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REVIEW ARTICLE

Applications and safety considerations of *Lactobacillus salivarius* as a probiotic in animal and human health

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Summary

The goals of this review are to summarize the current knowledge on the application of *Lactobacillus salivarius* as a probiotic in animals and humans, and to address safety concerns with its use on live hosts. Overall, several strains of *L. salivarius* are well established probiotics with multiple applications in animal health, particularly to reduce colonization by gastrointestinal pathogens, and to a lesser extent, as a production and quality aid. In humans, *L. salivarius* has been used to prevent and treat a variety of chronic diseases, including asthma, cancer, atopic dermatitis and halitosis, and to a much limited extent, to prevent or treat infections. Based on the results from primary research evidence, it seems that *L. salivarius* does not pose a health risk to animals or humans in the doses currently used for a variety of applications; however, there is a systematic lack of studies assuring the safety of many of the strains intended for clinical use. This review provides researchers in the field with up-to-date information regarding applications and safety of *L. salivarius*. Furthermore, it helps researchers identify knowledge gaps and potential opportunities for microbiological and clinical research.

Introduction

According the Food and Agriculture Organization of the United Nations (FAO 2006), probiotics are viable micro-organisms that, when administered in adequate amounts, promote or support a health benefit on the host. Probiotic bacteria typically used in animal and human health applications belong primarily to two groups, the bifidobacteria and the lactic acid bacteria (LAB) (Kanwar *et al.* 2016). The term LAB constitutes a phylogenetically homogeneous group in the order *Lactobacillales*, including environmental organisms, members of plant microbiota, commensals of humans and animals, and opportunistic or obligate pathogenic organisms (Gänzle 2015). *Lactobacillus salivarius* is a well-characterized bacteriocin producer. It has been frequently isolated from human, porcine and avian gastrointestinal tracts (GIT), human milk, and other sources, and several strains have gained attention as promising probiotics due to their ability to modulate gut microbiota, produce antimicrobial substances, stimulate protective immune response, inhibit faecal enzymatic activity and produce short chain fatty acids allowing an advisable acidification of the gut, among others (Messaoudi *et al.* 2013).

In this review, we summarize the current status of *L. salivarius* as a probiotic for animals and humans. We present applications in animal production and health and in different spheres of human health, and discuss potential safety concerns with the use of *L. salivarius*. Literature searches were conducted on Scopus® during June/July of 2016 and all relevant primary research abstracts identified in English, regardless of publication date, were retrieved in full text through the Texas Tech University Library System. Only studies that reported the use, application or safety of *L. salivarius* in animal or human health were considered for inclusion in this review after thorough revision of the corresponding abstracts and/or full articles.

Lactobacillus salivarius applications in animal health

Probiotics have been typically used as an alternative to low-dose antibiotics for animals. Most applications of *L.*

salivarius in animals focus on improving the immune status, and on reducing colonization by pathogenic bacteria in swine and poultry all in view of increasing animal production.

Swine production and health

Maré *et al.* (2006) used *in situ* fluorescence hybridization (FISH) and determined the adhesion sites of *Lactobacillus plantarum* 423 and *L. salivarius* 241 in pre- and postweaned piglets. The researchers found that the strains colonize different sections of the intestinal tract's mucus layers depending upon the piglet's age. *Lactobacillus plantarum* 423 adhered strongly to the ileum and posterior colon, while *L. salivarius* adhered to the duodenum in preweaned piglets. In postweaned piglets, high levels of 241 were recorded in the duodenum and posterior colon. Lowering of 25% of cells of *Enterococcus faecalis* was observed when preweaned piglets were challenged with 241, potentially having a competitive exclusion effect on *E. faecalis*, a commensal species in swine and a human and porcine opportunistic pathogen (Maré *et al.* 2006). Zhang *et al.* (2011a) administered neonatal piglets with 10^9 CFU per ml of *L. salivarius* B1 but did not observe significant changes in the composition of the intestinal microflora except for the *Bifidobacterium* counts at early lactation. However, B1 significantly improved the structure of the mucosal tissues (longer villi) and effectively enhanced the presence of intraepithelial lymphocytes and IgA-producing plasma cells in the intestinal tract, indicating that this strain can significantly promote maturation of intestinal mucosal immunity and elicit local immunomodulatory activities (Zhang *et al.* 2011a). In line with these results, Deng *et al.* (2013) evaluated the effect of the co-administration of *Bacillus subtilis* RJGP16 and *L. salivarius* B1 as potential probiotics to stimulate local immune responses. Newborn piglets were orally administered with different combination of probiotics (none; RJGP16; B1; RJGP16 and B1). Results 1 week postweaning showed that gene expression of interleukin (IL)-6 in the duodenum and ileum, and of porcine β-defensins-2 in the duodenum were significantly increased with coadministration. Furthermore, expression and release of toll-like receptor-2 and the number of IgA-producing cells significantly increased, demonstrating that cocolonization with these two probiotics can contribute to a variety of positive mucosal immune responses (Deng *et al.* 2013). Lastly, Rondón *et al.* (2013) evaluated the effect of a biopreparation of *L. salivarius* C65 (10^6 CFU per g) on production and health indicators in lactating piglets and found that average live weight (9·46 kg) improved significantly in animals treated with the probiotic compared to the control group (8·02 kg) at 5 weeks. The animals also had a better weight increase and daily live weight gain and a lower diarrhoea incidence, confirming the probiotic effects on animal performance (Rondón *et al.* 2013).

