhoea, including antibiotic associated and traveller's diarrhoea, vaginitis and functional gastrointestinal disorders [for review see (Allen *et al.* 2010; MacPhee *et al.* 2010; Girardin and Seidman 2011)]. Nowadays, various probiotic strains are commercialized with the aim to prevent or to treat these types of diseases. Human clinical trials and animal studies have shown that probiotics could have broader applications and they

could also be used to prevent, to treat or to relieve symptoms in cases of caries, periodontitis, allergies, atopic disease, respiratory infections, otitis, inflammatory bowel disease, Crohn's disease, colorectal cancer, acute gastroenteritis, lactose intolerance and cystitis [for review see (Stamatova and Meurman 2009b; Stamatova *et al.* 2009a; Ozdemir 2010; Gupta 2011; Meijer and Dieleman 2011; Yu 2011)].

In vitro and animal studies have allowed to elucidate some of the properties and modes of action of these beneficial micro-organisms. Their action can be directed at the host, the pathogen or both. Probiotics may modulate the host's innate or acquired immune response by products like metabolites, cell wall components and DNA, or they may increase intestinal mucin production (Mack *et al.* 1999). They may produce substances that inhibit pathogens – low-molecular-weight substances, low- and high-molecular-weight bacteriocins, antibiotics and microcins (Oelschlaeger 2010). These beneficial bacteria can also exert direct, mechanical effects on pathogens. These mechanical properties allow them to antagonize and to compete with them without affecting the metabolism neither of the host, nor of the pathogen. Competition involves adhesion, binding sites, nutrients and space (Lepargneur and Rousseau 2002).

The ability to adhere to the surface of epithelial cells or mucus enables probiotics to form a protective layer, thus blocking contact between pathogens and host cells. Adhesion ability also allows probiotics to compete with pathogenic bacteria for binding sites (Lepargneur and Rousseau 2002). If they bind to the same receptor and the affinity of the probiotic is higher, it has the ability to displace the pathogen. Furthermore, adherent probiotics occlude the access of recently arrived pathogens to the epithelium and thus exert competitive exclusion (O'Toole and Cooney 2008). Size can also be an important factor. Large-sized probiotic bacteria could exert better competitive exclusion of pathogens than small-sized ones by masking specific receptor sites for pathogenic micro-organisms on the cell surface by steric hindrance (Merk *et al.* 2005). Competition for nutrients results from the depletion by probiotics of nutrients from the environment that would otherwise be available for the pathogens. Additionally, they may bind and render unavailable to pathogens limited substances, such as iron (Oelschlaeger 2010). Probiotics and pathogens also compete for space that is essential for the multiplication of all micro-organisms (Alvarez-Olmos and Oberhelman 2001).

Another desirable mechanical property for probiotics is their capacity to aggregate among themselves (auto-aggregation), with other probiotics or with pathogens (co-aggregation). Through auto-aggregating or co-aggregating with other probiotics, an adequate mass is achieved which is necessary for these micro-organisms to manifest their beneficial effects (Collado *et al.* 2007). Aggregation also enables the formation of a barrier that protects the host's epithelium from colonization by pathogens. Moreover, the ability to co-aggregate with a pathogen allows the probiotics to entrap it (Boris *et al.* 1998; Re *et al.* 2000).

All properties and modes of action are probably involved in the global beneficial effect exerted by probiotics. The properties and modes of action differ among probiotics and are strain specific. Moreover, the mechanical properties are also location specific. Thus, a single probiotic cannot be a remedy for all diseases (Oelschlaeger 2010). This underlines the importance of choosing the appropriate strain for a given condition.

Upper respiratory tract infections (URTIs) represent the most common acute illness in the patient outsetting, and they account for 9% of all consultations in general practice (Bourke 2007). URTIs include rhinitis, rhinosinusitis, rhinopharyngitis, also called the common cold, pharyngitis, epiglottitis and laryngitis. We have also included otitis in this review. Even though otitis does not affect the respiratory tract, infections of the upper respiratory tract can extend to the ears through the Eustachian tubes. URTIs can have viral or bacterial origin. The most common viruses causing URTIs are rhinoviruses, coronaviruses, parainfluenza and influenza viruses. Among the bacteria causing URTIs, the most frequent pathogens are group A streptococci, *Mycoplasma pneumoniae*, *Chlamidia pneumoniae*, *Corynebacterium diphteriae*, *Staphylococcus aureus* and *Streptococcus pneumoniae* (Bourke 2007). In the case of pharyngitis, the origin of the infection is viral in 15–40% of cases in children and 30–60% of cases in adults. Bacterial origin accounts for 38–40% of cases in children and 5–10% of cases in adults (Pichichero 2007). URTIs of bacterial origin are commonly treated by antibiotics. As mentioned earlier, however, antibiotherapy presents drawbacks, especially the occurrence of resistant bacteria. An alternative method for the treatment of URTIs could be the use of probiotics.

