Editorial

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Probiotics to prevent tooth decay

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Introduction

Dental decay is a chronic degenerative infection with a multifactorial aetiology. Oral health education begins in the prenatal period with advice from the gynaecologist and the dentist. Use of fluorine, which can be administered in a variety of ways, is one of the main methods to prevent tooth decay. However, although it has proven protective effects, its administration is not always optimal, likely due to poor scientific knowledge and fear of dental fluorosis. Can the nutraceutical sciences can offer something different? Or something more? Overgrowth and disequilibrium of pathogenic microorganism species in the oral cavity can manifest as a variety of different oral diseases, including dental caries. *Streptococcus salivarius* strain M18 is a bacterial strain with clinically significant probiotic applications for curtailing this pathogenic bacterial growth.

The oral community and probiotics

The vast microbiome of bacteria, fungi and other organisms residing in the oral cavity contributes significantly to oral health. At any given point in time, approximately 700 different taxa of bacterial species simultaneously inhabit the human mouth [1]. Some of these taxa are 'good' bacteria that benefit the oral cavity (and the body as a whole), but some are 'bad' bacteria that exert detrimental effects. The 'bad' bacteria can be problematic by causing dental caries, periodontal disease, strep throat and a wide range of other diseases. The 'good' bacteria confer a host of beneficial effects on humans, including immunomodulation and the prevention of pathogen colonization [1]. Certain 'good' microorganisms can dampen the effects of 'bad' microbes, while providing many other positive effects for the oral cavity and the body as a whole. Because of the beneficial qualities of certain 'good' bacterial species, microbiologists have sought to utilize supplements containing these species for human consumption. A discrete dose of viable 'good' bacteria which confer benefits on the individual receiving the supplement is referred to as a probiotic.

An important benefit of probiotics is their ability to combat inflammatory diseases and infections. Different probiotics can exert beneficial effects in specific targeted areas of the digestive tract, beginning at the oral cavity and ending at the colon. Most primary research focuses on the gastrointestinal benefits of probiotics, but probiotics can exert a wide array of benefits on other parts of the body, such as the oral cavity. Compared to their gastrointestinal probiotic counterparts, oral probiotics are relatively new probiotic formulations capable of opposing 'bad' bacteria and disease in the oral region, which is very important from a health perspective. *S. salivarius* is a particularly important 'good' bacterial species that is the subject of extensive research and is utilized as a commercial oral

probiotic. This species, surely the most abundant in the oral cavity, is a spherical, gram-positive, oxidase-negative and catalase-negative bacterium. *S. salivarius* is one of the earliest colonizers of the epithelial lining of the human mouth and nasopharynx. The bacterium colonizes the dorsum of the tongue and the pharyngeal mucosa of infants, who acquire the bacterium from their mother within 2 days after birth. M18 is the best studied of the *S. salivarius* strains currently employed as probiotics to prevent dental decay.

Strain M18

The *S. salivarius* strain M18 exhibits a particular bacteriocin and enzymatic profile, secreting the bacteriocins A2, 9, MPS and M together with the enzymes urease and dextranase [2]. Salivaricin A2, MPS and 9 are all plasmid-encoded and capable of inhibiting *Streptococcus pyogenes* growth. While MPS, a 60 kDa peptide non-lantibiotic salivaricin, has an inhibitory effect specific to *S. pyogenes*, A2 and 9 are both broader in their actions and capable of inhibiting respiratory tract pathogens (i.e., *Haemophilus influenza, Moraxella catarrhalis* and *Corynebacterium* spp.). Salivaricin M is responsible for inhibiting mutans streptococci and likely *Actinomyces* (Fig. 1), and its expression is chromosomally regulated [2]. The release of urease and dextranase allows the strain to counteract oral acidity and to destroy dextran, a substrate which attaches to tooth enamel together with decay-promoting bacteria, allowing them to proliferate.

When M18 is introduced into the oral cavity, it must colonize specific oral regions and be tolerated by the human host. Once the bacteria have become established, they can then confer distinct oral health benefits on the human host. Colonization of the oral cavity by M18 is dose dependent and after 1×10^9 CFU/subject have been administered daily for 30 days, about 80–90% of treated subjects are colonized (Fig. 2). Despite colonization, there is little to no widespread perturbation of the oral microbiome. Instead of large-scale shifts in bacterial composition, the proportions of bacterial species only shift slightly. This experimental data supports the clinical safety of the strain, as extensive disturbances in the microbiota of healthy subjects are not desirable, significant alterations could potentially yield negative impacts on the probiotic consumers. Finally, in terms of oral persistence, M18 is detectable (1000 CFU/mL) in saliva of treated subjects still 27 days later the last administration [3].