Poultry and eggs production and health

The use of *L. salivarius* as a probiotic in poultry dates from over 15 years and several studies have reported the reduction in colonization by *Salmonella* Enteriditis (SE) attributed to the competitive exclusion effects of *L. salivarius*. Pascual *et al.* (1999) dosed strain CTC2197 by oral gavage together with SE directly into the proventriculus in 1-day-old chickens resulting in complete pathogen elimination after 21 days. The same results were obtained when the probiotic was administered in the feed and drinking water. The inclusion of CTC2197 in the first-day chicken feed revealed that a concentration of 10^5 CFU per g was enough to ensure the colonization of the GIT of the birds after 1 week, making it a suitable option to minimize *Salmonella* colonization in chickens (Pascual *et al.* 1999). Similarly, Zhang *et al.* (2007) found that feeding chickens an overnight culture (10^6–10^8 CFU per chick) of strains Salm-9, List40-1,8 or List40-41 reduced *Salmonella* carriage in caecal contents by 2·10, 2·52 and 2·20 log CFU per g respectively. The percentages of *Salmonella*-positive chickens after receiving these treatments were 35, 31 and 35%, respectively, compared with 84% for the control. A mixture of *Streptococcus cristatus* List40-13 and *L. salivarius* List40-41 reduced *Salmonella* carriage from 90 to 65% and from 88 to 31% in two feeding trials, and by 2·2 and 4·0 log CFU per g of caecal contents of chickens. This study showed that strains Salm-9, List40-18 and List40-41, and *S. cristatus* List40-13 were effective in significantly preventing *Salmonella* colonization of chickens (Zhang *et al.* 2007). In a study by Waewdee *et al.* (2012), broiler chicks were randomly assigned to six groups. At 1 day of age, each group received none, 10^4 or 10^{10} CFU per chick of *L. salivarius* LP 4.2-2 by either oral or cloacal route. At 2 days of age, all chicks except controls were challenged orally with 10^4 CFU per chick of SE. At 3 days of age, half the number of chicks in each group ($n = 20$ per group) were randomly selected for the detection of SE in caecal tonsils. The remaining chicks were allowed to grow until 9 days of age. The results showed that at 3 days of age, rates of SE infection were lower in all groups administered with LP 4.2-2. However, at 9 days of age, rates of SE infection were high in all groups, indicating that a single dose of *L. salivarius* could not prevent SE infection in all chicks but it could reduce the rate of infection in 3-day-old chicks (Waewdee *et al.* 2012). More recently, Sornplang *et al.* (2015) divided 150 newborn broiler chicks into five

groups: group 1 (control), given feed and water only; group 2 (positive control) given feed, water and SE infection; group 3 (L61 treated) given feed, water, SE infection followed by *L. salivarius* L61 treatment; group 4 (L55 treated) given feed, water, SE infection followed by *L. salivarius* L55 treatment; and group 5 given feed, water, SE infection followed by L61 + L55 combination treatment. After SE challenge, *L. salivarius* treatment lasted for 7 days. The results showed that L61 and L55 treatment increased the survival rate after SE infection, and upregulated heterophil phagocytosis and phagocytic index (Sornplang *et al.* 2015). Conversely, chick groups treated with *L. salivarius* showed lower SE recovery rate from caecal tonsils. The authors concluded that *Lactobacillus* may be used to prevent SE infection in young chicks when supplemented at an optimal time of posthatch to 2-day-old chicks because heterophils were more stimulated then (Sornplang *et al.* 2015).

An opposite result had been reported by Andreatti Filho *et al.* (2006). In their study, commercial 18-day-old incubating chicken embryos were inoculated with total or diluted caecal microbiota and *L. salivarius* cultures directly into the inner air sac. Two days after hatching, the chicks were challenged with SE, and 5 days later the presence of SE in caecum and liver was evaluated. The *in ovo* inoculation of total or diluted caecal microbiota, in addition to the *L. salivarius* (10^7 CFU per ml) treatment did not significantly decrease the colonization of SE in liver and caecum but resulted in hatchability of 65% or less, negatively impacting production (Andreatti Filho *et al.* 2006).

Not very many studies have evaluated quality measures, but Kalsum *et al.* (2012) showed that *L. salivarius* supplementation (10^8 CFU per ml) did not influence quail egg quality parameters and egg weight, but significantly improved total egg production and lowered cholesterol content in egg yolk, making a suitable feed additive for Japanese quail diets (Kalsum *et al.* 2012).