The aim of this review is to present the probiotic species that have been tested up to date in clinical trials for the prevention or treatment of URTIs and otitis (Table 1). All of them were bacteria, and no yeast species were found. We have mainly focused on the species with mechanical properties, but probiotics with both mechanical properties and immune stimulatory effects were also included. Trials conducted on subjects under heavy physical training, as well as trials employing synbiotics, were excluded. *In vitro* and animal studies have explained some of the mechanical properties and modes of action of the tested probiotics. Table 2 summarizes the mechanical properties of these probiotics in the upper respiratory tract, as well as at other sites of the body. This

Table 1 Effect of probiotics used in clinical trials for the prevention or treatment of upper respiratory tract infections (URTIs) and otitis

Probiotic	Strain	Population	Effect of treatment	Reference
Lactobacillus rhamnosus	GG	Healthy children	Reduction of RTI (otitis media, sinusitis, bronchitis and pneumonia) and antibiotic treatment	Hatakka *et al.* (2001)
Lact. rhamnosus	GG	Healthy children	Reduction of the risk of RTI Reduction of the number of days with respiratory symptoms	Hojsak *et al.* (2010)
Lact. rhamnosus *Bifidobacterium breve* *Propionibacterium freudenreichii* subsp. *shermanii*	GG 99 JS	Otitis-prone children	No decrease in the occurrence or recurrence of acute otitis media Tendency to decrease recurrent RTI No decrease in the nasopharyngeal carriage of *S. pneumoniae*, *H. influenzae* Increased prevalence of *M. catarrhalis*	Hatakka *et al.* (2007)
Lact. rhamnosus *Streptococcus thermophilus* *Lact. acidophilus* *Bifidobacterium sp.* (Actifit plus, Emmi Schweiz AG)	GG 145 B420	Healthy adults	Reduction of the nasal colonization with pathogens (*Staphylococcus aureus and S. pneumoniae*)	Gluck and Gebbers (2003)
Lact. rhamnosus *Bif. animalis* subsp. *lactis*	GG Bb-12	Healthy infants	Reduction of the incidence of recurrent RTI Reduction of the risk of acute otitis media Reduction of the need for antibiotic treatment	Rautava *et al.* (2009)
Bif. animalis subsp. *lactis*	Bb-12	Healthy new-born infants	Decrease in respiratory infections No significant difference in otitis media No significant difference in use of antibiotics	Taipale *et al.* (2011)
Bif. animalis subsp. *lactis*	Bb-12	Healthy children	Significant difference in respiratory illness	Weizman *et al.* (2005)
Lact. acidophilus *Bif. animalis* subsp. *lactis*	NCFM Bi-07	Healthy children	Reduction of fever, rhinorrhoea, cough incidence Reduction of antibiotic prescription	Leyer *et al.* (2009)
Lact. delbrueckii subsp. *bulgaricus* *Strep. thermophilus*	OLL1073R-1 OLS3059	Healthy adults and elderly	Decreased risk of catching the common cold or influenza virus	Makino *et al.* (2010)
Lact. paracasei subsp. *paracasei* *Strep. thermophilus* *Lact. delbrueckii* subsp. *bulgaricus* (Actimel)	DN-114001	Healthy free-living elderly	Reduction of duration of URTIs, specifically rhinopharyngitis	Guillemard *et al.* (2010)
Lact. paracasei subsp. *paracasei* *Strep. thermophilus* *Lact. delbrueckii* subsp. *bulgaricus* (Actimel)	DN-114001	Healthy children	Decrease in incidence of URTIs	Merenstein *et al.* (2010)
Lact. plantarum *Lact. paracasei*	HEAL 9 (DSM 15312) 8700:2 (DSM 13434)	Healthy adults	Reduction of incidence and duration of common cold episodes Reduction of severity of symptoms	Berggren *et al.* (2011)
Lact. gasseri *Bif. longum* *Bif. bifidum* (Tribion harmonis)	PA 16/8 SP 07/3 MF 20/5	Healthy adults	Reduction in the duration of common cold episodes Reduction in the severity of symptoms	Vrese *et al.* (2005)

(*Continued*)

Table 1 *(Continued)*

Probiotic	Strain	Population	Effect of treatment	Reference
Corynebacterium Co304	Isolated from nasal mucus of a healthy volunteer	Healthy adults	Prevents and eliminates colonization of the nasal cavity by *Staph. aureus*	Uehara *et al.* (2000)
Strep. sanguinis	89a, NCIMB 40104	Children with fluid in the middle ear	Complete or almost complete resorption of middle ear fluid	Skovbjerg *et al.* (2009)
Strep. sanguinis *Strep. mitis* (Bactonormal, Essum AB, Sweden)		Pharyngotonsillitis-prone patients	Decrease in the recurrence of streptococcal tonsillitis	Roos *et al.* (1993a)
Strep. sanguinis *Strep. mitis*		Tonsillitis-prone patients	Decrease in the recurrence of tonsillitis	Roos *et al.* (1993b)
Strep. sanguinis *Strep. mitis*		Pharyngotonsillitis-prone patients	Decrease in the recurrence of tonsillitis	Roos *et al.* (1996)
Strep. mitis *Strep. sanguinis*		Patients with acute pharyngotonsillitis	Decrease in the recurrence rate of group A streptococci	Falck *et al.* (1999)
Strep. sanguinis *Strep. mitis* *Strep. oralis*	Isolated from the opening of the Eustachian tubes of healthy children	Otitis media-prone children	Decrease in the recurrence of otitis media	Roos *et al.* (2001)
Strep. sanguinis *Strep. mitis* *Strep. oralis*	Isolated from the nasopharynges of healthy children	Otitis media-prone children	No significant effect	Tano *et al.* (2002)

review may be of help in the identification of novel strains among the cited species as a new way of management of infectious disease.