The oral health benefits of strain M18

Once it has become established in the oral cavity, *S. salivarius* M18 can exert beneficial probiotic health effects on the human host, as demonstrated in *in vitro*, *in vivo* and clinical experiments. One particularly significant benefit is the reduction in dental caries, gingivitis and periodontitis. Dental caries is one of the most common childhood diseases and is characterized by the breakdown of tooth enamel and

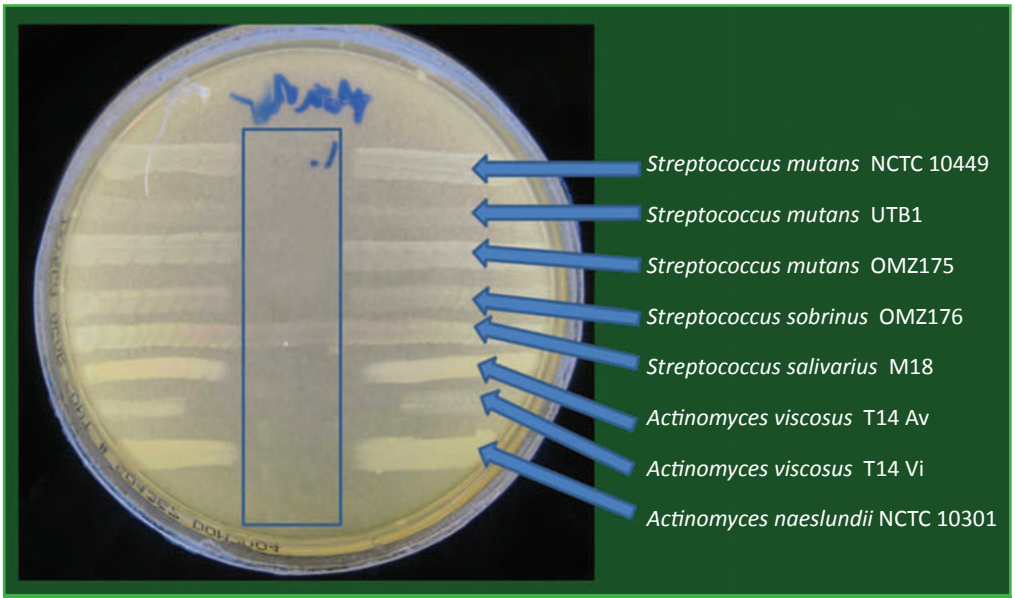

Figure 1 - *Streptococcus salivarius* M18: deferred antagonism

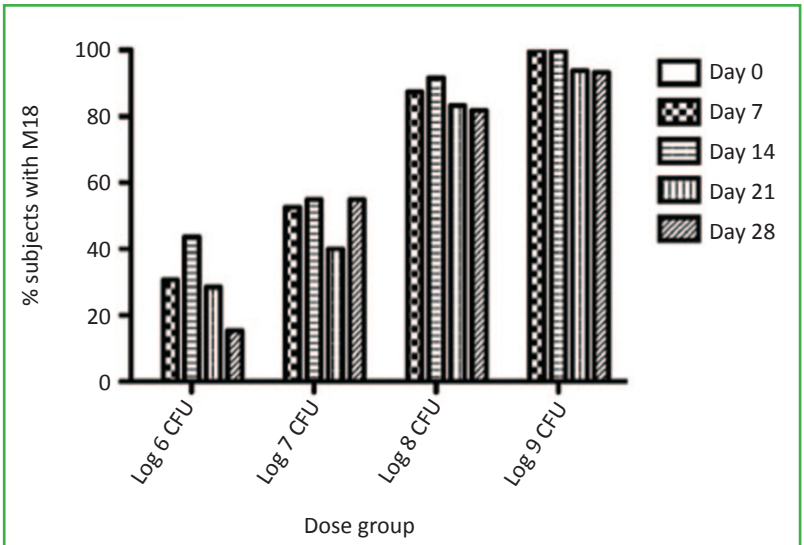

Figure 2 - *Percentage of subjects with strain M18 detected in saliva samples*

dentine due to 'bad' bacteria. These 'bad' bacteria release organic acids that reduce the pH of the oral cavity. The lowered environmental pH causes the dissolution of hydroxyapatite matrices of enamel and dentine. Typically, a combination of mutans streptococci (particularly *Streptococcus mutans* and *Streptococcus sobrinus*) and individual factors (i.e., saliva composition, fluoride exposure, dietary and hygiene habits, etc.) can stimulate this decrease in the pH of the oral cavity. Treatment with M18 effectively reduces a patient's risk of developing dental caries through a molecular mechanism that increases oral pH and reduces plaque formation. In one study, the risk of a patient developing dental caries was assessed using Cariogram, a software program that identifies the relative risk of developing caries based on nine pathological and protective factors, coupled with the expertise of the dentist. According to Cariogram, when children at high risk of developing caries were treated for 90 days with M18 they were less likely to develop dental caries. In the untreated control group, on average, there was a 20% chance of avoiding cavities at day zero, which percentage only slightly increased to 37% after 90 days. In the M18-treated group, subjects also on average had a 20% chance of avoiding new cavities, but this percentage significantly increased to 70% after 90-day treatment with M18. The amount of plaque and mutans streptococci both decreased by approximately 50% and 75%, respectively, in the treated group, while the untreated control group did not exhibit any differences in amount of plaque or mutans streptococci [4].

Treatment of dental caries using the M18 probiotic appears to yield greater benefit and effectiveness in certain groups of patients. Those with high plaque scores benefit more from M18 treatment as they exhibit higher levels of plaque reduction. Additionally, patients colonized by M18 demonstrate greater plaque reduction compared to those not colonized but merely exposed to the bacterial probiotic. Similarly, M18-colonized patients exhibit a greater reduction in *S. mutans*. Studies indicate that higher levels of colonization result in greater reductions in this caries-causing