Probiotic status of *L. salivarius*: results from animal models and *in vitro* studies

Several animal models and other experimental studies have aimed at determining whether *L. salivarius* has probiotic activity with potential applications in animals and human health and at deciphering the mechanisms by which this bacterium may exert probiotic activity. The following subsections summarize the main results with potential applications to human health.

Immunomodulatory and anti-inflammatory effects

In a study by Li *et al.* (2010), the researchers investigated the effect of potential probiotics in response to antigen challenge in an ovalbumin (OVA)-sensitized asthma model in BALN/c mice. Oral treatment with live *L. salivarius* PM-A0006 (10^6–10^7 CFU) significantly attenuated the influx of eosinophils to the airway lumen and reduced the levels of serum OVA-specific immunoglobulin E and eotaxin in BAL fluid of antigen-challenged animals. Furthermore, PM-A0006 decreased allergen-induced airway hyper-responsiveness and elevated the levels of interferon (IFN)-γ. These results showed that strain PM-A0006 could have therapeutic probiotic potential for treatment of allergic airway disease. In a study with human subjects, Drago *et al.* (2015) evaluated the characteristics of *L. salivarius* LS01 and *Bifidobacterium breve* BR03 and their immunomodulatory activity in asthmatic subjects. The authors concluded that these bacteria have promising probiotic properties and beneficial immunomodulatory activity after their combination decreased the secretion of proinflammatory cytokines, leading to an intense increase in IL-10 production, aiding to maintain the physiological profile of the immune response in mucosal lymphoid tissue (Drago *et al.* 2015).

Feighery *et al.* (2008) observed that following oral treatment with strain UCC118, faecal microbial analysis indicated that viable intact bacteria reached the colons of IL-$10^{-/-}$ mice and dextran sodium sulphate-treated mice. However, neither prophylactic nor therapeutic UCC118 treatment significantly prevented or attenuated inflammation in either model. In all studies, the probiotic-treated mice had comparable cytokine responses as the vehicle-treated animals. Colonic mucosa from UCC118-treated mice had unchanged trans-epithelial electrical resistance values and mannitol fluxes compared with controls. Finally, in two different mouse colitis models examined, the data suggested that this *L. salivarius* strain has limited potential as a prophylactic or therapeutic treatment for inflammatory bowel disease. However, a previous study about colitis reported opposite conclusions. Peran *et al.* (2005) investigated the intestinal anti-inflammatory effect and mechanism of *L. salivarius* CECT5713 (10^8 CFU orally per day) for 3 weeks in the trinitrobenzenesulfonic acid (TNBS) model of rat colitis. One week after colitis induction, all animals were killed and colonic damage was evaluated. Treatment of colitic rats resulted in amelioration of the inflammatory response. Anti-inflammatory and histological improvements were confirmed by a significant reduction in colonic myeloperoxidase activity, a marker of neutrophil infiltration. The beneficial effect was associated with an increase in the colonic glutathione content, which is depleted in colitic rats as a consequence of the oxidative stress induced by the inflammatory process. In addition, the treatment of colitic rats resulted in a significant reduction in colonic tumour necrosis factor (TNF)-α levels and in a lower colonic nitric oxide

synthase expression. The authors concluded that administration of the probiotic *L. salivarius* CECT5713 facilitates the recovery of the inflamed tissue in the TNBS model of rat colitis (Peran *et al.* 2005).

Acute liver disease

Lv *et al.* (2014) investigated the effect of the intragastric administration of five LAB on acute liver failure in rats. Rats were given intragastric supplements of *L. salivarius* LI01, *L. salivarius* LI02, *Lactobacillus paracasei* LI03, *L. plantarum* LI04 or *Pediococcus pentosaceus* LI05 for 8 days. Acute liver injury was induced on the eighth day. The results indicated that pretreatment with *L. salivarius* LI01 or *P. pentosaceus* LI05 significantly reduced elevated alanine amino-transferase and aspartate amino-transferase levels, prevented the increase in total bilirubin, reduced the histological abnormalities of both the liver and the terminal ileum, decreased bacterial translocation, increased the serum level of IL-10 and/or IFN-γ, and resulted in a caecal microbiome that differed from that of the liver injury control. The authors indicated that the excellent characteristics of *L. salivarius* LI01 and *P. pentosaceus* LI05 enable them to serve as potential probiotics in the prevention or treatment of acute liver failure (Lv *et al.* 2014).