Probiotics with Effects in Upper Respiratory Tract Infections

We have identified 21 clinical trials addressing the effect of probiotics in URTIs and otitis. Four trials have shown no significant difference in the outcome measures between the probiotic and placebo groups (Tano *et al.* 2002; Weizman *et al.* 2005; Hatakka *et al.* 2007; Taipale *et al.* 2011). Moreover, the clinical trial conducted by Hatakka and colleagues showed an increase in the prevalence of *Moraxella catarrhalis* in the probiotic group. All the other trials reported a beneficial effect of the probiotic and improvement in specific sickness-related outcome measures.

A variety of probiotic strains have been used in these clinical trials. We have chosen to include three probiotic species (*Lactobacillus casei*, *Lact. helveticus* and *Lactococcus lactis*) to this review, which have not been tested in clinical trials for their effect on URTIs. However, *in vitro* studies have shown that they possess mechanical properties that allow them to antagonize respiratory tract pathogens. They could thus be potential probiotic candidates for future clinical studies.

Lactobacillus rhamnosus

The most commonly used strain of *Lact. rhamnosus* in URTI trials is GG. Alone or in association with *Bifidobacterium animalis* subsp. *lactis* Bb-12, this probiotic reduced the incidence of respiratory infections and acute otitis media in children, as well as the use of antibiotics (Hatakka *et al.* 2001; Rautava *et al.* 2009). The combination of these two probiotics may reduce the colonization by respiratory pathogens through local inhibition, as well as through immunomodulation throughout the common mucosa-associated immune system (Rautava *et al.* 2009). Hojsak and colleagues demonstrated not only a reduced risk of URTIs upon consumption of *Lact. rhamnosus* GG, but also a reduction in the total number of days with respiratory symptoms (Hojsak *et al.* 2010). In combination with *Strep. thermophilus*, *Lact. acidophilus* 145 and *Bifidobacterium* sp B420, *Lact. rhamnosus* GG was shown to reduce the nasal colonization with pathogens such as *Staph. aureus* and *Strep. pneumoniae* in adults. An immunostimulatory mechanism may be involved (Gluck and Gebbers 2003).

In another clinical trial, however, strain GG in association with *Bif. breve* 99 and *Propionibacterium freudenreichii* subsp. *shermanii* JS did not show beneficial effects. No decrease in the occurrence or recurrence of acute otitis media or in the nasopharyngeal carriage of

Table 2 Mechanical effects of probiotics used in clinical trials for the prevention or treatment of upper respiratory tract infections and otitis. Most of these properties have been demonstrated *in vitro*

Probiotic	Mechanical properties	Site of action	Reference
Lactobacillus rhamnosus	Auto-aggregation	/	Pascual *et al.* (2008)
	Co-aggregation	resp., oral cav., intest., urog., intest.	Collado *et al.* (2007), Pascual *et al.* (2008), Twetman *et al.* (2009)
	Adherence	resp., oral cav., intest., urog.	Tuomola and Salminen (1998), He *et al.* (2001), Haukioja *et al.* (2006), Morelli *et al.* (2006), Pascual *et al.* (2008), Stamatova *et al.* (2009a), Guglielmetti *et al.* (2010b)
	Competitive exclusion	vag.	Reid *et al.* (1987), Roos *et al.* (2001), Sookkhee *et al.* (2001), Guglielmetti *et al.* (2010a)
	Competition by steric hindrance	intest.	Lee and Puong (2002)
	Competition for binding sites	vag., intest.	Princivalli *et al.* (2009)
	Competition for adhesion	intest.	Forestier *et al.* (2001), Gopal *et al.* (2001), Coudeyras *et al.* (2008)
Lact. acidofilus	Auto-aggregation	/	Boris *et al.* (1998)
	Co-aggregation	oral cav., vag.	Boris *et al.* (1998), Twetman *et al.* (2009)
	Adherence	intest., vag.	Chauviere *et al.* (1992), Coconnier *et al.* (1992), Bernet *et al.* (1994), Tuomola and Salminen (1998), Gopal *et al.* (2001), Zarate and Nader-Macias (2006)
	Competitive exclusion	vag.	Zarate and Nader-Macias (2006)
	Competition by steric hindrance	vag.	Reid *et al.* (1987)
	Competition for binding sites	vag.	Boris *et al.* (1998)
Bifidobacterium animalis subsp. *lactis*	Auto-aggregation	/	Gopal *et al.* (2001)
	Co-aggregation	intest.	Collado *et al.* (2007)
	Adherence	intest.	Gopal *et al.* (2001)
	Competitive exclusion	intest.	Candela *et al.* (2008)
Lact. delbrueckii subsp. *bulgaricus*	Auto-aggregation	/	Aslim *et al.* (2007)
	Co-aggregation	intest.	Aslim *et al.* (2007)
	Adherence	oral cav., intest.	Greene and Klaenhammer (1994), Stamatova *et al.* (2009a)
	Competition for adhesion	intest.	Banerjee *et al.* (2009)
Bif. longum	Auto-aggregation	/	Vlkova *et al.* (2008)
	Co-aggregation	/	Vlkova *et al.* (2008)
	Adherence	intest.	Re *et al.* (2000), Candela *et al.* (2008)
	Competitive exclusion	intest.	Candela *et al.* (2008)
Lact. plantarum	Auto-aggregation	/	Vizoso Pinto *et al.* (2007)
	Co-aggregation	oral cav., intest.	Vizoso Pinto *et al.* (2007), Twetman *et al.* (2009)
	Adherence	intest.	Klarin *et al.* (2005), Vizoso Pinto *et al.* (2007), Ramiah *et al.* (2008)
	Competitive exclusion	intest.	Candela *et al.* (2008)
	Competition for adhesion	intest.	Ramiah *et al.* (2008)
Streptococcus salivarius	Adherence	resp.	Guglielmetti *et al.* (2010a), Taverniti *et al.* (2012)
	Competitive exclusion	resp.	Guglielmetti *et al.* (2010a)
Corynebacterium Co304	Aggregation	/	Uehara *et al.* (2000)
	Competition for adhesion	nasal cav.	Uehara *et al.* (2000)
	Abiotic action	nasal cav.	Uehara *et al.* (2000)