bacterium in saliva, and thus an overall reduction in the development of dental caries. This significantly decreased risk of developing dental caries due to a 90-day *S. salivarius* M18 regimen can be attributed to several proteins produced by the strain. As previously mentioned, M18 releases salivaricin M, which limits the growth of the caries-causing bacterial species *S. mutans* and *S. sobrinus*. Moreover, the strain secretes dextranase and urease. While dextranase catalyzes the breakdown of dextran, urease facilitates the hydrolysis of urea. As dental plaques are rich in dextran, dextranase can help solubilize the plaques that contribute to the breakdown of tooth enamel and dentine. Similarly, urease can increase the pH of saliva by breaking down urea into carbon dioxide and ammonia, and thus prevent hydroxyapatite dissolution. Therefore, dextranase and urease are two M18 enzymes that are effective in decreasing rates of dental caries by reducing plaque accumulation and plaque acidification, respectively [5].

Gingivitis is characterized by inflammation of the gingiva as a result of excess plaque, while periodontitis is a more severe form of gum disease that involves the gingiva pulling away from the teeth. Periodontal disease can be caused by several bacterial species, including *Porphyromonas gingivalis*, *Aggregatibacter actinomycetemcomitans* and *Fusobacterium nucleatum* [3]. These bacteria induce

inflammation of the gums by releasing multiple cytokines, including IL-6 and IL-8. The accumulation of plaque on the surfaces of human dentition, especially near the gingiva, also contributes to the development of gingivitis and periodontitis. When the M18 strain is administered to patients, measures of gingivitis including supra-gingival plaque, gingival inflammation, sulcular bleeding and probing pocket depth using a Williams periodontal probe are all significantly reduced compared to baseline levels prior to consuming the probiotic. How does strain M18 work on periodontal disease? The M18 strain is capable of reducing *P. gingivalis*, *A. actinomycetemcomitans* and *F. nucleatum*-induced IL-6 and IL-8 levels, which are important indicators of the level of inflammation in periodontal disease. Moreover, as *S. salivarius* M18 reduces plaque in human hosts, this can also reduce the gingival inflammation involved in gingivitis. In summary, the probiotic M18 reduces levels of gingival inflammation and plaque, which in turn leads to a reduction in the severity measures of gingivitis and periodontitis [6].

Conclusions

On the basis of the available scientific literature, strain M18 can be considered, alone or as add-on therapy to fluorine administration, as a safe and effective nutraceutical tool to counteract tooth decay, gingivitis and periodontitis.

Conflict of interest

Francesco Di Pierro is owner of Velleja Research.

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