Cancer and carcinogenesis

When it comes to cancer, several animal studies have attempted to clarify the effect, if any, of *L. salivarius* as a probiotic and its potential extrapolations to human health. Zhang *et al.* (2011b) investigated the impact of an important carcinogen, 4-nitroquinoline-1-oxide (4NQO) on colonic microflora and the efficacy of *L. salivarius* Ren to counteract its effects. A total of 27 GI bacterial strains were identified as being affected by treatment with 4NQO or with Ren. These results suggested that Ren may be a potential probiotic, efficiently acting against the initial infection with, and the growth of, potential pathogenic bacteria including *Helicobacter* and *Desulfovibrio* (Zhang *et al.* 2011b). In a follow-up study by Zhang *et al.* (2013b), the results indicated that oral administration of Ren or its secretions could effectively suppress 4NQO-induced oral carcinogenesis in the initial and postinitial stage, and the inhibition was dose-dependent. A significant decrease in neoplasm incidence was detected in rats fed with a high dose of Ren (10^{10} CFU per kg body weight per day). *In vivo* evidence indicated that the *L. salivarius* strain inhibited 4NQO-induced oral cancer by protecting DNA against oxidative damage and down-regulating cyclooxygenase-2 expression. Ren treatment significantly decreased the expression of proliferating cell nuclear antigen and induced apoptosis in a dose-dependent manner. These findings suggested that *L. salivarius* Ren may act as a potential agent for oral cancer prevention (Zhang *et al.* 2013b).

Another study by Zhang *et al.* (2015) investigated the impact of Ren in modulating colonic microbiota structure and colon cancer incidence in a rat model after injection with 1,2-dimethylhydrazine (DMH). The results showed that oral administration of Ren could effectively suppress DMH-induced colonic carcinogenesis. A significant decrease in cancer incidence (87·5–25·0%) was observed in rats fed with 10^{10} CFU per kg body weight per day. It was demonstrated that injection with DMH significantly altered the rat gut microbiota, and that Ren counteracted the adverse effects and promoted reversion of the gut microbiota close to the healthy state. Injection of DMH significantly increased the amount of *Ruminococcus* and *Clostridiales*, and decreased *Prevotella* levels. Administration of Ren reduced the amount of *Ruminococcus*, *Clostridiales* bacteria and *Bacteroides dorei*, and increased the amount of *Prevotella*. These findings suggested that Ren is a potential agent for colon cancer prevention (Zhang *et al.* 2015). Lastly, Zhu *et al.* (2014) found that Ren prevents early colorectal carcinogenesis in a DMH-induced rat model. The authors investigated the impact of Ren on modulating colonic microflora structure and influencing host colonic health in a rat model with colorectal precancerous lesions. Male F344 rats were injected with DMH and treated with Ren at two doses (10^8 and 10^{10} CFU per kg body weight) for 15 weeks. A distinct segregation of colonic microflora structures was observed in the Ren-treated group. The abundance of one *Prevotella*-related strain associated with high butyrate production was increased, and the abundance of one azoreductase-producing strain of *Bacillus* was decreased by the treatment, hence reducing the concentration of azoreductase, an enzyme involved in the initial stages of carcinogenesis (Zhu *et al.* 2014). Overall, *L. salivarius* Ren improved the colonic microflora structures and the luminal metabolism in addition to preventing the early colorectal carcinogenesis in the DMH-induced rat model, suggesting once again that this strain could potentially be used as a probiotic for the prevention of colorectal cancer (Zhu *et al.* 2014).

Lactobacillus salivarius applications in human health

The probiotic effects of *L. salivarius* in humans have been explored and exploited in multiple applications, ranging from alternatives to control oral malodor (bad breath, halitosis) to treating chronic diseases and chronic infections in children and adults. In the following sections, we address these major applications.

Periodontal health and dental caries

In a study assessing the effect of *L. salivarius* on oral microbiomes, Suzuki *et al.* (2012) evaluated the use of oil drops containing *L. salivarius* WB21 on periodontal health and oral microbiota producing volatile sulphur compounds (VSCs). Oral assessment and saliva collection were performed on days 1 and 15 on 42 human subjects. In treatment and control groups, the average probing depth, number of periodontal pockets and the percentage of bleeding on probing (BOP) decreased while stimulated salivary flow increased on day 15. The numbers of *Prevotella intermedia*, which correlates with H_2S concentration in mouth air, increased in the placebo group but did not change in the experimental group. *Porphyromonas gingivalis*, *P. intermedia*, *Tannerella forsythensis* and *Fusobacterium nucleatum* decreased in the experimental group. Thus, oil drops containing *L. salivarius* WB21 improved BOP and inhibited VSC-producing periodontopathic bacteria (Suzuki *et al.* 2012). Furthermore, Nissen *et al.* (2014) investigated the effect of *L. salivarius* and *Lactobacillus gasseri* on the expression of the two major virulence factors of *Aggregatibacter actinomycetemcomitans*, a Gram-negative species highly implicated in localized aggressive periodontitis. Neither lactobacilli affected the growth, but strongly attenuated the expressions of both cytolethal distending toxin (CdtB) and leukotoxin (LtxA) (Nissen *et al.* 2014). These findings may indicate that lactobacilli can reduce the virulence of putative opportunistic oral pathogens, and may provide insights for future therapeutic approaches for the respective diseases (Nissen *et al.* 2014). However, the ability of *L. salivarius* W24 to incorporate into and to affect the compositional stability and cariogenicity of oral microbial communities has been reported by Pham *et al.* (2009). The study indicated that W24 may increase the cariogenic potential of the oral microbial community by establishing itself into the oral community, even more intensely at low pH and in a sucrose-supplemented medium (Pham *et al.* 2009). The results of Pham *et al.* (2009) are supported by those of Matsumoto *et al.* (2004), who found that *L. salivarius* strain LS1952R possesses an inherent cariogenic activity following adherence to the tooth surface in a rat model, and by those of Seppä *et al.* (1989) who found that *L. salivarius* is even more cariogenic in a gnotobiotic rat model than *Streptococcus mutans*.