(*Continued*)

Table 2 *(Continued)*

Probiotic	Mechanical properties	Site of action	Reference
Lact. paracasei	Co-aggregation	oral cav.	Twetman *et al.* (2009)
	Adherence	intest., vag.	Zarate and Nader-Macias (2006), Jankowska *et al.* (2008)
	Competition for adhesion	intest.	Jankowska *et al.* (2008)
	Competitive exclusion	vag.	Zarate and Nader-Macias (2006)
Lact. casei	Adherence	oral cav., intest.	Tuomola and Salminen (1998)
	Competitive by steric hindrance	resp., intest.	Lee and Puong (2002)
Lact. helveticus	Adherence	upper resp.	Guglielmetti *et al.* (2010b)
	Competition for adhesion	upper resp.	Guglielmetti *et al.* (2010b)
	Competitive exclusion	upper resp.	Guglielmetti *et al.* (2010b)
Strep. thermophilus	Adherence	intest.	Perea Velez *et al.* (2007), Khalil (2009)
Lactococcus lactis	Adherence	upper resp., intest.	Kimoto *et al.* (1999), Guglielmetti *et al.* (2010b)
Strep. sanguinis	Adherence	oral cav.	Okahashi *et al.* (2010, 2011)
Strep. mitis	Adherence	oral cav.	Hoogmoed *et al.* (2008)

resp., respiratory tract; oral cav., oral cavity; intest., intestinal tract; urog., urogenital tract; nasal cav., nasal cavity.

Strep. pneumoniae and *Haemophilus influenzae* was observed in the probiotic group. Moreover, the presence of *Mor. catarrhalis* was increased (Hatakka *et al.* 2007).

The mechanical properties of *Lact. rhamnosus* are among the most extensively studied among probiotics. Different strains of this probiotic have mechanical effects on respiratory tract, intestinal, urogenital and oral cavity pathogens. *Lactobacillus rhamnosus* strains show the following mechanical properties: auto-aggregation, co-aggregation, adherence, competitive exclusion, competition by steric hindrance, competition for adhesion and competition for binding sites.

In vitro, *Lact. rhamnosus* GG has a good binding capacity to human pharyngeal cells (Guglielmetti *et al.* 2010b). It antagonizes respiratory tract pathogens such as *Strep. pyogenes* (Guglielmetti *et al.* 2010b). *In vitro* studies have shown that strain GG has no antimicrobial activity against group A streptococci. Its antagonistic activity may be exerted by inhibiting cell invasion by pathogens, probably by competition for Fn binding sites – a fibronectin required for efficient entry into epithelial cells (Princivalli *et al.* 2009). Strains L17 and N8, isolated from the oral cavities of healthy Thai volunteers, antagonize *Staph. aureus* (Sookkhee *et al.* 2001).

Lactobacillus rhamnosus LB21 has the capacity to co-aggregate with cariogenic pathogens (*Strep. mutans* and *Strep. sobrinus*) (Twetman *et al.* 2009). Strain GG co-aggregates with intestinal pathogens (*Escherichia coli* and *Salmonella enterica*) (Collado *et al.* 2007) and interferes with their adhesion through steric hindrance (Lee and Puong 2002). This strain adheres to buccal epithelial cells and to saliva-coated surfaces (Haukioja *et al.* 2006; Stamatova *et al.* 2009a), to human epithelial intestinal cells (Tuomola and Salminen 1998), to the colon (Morelli *et al.* 2006) and to intestinal mucus (He *et al.* 2001). Strains DR20 and Lcr35 also adhere to human intestinal epithelial cells, and they compete with intestinal pathogens for adherence to these cells (Forestier *et al.* 2001; Gopal *et al.* 2001). Another strain that adheres to human epithelial intestinal cells is LC-705 (Tuomola and Salminen 1998). Strain Lcr35 competes with vaginal pathogens for adhesion to cervical and vaginal cells (Coudeyras *et al.* 2008). *Lactobacillus rhamnosus* L60 adheres to vaginal epithelial cells and co-aggregates with vaginal pathogens (*E. coli*, *Gardnerella vaginalis* and *Candida albicans*) (Pascual *et al.* 2008). Strain GR-1 adheres to squamous uroepithelial cells and competes with uropathogens by competitive exclusion (Reid *et al.* 1987; Reid 2001).

Lactobacillus acidophilus

A clinical trial involving *Lact. acidophilus* NCFM alone or in combination with *Bif. animalis* subsp. *lactis* Bi-07 shows that this probiotic reduces influenza-like symptoms (fever, rhinorrhoea, cough incidence and duration of antibiotic prescription). No explanation was given by the authors to explain this effect (Leyer *et al.* 2009). Strain 145 of *Lact. acidophilus* along with *Lact. rhamnosus* GG, *Strep. thermophilus* and *Bifidobacterium* sp B420 reduced the nasal colonization with pathogens such as *Staph. aureus* and *Strep. pneumoniae* in adults possibly by an immunostimulatory mechanism (Gluck and Gebbers 2003).

Lactobacillus acidophilus strains possess numerous mechanical properties, one of which is auto-aggregation (Boris *et al.* 1998). Strain CCUG 5917 can co-aggregate with cariogenic bacteria (*Strep. mutans* and

Strep. sobrinus) (Twetman *et al.* 2009). Strains isolated from a healthy woman's vagina can co-aggregate with vaginal pathogens (*E. coli*, *G. vaginalis* and *C. albicans*) and compete with them for binding to glycogen receptors of the vaginal cells (Boris *et al.* 1998). *Lactobacillus acidophilus* CRL 1259 adheres to vaginal epithelial cells and competitively excludes *Staph. aureus* (Zarate and Nader-Macias 2006). Strains LB, BG2FO4, LA-1, HNO17, LC1 and NCFM/N2 show adherence to human epithelial intestinal cells in culture (Chauviere *et al.* 1992; Coconnier *et al.* 1992; Bernet *et al.* 1994; Tuomola and Salminen 1998; Gopal *et al.* 2001). Strains BG2FO4, LA-1 and HNO17 also adhere to intestinal mucus (Chauviere *et al.* 1992; Bernet *et al.* 1994; Gopal *et al.* 2001). Strain LB inhibits the adhesion of diarrhoeagenic enterotoxic *E. coli* to the brush border of intestinal cells (Coconnier *et al.* 1993) and competes with this pathogen by steric hindrance for attachment to enterocytic pathogen receptors (Reid *et al.* 1987). Strain HN017 inhibits colonization of the intestinal monolayer by *E. coli* (Gopal *et al.* 2001). *Lactobacillus acidophilus* strain UO 001 inhibits the growth of certain enteropathogens (*Salmonella*, *Listeria* and *Campylobacter*) (Fernandez *et al.* 2003).

Streptococcus salivarius

Streptococcus salivarius BLIS K12 Throat Guard is a probiotic product described as a natural remedy for the common cold and flu. *Streptococcus salivarius* K12 reduces halitosis (Burton *et al.* 2006; Masdea *et al.* in press) and is commercialized as a probiotic against oral malodour.

Strain K12 has the capacity to adhere to human epithelial pharyngeal cells *in vitro*. It antagonizes *Strep. pyogenes* through exclusion and competition (Guglielmetti *et al.* 2010a). This strain also inhibits the growth of *C. albicans in vitro* and protects mice from oral cadidosis (Ishijima *et al.* 2012). *In vitro*, alone or in combination with *Lact. helveticus* MIMLh5, strain ST3 adheres to pharyngeal epithelial cells, antagonizes *Strep. pyogenes* and modulates host innate immunity by inducing potentially protective effects (Taverniti *et al.* 2012). Strain NCC1561 modulates the growth of oral bacteria *in vitro* (Comelli *et al.* 2002).

Bifidobacterium animalis subsp. *lactis*

Bifidobacterium animalis subsp. *lactis* Bb-12 alone or in combination with *Lact. rhamnosus* GG reduced respiratory infections (Rautava *et al.* 2009; Taipale *et al.* 2011). In the trial conducted by Rautava and collaborators, the association of the two probiotics also reduced the risk of early acute otitis media, as well as the use of antibiotics. This effect may have been mediated via both reduction of colonization by pathogens by local inhibition and immunomodulation throughout the common mucosa-associated immune system (Rautava *et al.* 2009). However, Taipale and co-authors reported no difference in the incidence of otitis media or in the use of antibiotics (Taipale *et al.* 2011). A significant difference in the rate and duration of respiratory illnesses between the probiotic and the placebo group upon administration of *Bif. animalis* subsp. *lactis* Bb-12 was also absent in the trial conducted by Weizman and colleagues (Weizman *et al.* 2005). *Bifidobacterium animalis* subsp. *lactis* Bi-07 was administered alone or in combination with *Lact. acidophilus* NCFM in another clinical trial. This resulted in the reduction of influenza-like symptoms and the duration of antibiotic use (Leyer *et al.* 2009).

Bifidobacterium animalis subsp. *lactis* has the ability to auto-aggregate. Strain DR10 adheres to the brush border of intestinal epithelial cells and to intestinal mucus (Gopal *et al.* 2001). *Bifidobacterium animalis* subsp. *lactis* Bb-12 co-aggregates with *E. coli* and *Salm. enterica* (Collado *et al.* 2007), and strain Bar30 exerts competitive exclusion against these intestinal pathogens (Candela *et al.* 2008).

Lactobacillus delbrueckii subsp. *bulgaricus*

A clinical trial involving *Lact. delbrueckii* subsp. *bulgaricus* OLL1073R-1 in association with *Strep. salivarius* subsp. *thermophilus* OLS3059 showed a reduced risk of catching the common cold when probiotics were ingested. Subsequent *in vitro* studies showed that this probiotic has immunostimulatory effects (Makino *et al.* 2010).