Although the results of the previous studies indicated that some strains of *L. salivarius* may be cariogenic, other strains have been studied for their potential to prevent caries. Nishihara *et al.* (2014) evaluated the effects of *L. salivarius* on caries risk factors. The participants took tablets (10^9 CFU per day) containing *L. salivarius* WB21, *L. salivarius* TI 2711, Ovalgen® DC (antibody against a glucosyltransferase from *S. mutans*) or xylitol. The levels of mutans streptococci seemed to decrease in the WB21, TI2711 and Ovalgen® DC groups compared to the xylitol group, with no significant differences between the treatment groups. Lactobacilli levels significantly increased in the WB21 and TI 2711 groups compared to the other groups. The salivary buffering capacity significantly increased in the TI2711 group and Ovalgen® DC group compared to the xylitol group. The short-term administration trial showed that the *L. salivarius* WB21-containing tablets significantly decreased the number of mutans streptococci and may increase resistance to caries risk factors (Nishihara *et al.* 2014). With a similar study design, Mayanagi *et al.* (2009) evaluated whether the oral administration of lactobacilli could change the bacterial population in supra/subgingival plaque. Healthy volunteers without severe periodontitis were randomized into two groups to receive *L. salivarius* WB21 (10^9 CFU per day) or placebo for 8 weeks. The numerical sum of five selected periodontopathic bacteria in the test group was decreased significantly in subgingival plaque at 4 weeks. Multivariate analysis showed that significantly higher odds were obtained for the reduction in *Tannerella forsythia* in subgingival plaque of the test group at both four and 8 weeks (Mayanagi *et al.* 2009). Overall, the oral administration of probiotic lactobacilli reduced the numerical sum of five selected periodontopathic bacteria and could contribute to the beneficial effects on periodontal conditions (Mayanagi *et al.* 2009).

Halitosis

The use of *L. salivarius* has also seen applications for the treatment of halitosis and mouth malodor. Iwamoto *et al.* (2010) evaluated whether oral administration of lactobacilli alters the degree of halitosis and clinical conditions associated with halitosis. Twenty patients with genuine halitosis were given 10^9 *L. salivarius* WB21 and xylitol in tablet form daily. Oral administration of lactobacilli primarily improved physiological halitosis at 2 weeks and showed beneficial effects on BOP from the periodontal pocket (Iwamoto *et al.* 2010). A follow-up study in Japan (Suzuki *et al.* 2014) evaluated the effect of an intervention using lactobacilli on oral malodor with a 14-day, double-blind, placebo-controlled, randomized crossover trial of tablets containing *L. salivarius* WB21 (10^9 CFU per day) or a placebo taken orally by patients with oral malodor. Organoleptic test scores significantly decreased in both the probiotic and placebo periods compared with the respective baseline scores (Suzuki *et al.* 2014). Bacterial quantitative analysis found significantly lower levels of ubiquitous bacteria and *F. nucleatum* in the probiotic period, indicating that daily oral consumption of tablets

containing probiotic lactobacilli could help control oral malodor- and malodor-related factors (Suzuki *et al.* 2014).

Atopic dermatitis

One of the major applications of *L. salivarius* in humans has been the treatment of atopic dermatitis (AD). Wu *et al.* (2012) conducted a double-blind, randomized, clinical trial to compare the effects of *L. salivarius* and fructo-oligosaccharide (synbiotic) to those of fructo-oligosaccharide alone (FOS, prebiotic) on children with moderate to severe AD. Sixty children aged 2–14 years AD [SCORing AD (SCORAD) >25] were randomly assigned to a treatment (synbiotic) or a control (prebiotic). They received one capsule twice daily for 8 weeks containing *L. salivarius* plus FOS (treatment) or FOS only (control). At 8 weeks, the treatment group SCORAD values were significantly lower than the controls and this difference remained at 10 weeks. At 8 weeks, treatment group's AD intensity was significantly lower. Furthermore, medication use frequency and eosinophil cationic protein levels were significantly reduced in the treatment group at 8 weeks compared with 4 weeks (Wu *et al.* 2012). The authors concluded that the combination was effective for treatment but cautioned about evaluating the effect for a longer period of time (Wu *et al.* 2012). Niccoli *et al.* (2014) showed similar results in a study performed with children ages 1–11 years. *Lactobacillus salivarius* LS01 seemed to be able to improve the quality of life of children affected by AD and, as a consequence, it may have promising clinical and research implications (Niccoli *et al.* 2014). Furthermore, a randomized, double-blind, placebo-controlled study with adults evaluated the clinical efficacy of the probiotic strain LS01 in the treatment of AD (Drago *et al.* 2011). Patients treated with probiotics showed a statistical improvement of SCORAD after 16 weeks. A statistically relevant decrease of staphylococci in faeces of the probiotic-treated group was also observed at the end of treatment. The authors concluded that this strain could have an important role in modulating Thl/Th2 cytokine profiles and could be considered as an important adjunctive therapy in the treatment of adult AD (Drago *et al.* 2011). A follow-up study by Drago *et al.* (2014) evaluated the efficacy of a highly concentrated *L. salivarius* LS01 preparation containing a gelling complex formed by *Streptococcus thermophilus* ST10 and tara gum in the treatment of AD. A significant improvement in SCORAD index was observed in the probiotic group. A slight decrease in faecal *Staphylococcus aureus* count was observed in probiotic-treated patients (Drago *et al.* 2014). The addition of tara gum and *S. thermophilus* ST10 seemed to improve the overall efficacy of the probiotic strain, in particular shortening the time required for the onset of the positive effects (Drago *et al.* 2014).