In vitro, *Lact. delbrueckii* subsp. *bulgaricus* strains B3 and G12 have the capacity to auto-aggregate and to co-aggregate with *E. coli* (Aslim *et al.* 2007). A number of strains of the laboratory collection of LB Lactis, Bulgaria, adhere to saliva-coated surfaces (Stamatova *et al.* 2009a). Strain 1489 can bind to intestinal epithelial cells (Greene and Klaenhammer 1994) and strain B-30892 inhibits the cytotoxic effects and adhesion of pathogenic *Clostridium difficile* to these cells (Banerjee *et al.* 2009).

Lactobacillus paracasei

Lactobacillus paracasei 8700:2, in association with *Lact. plantarum* HEAL 9, lowered the incidence of common cold episodes and reduced the severity of pharyngeal symptoms. No explanation was given by the authors for this effect (Berggren *et al.* 2011). *Lactobacillus casei* DN-114001, named *Lact. paracasei* subsp. *paracasei* according to the current nomenclature, is contained in Actimel in association with *Strep. thermophilus* and *Lact. delbrueckii* subsp. *bulgaricus*. The consumption of

this commercially available fermented probiotic dairy drink reduced the duration of URTIs, specifically rhinopharyngitis (Guillemard *et al.* 2010; Merenstein *et al.* 2010). Even though no explanation of this effect was given by the authors, Guillemard and colleagues did not observe modulation of immune parameters (natural killer cell activity, cytokine secretion) (Guillemard *et al.* 2010).

Lactobacillus paracasei F19 co-aggregates with cariogenic bacteria (*Strep. mutans* and *Strep. sobrinus*) (Twetman *et al.* 2009). Strains D6, D14 and N14, isolated from the oral cavities of healthy volunteers, possess high capacity to antagonize important oral pathogens, including *Staph. aureus* (Sookkhee *et al.* 2001). Strain IBB2588 adheres to human epithelial intestinal cells and competes for adhesion with *Salm. enterica in vitro* (Jankowska *et al.* 2008). Strain CRL 1289 adheres to vaginal epithelial cells *in vitro* and exerts competitive exclusion against *Staph. aureus* (Zarate and Nader-Macias 2006).

Lactobacillus plantarum

A clinical trial conducted with *Lact. plantarum* HEAL 9 in association with *Lact. paracasei* 8700:2 showed a reduced incidence and duration of common cold episodes and a reduction in the severity of pharyngeal symptoms. No explanation for this effect was given by the authors (Berggren *et al.* 2011).

Lactobacillus plantarum 299v co-aggregates with cariogenic bacteria (*Strep. mutans* and *Strep. sobrinus*) (Twetman *et al.* 2009). Strain 299v also adheres *in vitro* to mucosal colonic cells and to the rectal mucosa of patients (Klarin *et al.* 2005). Strain BFE 1685 has the capacity to auto-aggregate, to adhere to human epithelial intestinal cells and to co-aggregate with intestinal pathogens (Vizoso Pinto *et al.* 2007). *Lactobacillus plantarum* Bar10 exerts competitive exclusion and displacement of *Salm. typhimurium* and *E. coli* (Candela *et al.* 2008). Strain 423 adheres to human epithelial intestinal cells and competes for adhesion with *Clostridium sporogenes* and *Enterococcus faecalis* (Ramiah *et al.* 2008).

Streptococcus thermophilus

In association with *Lact. delbrueckii* subsp. *bulgaricus* OLL1073R-1, *Strep. thermophilus* OLS3059 reduced the risk of catching the common cold in healthy adults and elderly. Subsequent *in vitro* studies showed that this probiotic has immunostimulatory effects (Makino *et al.* 2010). *Streptococcus thermophilus* in combination with *Lact. rhamnosus* GG, *Lact. acidophilus* 145 and *Bifidobacterim* sp B420 reduced the nasal colonization with pathogenic bacteria. An immunostimulatory effect is suspected for this action (Gluck and Gebbers 2003).

Streptococcus thermophilus CHCC 3534 adheres relatively well to intestinal epithelial cells (Perea Velez *et al.* 2007) and to intestinal mucus (Khalil 2009).

Bifidobacterium longum

Bifidobacterium longum SP 07/3, in combination with *Lact. gasseri* PA 16/8 and *Bif. bifidum* MF 20/5, reduced the duration of common cold episodes and the severity of symptoms in a clinical trial. This may be due to immune stimulatory effects (Vrese *et al.* 2006).

Bifidobacterium longum I10 possesses the capacity to auto-aggregate and to co-aggregate with *Clostridia in vitro* (Vlkova *et al.* 2008). Strain Bar33 adheres to the brush border of intestinal cells and to intestinal mucus and exerts competitive exclusion against *E. coli* and *Salmonella* (Candela *et al.* 2008). Strains isolated from gastric juice adhered to intestinal epithelial cells (Re *et al.* 2000).

Bifidobacterium bifidum

Bifidobacterium bifidum MF 20/5, in association with *Lact. gasseri* PA 16/8 and *B. longum* SP 07/3, reduced the duration of episodes of common cold and reduced the severity of symptoms by an immunostimulatory effect (Vrese *et al.* 2006). *Bifidobacterium bifidum* I4 has the capacity to auto-aggregate as well as to co-aggregate with *Clostridia in vitro* (Vlkova *et al.* 2008).

Corynebacterium Co304

A study on volunteers followed by *in vitro* analysis shows that *Coryne.* Co304, isolated from the nares of a healthy volunteer, prevented and eliminated colonization of the nasal cavity by pathogens such as *Staph. aureus* using a non-bacteriocin-like mechanism. This strain possesses aggregation capacity and possibly competes with *Staph. aureus* for an attachment molecule (Uehara *et al.* 2000).

Streptococcus sanguinis

Streptococcus sanguinis has been tested in seven clinical trials. In one clinical trial with strain 89a, NCIMB 40104, a complete or almost complete resorption of middle ear fluid was observed in children with secretory otitis media. The authors suggested that stimulation of antibacterial immune effector mechanisms, rather than bacterial interference, might be responsible for the observed clinical effect (Skovbjerg *et al.* 2009). In association with *Strep. mitis*, *Strep. sanguinis* decreased the recurrence rate of group A streptococci in patients with acute streptococcal

pharyngotonsillitis (Falck *et al.* 1999). This combination of probiotics also decreased the recurrence of tonsillitis in patients suffering from recurrent acute streptococcal tonsillitis or pharyngotonsillitis (Roos *et al.* 1993a,b, 1996). A combination of *Strep. sanguinis*, *Strep. mitis* and *Strep. oralis* strains isolated from the opening of the Eustachian tubes of healthy children decreased the recurrence of otitis media (Roos *et al.* 2001). In another clinical trial, the same combination of probiotic species, isolated from the nasopharynges of healthy children, had no beneficial effect (Tano *et al.* 2002).

In vitro, *Strep. sanguinis* strains isolated from the opening of the Eustachian tubes of healthy children have the capacity to antagonize pathogens including *Strep. pneumoniae*, *H. influenza*, *Mor. catarrhalis* and *Strep. pyogenes* (Roos *et al.* 2001). Strain SK36 binds to human oral epithelial cells and to saliva (Okahashi *et al.* 2010, 2011). Strain KTH-4 inhibits *Aggregatibacter actinomycetemcomitans*, an oral bacterium found in infections of the oral cavity, mainly periodontitis (Sliepen *et al.* 2009).

Streptococcus mitis

The effect of *Strep. mitis* on URTIs has been tested in six clinical trials. A combination of *Strep. mitis*, *Strept. oralis* and *Strept. sanguinis* strains, isolated from the nasopharynges of healthy children, were used by Tano and colleagues. This clinical trial did not show significant outcomes (Tano *et al.* 2002). On the contrary, upon administration of *Strep. mitis*, *Strep. oralis* and *Strep. sanguinis* strains isolated from the opening of the Eustachian tubes of healthy children, Roos and colleagues observed a decrease in the recurrence rate of group A streptococci in children suffering from recurrent otitis media (Roos *et al.* 2001). Four clinical trials were conducted with a combination of *Strep. mitis* and *Strep. sanguinis* strains. A decrease in the recurrence of tonsillitis was observed in patients with acute recurrent tonsillitis or pharyngotonsillitis (Roos *et al.* 1993a,b, 1996), as well as a decrease in the recurrence rate of group A streptococci (Falck *et al.* 1999). *In vitro*, strain BMS reduces the adhesion of and inhibits *Prevotella gingivalis* (Hoogmoed *et al.* 2008).

Streptococcus oralis

The effect of *Strep. oralis* has been studied in two clinical trials both employing a combination of *Strep. oralis*, *Strep. sanguinis* and *Strep. mitis*. One of the clinical trials had a positive outcome, and a significant decrease in the recurrence rate of group A streptococci was observed in the treated group. This trial used strains isolated from the opening of the Eustachian tubes of healthy children (Roos *et al.* 2001). The second clinical trial employed strains isolated from the nasopharynges of healthy children and showed no positive outcomes (Tano *et al.* 2002).

In vitro, *Strep. oralis* strains Parker and Booth, isolated from the nasopharynges of patients undergoing adenoidectomy for either hypertrophy or recurrent otitis media, have been analysed. These strains have the capacity to antagonize and inhibit the growth of pathogens in the nasopharynx including *Strep. pneumoniae*, *H. influenza*, *Mor. catarrhalis* and *Strep. pyogenes* (Bernstein *et al.* 2006).

Lactobacillus casei

Lactobacillus casei has not been tested in a clinical trial for its effect against URTIs. *In vitro*, *Lact. casei* Shirota exhibits a high binding capacity to saliva-coated surfaces and survives in saliva (Haukioja *et al.* 2006). This strain also possesses an antagonist activity against *Strep. pyogenes* by exclusion (Guglielmetti *et al.* 2010b).

Lactobacillus casei Fyos adheres to human intestinal epithelial cells (Tuomola and Salminen 1998) and strain Shirota competes with intestinal pathogens (*E. coli*, *Salm. enterica*) probably by steric hindrance (Lee and Puong 2002).

Lactobacillus helveticus

Lactobacillus helveticus MIMLh5 adheres to human pharyngeal cells *in vitro*. It antagonizes *Strep. pyogenes* through exclusion and competition for adhesion sites on cells (Guglielmetti *et al.* 2010b). This probiotic has not been used in clinical trials against URTIs.

Lactococcus lactis

Lactococcus lactis subsp. *cremoris* Viili possesses a high binding capacity to human pharyngeal cells and antagonizes *Strep. pyogenes* (Guglielmetti *et al.* 2010b).

Strain NIAI527 adheres to colonic cells and to human intestinal mucus (Kimoto *et al.* 1999). A strain not specified by the authors reduced the adhesion and viability of *Staph. aureus* (Vesterlund *et al.* 2006). Clinical trials have not been conducted with this probiotic up to date.