Infant and children's health

Moles *et al.* (2015) studied the effect of administering human milk probiotics *B. breve* PS12929 and *L. salivarius* PS12934 on their presence in faeces of pretermed infants. For this purpose, five preterm infants received two daily doses (10^9 CFU) of a 1 : 1 mix of the probiotics. The phylum *Firmicutes* dominated in nearly all faecal samples while *L. salivarius* PS12934 was detected in all the infants at numerous sample collection points and *B. breve* PS12929 appeared in five faecal samples (Moles *et al.* 2015). A noticeable decrease in the faecal calprotectin, an inflammatory biomarker, suggested that the probiotic combination has a protective effect on the GI health of the pretermed infants (Moles *et al.* 2015). Other reports have presented similar results in terms of *L. salivarius* having a positive effect in modulating inflammatory responses *in vivo*. For example, Rajkumar *et al.* (2015) investigated the effect of supplementation with *L. salivarius* UBL S22 with or without the prebiotic FOS on serum lipid profiles, immune responses, insulin sensitivity and gut lactobacilli in 45 healthy young individuals. After 6 weeks, a significant reduction in total cholesterol, low-density lipoprotein cholesterol and triglycerides, and an increase in high-density lipoprotein cholesterol were observed in the probiotic as well as in the synbiotic group; however, the results of total cholesterol and LDL were more pronounced in the synbiotic group (Rajkumar *et al.* 2015). Similarly, when compared to the placebo group, the serum concentrations of inflammatory markers such as high sensitivity C-reactive protein, IL-6, IL-1b and TNF-α were significantly reduced in both experimental groups, but the reduction in the synbiotic group was more pronounced. Also, an increase in faecal counts of total lactobacilli and a decrease in total coliforms and *Escherichia coli* were observed in both experimental groups after 6 weeks of ingestion (Rajkumar *et al.* 2015). Overall, the combination of *L salivarius* with FOS was observed to be more beneficial than *L salivarius* alone (Rajkumar *et al.* 2015).

Other applications in human health

Not very many studies have reported on the effect of *L. salivarius* probiotic activity on the prevention or treatment of infectious diseases. Very recently, Fernández *et al.* (2016) evaluated the potential of *L. salivarius* PS2 to prevent infectious mastitis when orally administered during late pregnancy to women who had experienced infectious mastitis after previous pregnancies. Women in the probiotic group ($n = 55$) ingested 10^9 CFU of PS2 daily from approximately week 30 of pregnancy until delivery. Overall, 44 of 108 women (41%) developed mastitis; however, the percentage of women with mastitis

in the probiotic group (25%, $n = 14$) was significantly lower than in the control group (57%, $n = 30$) (Fernández *et al.* 2016). When mastitis occurred, the milk bacterial counts in the probiotic group were significantly lower than those obtained in the placebo group, indicating that oral administration of *L. salivarius* PS2 during late pregnancy appears to be an efficient method to prevent infectious mastitis (Fernández *et al.* 2016).