Discussion

In a time when the drawbacks and the risks of unjustified antibiotic treatment have been understood, patients and doctors may be turning to probiotics as a safer means for prevention and treatment of disease. Here, we have presented the probiotics that have been employed in clinical trials aiming to prevent or treat URTIs and otitis.

Many of these bacteria have not only mechanical but also immune stimulatory effects. The discussion of their immune properties is a vast topic and was not the subject of the present review. In the following sections, only the mechanical properties of probiotics are discussed.

Most of the probiotics reported in this review are lactic acid bacteria and belong to the *Lact.*, *Lactococcus* and *Bifidobacterium* families, but there are also several *Strep.* species.

A very important aspect that should be considered before developing a probiotic product is safety. A large number of lactic acid bacteria are considered as safe. They have been approved by EFSA for their introduction into the food chain and have been granted a positive QPS status. 'Qualified Presumption of Safety' is a safety assessment system based on four parameters: establishing the identity, body of knowledge, possible pathogenicity and end use of the micro-organism. Organisms that are granted a QPS status do not raise safety concerns and can be used as probiotic substances without further safety assessment other than satisfying any qualifications specified. The lack of a positive QPS status does not imply that a micro-organism is hazardous; however, it must undergo full safety assessment (EFSA 2007). A micro-organism might not be approved by EFSA but may have gained a GRAS (Generally Recognized as Safe) status by the FDA in the USA. This is the case for *Strep. salivarius*. It has also been approved as a food ingredient in both Australia and New Zealand. Its safety has recently been assessed in a clinical trial which shows that the intake of this bacterium is well tolerated by humans (Burton *et al.* 2011).

Most of the species effective against URTIs have been granted a positive QPS status. In very rare cases and in the presence of predisposing factors such as underlying disease, immunocompromised status or early age, some of the presented species have been associated with infection (EFSA 2007). It is important to underline that these cases are extremely rare and are not related to the consumption of probiotics. As an example, *Strep. mitis*, *Strep. oralis* and *Strep. sanguinis* have been responsible for rare cases of infectious endocarditis (Miyata *et al.* 2007; Nyawo *et al.* 2007; Renton *et al.* 2009). *Lactococcus lactis* can also very rarely cause severe infections, such as infectious endocarditis (Halldorsdottir *et al.* 2002). This commonly consumed bacterium is a dairy starter and its consumption in large quantities in cheese and fermented milks is generally safe. It has, however, been denied a QPS status by EFSA.

The large majority of the here-cited clinical trials conducted against URTIs and otitis show positive outcomes. Among the 21 clinical trials presented in this review, 17 had a positive outcome and only four showed no beneficial effect of the use of probiotics. Moreover, six clinical trials have been conducted with *Strep. mitis*, *Strep. oralis* and *Strep. sanguinis*. They have all shown positive outcomes without any clinical complications.

In the search of a probiotic strain for a given condition, the aim of the probiotic preparation should be clearly defined. Prevention of infection or its treatment may necessitate different mechanical properties. In the case of prevention, probiotics should establish a microbiota capable to inhibit and/or block colonization by pathogens. In the case of treatment, however, probiotics will need to confront an already established population of pathogens. In both cases, aggregation and adherence are required properties for the probiotic. In the absence of pathogens, the probiotic would adhere to the host cells and protect them by blocking the access of pathogens. When a pathogen arrives, they could trap it by co-aggregating with it and thus eliminate it. It is possible that probiotics do not confer complete protection against infection. However, they could help fight pathogens and thus reduce the duration and the severity of symptoms. In an already infected organism, a desirable ability for a probiotic is to exert competitive exclusion. A higher affinity for binding sites would allow them to exclude attached pathogens that could subsequently be eliminated through co-aggregation. On the other hand, strong adherence to host cells would allow the probiotic to occupy any free space on the epithelial surface. Thus, they could slow down the multiplication of the pathogens by competing with them for space and nutrients. In the case of URTIs, most clinical trials are destined to prevent disease in the healthy population or to prevent recurrence in disease-prone patients.

Conclusion

This review aimed to underline the importance of the mechanical properties of probiotics in their action against pathogens with a special interest on the probiotics that have shown to be effective in the prevention of URTIs and otitis. The properties of a probiotic being strain specific, what we know up to date is only a small part of the potential offered by these 'friendly bacteria'. This review might help the choice of probiotic species for the development of novel probiotic applications. Of the 16 here-cited species, almost half are lactic bacteria belonging to the *Lact.* genus (*Lact. rhamnosus*, *Lact. acidophilus*, *Lact. delbrueckii*, *Lact. paracasei*, *Lact. plantarum*, *Lact. casei* and *Lact. helveticus*) and the *Bifidobacterium* genus (*Bif. animalis*, *B. longum* and *B. bifidum*). Some species, as *Lact. rhamnosus* and *Lact. acidophilus*, have been extensively studied compared to others that have been tested in few clinical trials. These extensively studied species have

well-documented mechanical properties and seem to offer a bigger chance of success in the search of new strains. The mechanical properties of the less studied probiotics are less well known, but their potential should not be underestimated. Finally, it could also be interesting to investigate the less frequently used in URTI studies *Streptococcus* genus (*Strep. salivarius*, *Strep. mitis*, *Strep. oralis* and *Strep. sanguinis*).

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