Treatment with *L. salivarius* has seen application in other health fields, however, not always successfully or with the expected results. Gleeson *et al.* (2012) examined the effects of a probiotic supplement during 4 months of spring training in men and women engaged in endurance-based physical activities on the incidence of upper respiratory tract infections (URTI) and mucosal immune markers. Sixty-six highly active individuals were randomized to probiotic or placebo and, under double-blind procedures, received probiotic (PRO, *L. salivarius*, 10^{10} CFU) or placebo (PLA) daily for 16 weeks. Fifty-four subjects completed the study ($n = 27$ PRO, $n = 27$ PLA). The proportion of subjects on PRO who experienced one or more week with URTI symptoms was not significantly different from that of those on PLA. The number of URTI episodes was similar in the two groups. Blood leucocyte, neutrophil, monocyte and lymphocyte counts; saliva IgA; and lysozyme concentrations did not change over the course of the study and were not different on PRO compared with PLA (Gleeson *et al.* 2012). Consequently, the authors concluded that regular ingestion of *L. salivarius* does not appear to be beneficial in reducing the frequency of URTI in an athletic cohort and does not affect blood leucocyte counts or levels of salivary antimicrobial proteins during a spring period of training and competition (Gleeson *et al.* 2012). In a study by Larsen *et al.* (2013) with obese adolescents, the researchers found that administration of *L. salivarius* Ls-33 might modify the faecal microbiota in the cohort in a way not related to metabolic syndrome, a condition typically associated with the population under study. The ratio of *Bacteroides*/*Prevotella*/*Porphyromonas* group to *Firmicutes*-belonging bacteria, including *Clostridium*, was significantly increased after administration of Ls-33. However, the cell numbers of faecal bacteria, including *Enterobacteriaceae*, *Enterococcus*, the *Lactobacillus* and *Bifidobacterium* were not significantly altered by the intervention (Larsen *et al.* 2013).

Safety concerns of *L. salivarius* as a probiotic

General considerations

Safety assessments for specific *L. salivarius* strains are very limited in the scientific literature. Strain CECT5713, originally isolated from human milk, is the most widely studied in this regard. Maldonado *et al.* (2010) evaluated the safety of a follow-on formula with CECT5713 in 6-month-old children. The antibiotic susceptibility profile of the strain was deemed safe. No adverse effects associated with the consumption (10^6 CFU per day for 6 months) of the probiotic formula were reported. In addition, clinical parameters did not differ between control and treatment groups. Consumption of the formula led to an increase in the faecal lactobacilli content. Furthermore, probiotic consumption induced a significant increase in the faecal concentration of butyric acid at 6 months (Maldonado *et al.* 2010). The authors concluded that a follow-on formula with *L. salivarius* CECT5713 is safe and well tolerated in 6-month-old infants (Maldonado *et al.* 2010). An animal model study by Lara-Villoslada *et al.* (2007) evaluated the oral toxicity of CECT5713 in mice. Fifty Balb/C mice were divided into five groups. Three groups were treated orally with different doses of CECT5713: 10^8, 10^9 or 10^{10} CFU per mouse per day for 28 days. Oral administration of CECT5713 to mice had no adverse effects on mouse body weight or food intake. No bacteraemia was shown and there was no treatment-associated bacterial translocation to the liver or spleen. Intraperitoneal administration caused a significant bacterial translocation to the liver and spleen, but not to the blood. However, this translocation was not related to illness or death at either 2 or 5 days (Lara-Villoslada *et al.* 2007). These results suggest that strain CECT5713 is nonpathogenic for mice, even in doses 10 000 times higher (expressed per kilograms of body weight) than those normally consumed by humans. Thus, this strain is likely to be safe for human consumption (Lara-Villoslada *et al.* 2007). With a similar study design and methods, Zhang *et al.* (2013a) concluded that the strain Ren is likely to be safe for human consumption as well.

A technical panel of the European Food Safety Authority (EFSA Panel on Additives and Products or Substances used in Animal Feed (FEEDAP) 2012) was asked to deliver a scientific opinion on the safety for the target animals, consumer, user and environment, and on the efficacy of two specific bacterial strains of *L. salivarius* CNCM I-3238–ATCC 11741 and *L. casei* ATCC PTA-6135, when used as technological additives to improve the ensiling process at a proposed dose of $1{\cdot}3 \times 10^7$ and $1{\cdot}3 \times 10^6$ CFU per kg fresh material respectively. Both species were considered by EFSA to be suitable for the qualified presumption of safety approach (EFSA Panel on Additives and Products or Substances used in Animal Feed (FEEDAP) 2012). As the identity of the strains has been clearly established and as no antibiotic resistance of concern was detected, their use in the production of silage is considered safe for livestock species, consumers of products from animals fed the treated silage and for

the environment (EFSA Panel on Additives and Products or Substances used in Animal Feed (FEEDAP) 2012). Another EFSA scientific panel (EFSA Panel on Additives and Products or Substances used in Animal Feed (FEEDAP) 2015) concluded that Biomin®C3, a preparation of several strains of *Enterococcus faecium*, *Bifidobacterium animalis* and *L. salivarius* is a safe product. It is currently authorized in the European Union for use in feed for fattening of chickens. A tolerance study using water as the delivery system showed that consumption of 100 times the currently authorized maximum dose in feed did not cause adverse effects in chickens for fattening. Thus, delivery of comparable doses of the additive via water for drinking is considered to be as safe for chickens for fattening as delivery via feed (EFSA Panel on Additives and Products or Substances used in Animal Feed (FEEDAP) 2015). The conclusions on safety for chickens for fattening, including the need for a maximum dose, would also apply to chickens reared for laying and minor avian species to the point of lay (EFSA Panel on Additives and Products or Substances used in Animal Feed (FEEDAP) 2015).

Lastly, none of the primary literature reports included in this review particularly indicated negative effects of the *L. salivarius* strains used in the corresponding study, with the notable exception of caries research, which has shown that certain strains of *L. salivarius* are truly cariogenic.

Antimicrobial resistance of *L. salivarius*

The *in vitro* susceptibility testing of human isolates of *Lactobacillus* species has been limited to a variety of small studies often using diverse methodologies (Goldstein *et al.* 2015). The taxonomic complexity of this genus makes study and generalizations difficult. Some species of lactobacilli are intrinsically resistant to vancomycin and aminoglycosides (Goldstein *et al.* 2015). In fact, FAO (2006) has recommended the establishment of standardized assays for the determination of drug insensitivity or resistance profiles in lactobacilli and bifidobacteria (FAO 2006). However, it does not seem like those guidelines have been published by FAO or any other international public health agency that harmonize antimicrobial resistance testing for LAB. The spread of AMR determinants among LAB has been reported in China (Nawaz *et al.* 2011) in fermented food products, and in Malaysia (Wong *et al.* 2015) in domestic and imported probiotic dietary supplements. The authors caution that the possibility that AMR gene determinants can be transferred to susceptible bacteria remains active (Wong *et al.* 2015).

The antimicrobial susceptibility patterns for *L. salivarius* isolated from multiple sources have been reported, however scarcely. For example, Blandino *et al.* (2008) studied the AMR profiles of LAB isolated from probiotic products in Italy and found that one strain of *L. salivarius* was atypically resistant to erythromycin. The authors indicated that this was a nonintrinsic case of resistance, compared to the resistance level of other LAB in the study (Blandino *et al.* 2008). Conversely, Langa *et al.* (2012) reported that *L. salivarius* CECT 5713 isolated from human milk was sensitive to most antibiotics tested and no transmissible genes potentially involved in antibiotic resistance were detected (Langa *et al.* 2012). Lastly, Cauwerts *et al.* (2006) reported that acquired resistance to tetracycline and minocycline were extremely high for *L. crispatus*, *L. reuteri*, *L. gallinarum* and *L. salivarius* subsp. *salivarius* (75–100%) isolated from cloacal swabs of broiler chickens derived from 20 different farms in Belgium (Cauwerts *et al.* 2006). In several strains, resistance against the tetracycline antibiotics was associated with the presence of the resistance genes *tet*(K), *tet*(L), *tet*(M), *tet*(W) and *tet*(Z). These findings may indicate that intestinal *Lactobacillus* species may act as a pool of antimicrobial resistance genes (Cauwerts *et al.* 2006).

Lactobacillus salivarius as a human pathogen

The scientific community is also concerned with *Lactobacillus* species as potential human pathogens because they have been implicated as the aetiological agent in cases of bacteraemia, cholecystitis, dental abscess/caries, endocarditis, meningitis, peritonitis, prosthetic knee infection and pyelonephritis (Goldstein *et al.* 2015). In a retrospective study of bacteraemia cases associated with *Lactobacillus* species in a university hospital in Taiwan, Lee *et al.* (2015) found that the most commonly isolated species from 89 patients was *L. salivarius* (21), *L. paracasei* (16) and *L. fermentum* (13). There were no significant differences in mortality among patients with bacteraemia due to different *Lactobacillus* spp. Minimum inhibitory concentrations were highest for glycopeptides, cephalosporins and fluoroquinolones and were lowest for carbapenems and aminopenicillins (Lee *et al.* 2015). *Lactobacillus* bacteraemia was associated with a high mortality rate, and patient outcome was associated with underlying malignancy, including diabetes, liver cirrhosis, recent chemotherapy and abdominal surgery (Lee *et al.* 2015).

Conclusions

Primary evidence results from animal models, experimental studies *in vitro* and human population studies unequivocally demonstrate that multiple strains within *L. salivarius* have and exerted probiotic effects on animal and human hosts. In animals, the probiotic is capable of

improving the immune status and reducing colonization by pathogenic bacteria, particularly by *Salmonella*; in humans, probiotic strains have been used for the treatment of multiple chronic diseases, including asthma, cancer, colitis and AD. *Lactobacillus salivarius* acts mostly by modulation of local immune responses and by modifying the ratio of different commensal lactic acid and other bacteria in the GI tract of the host.

Despite having been associated with the spread of antimicrobial resistance and having an opportunistic pathogen status, *L. salivarius* seems to be safe for consumption by animals and humans. However, there is still a lack of information on the safety of many of the strains currently used for experimental treatment of disease, or as prophylactics in animal husbandry. However, the results of those investigations *de facto* demonstrate that this LAB is safe at the doses studied. Further research efforts should focus on the complete phenotypic and genotypic characterization of multiple *L. salivarius* strains so that probiotic studies can be more accurately compared to one another. Additionally, long-term safety assessments of fermented and functional food products containing *L. salivarius* need to be performed to determine any potential adverse health effect throughout time.

Conflict of Interest

Dr Brashears and Dr Nightingale are partial owners of NextGen Innovations, a company that produces and sells probiotic cultures for commercial use.

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