BLIS-producing probiotics targeting the oral cavity

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Consumers seeking health-promoting dietary supplements have long been conditioned to the regular ingestion of yoghurt as a convenient source of living beneficial microbes (*viz*. probiotics). Conventional probiotics have typically been bacteria of intestinal origin (especially lactobacilli and bifidobacteria) and their application has principally been to provide relief for maladies of the gastrointestinal tract. However, the realisation that much human illness can be linked either directly (dental caries, periodontal disease and candidosis) or indirectly (cardiovascular disease and perhaps even obesity) to the development of oral microbiota disequilibria has diverted much of the thrust of contemporary probiotic research towards the establishment and maintenance of a healthy oral microbiota. Step one was to determine whether conventional intestinal probiotics could influence the oral microbiota, but these (perhaps unsurprisingly) have no oral persistence and any oral cavity health benefits are transitory and largely attributable to immune stimulation. Enter, *Streptococcus salivarius* K12 – the world's first purposely-selected oral probiotic, a bacterium derived from the oral microbiota of a healthy human and shown to colonise the oral cavity and to express a wide variety of anti-competitor molecules, termed BLIS (bacteriocin-like inhibitory substances) capable of targeting oral pathogens and also thought to have a role in the stabilisation of a healthy oral microbiota.

The origins of BLIS-producing oral probiotics

The genesis of the senior author's notion that harmless bacteriocin-producing oral streptococci could potentially be used to counter oral infections by pathogenic streptococci can be traced to an undergraduate practical class given by Dr Rose Mushin of the Department of Microbiology at the University of Melbourne in 1966. Following a study leave with Professor Rene Dubos at The Rockefeller University, Rose had embraced Dubos's (1963) concept of bacterial interference[1] (the antagonism of certain human pathogens by the normal microbiota of the body) and in her own research she endeavoured to implant colicin-producing *Escherichia coli* in mice and human subjects (that is, microbiology students) as a strategy for countering infection by intestinal pathogens[2] (Figure 1).

In Dr Mushin's third year class was John Tagg, for nine years habituated to the daily prophylactic consumption of penicillin following an episode of rheumatic fever at age 11. Motivated by the hypothesis that bacterial interference might be used as an alternative to penicillin to counter *Streptococcus pyogenes* pharyngitis, Tagg undertook an MSc with Dr Mushin, the theme being the role of pyocins in strain typing and as agents of bacterial antagonism in *Pseudomonas aeruginosa*[3] (Figure 2). Dr Mushin's retirement and Tagg's now-heightened desire to study bacterial interference in a streptococcal context led to his move to the Department of Pathology at Monash University for PhD studies of the autoimmune basis of rheumatic fever and an opportunity to initiate his quest for a candidate probiotic streptococcus[4]. It was here that streptococcin A-FF22, the

first of the streptococcal bacteriocins, was isolated from a *S. pyogenes*, and later shown to be a lantibiotic, similar to the well-known food preservative nisin[5]. This finding, together with the encouragement of Dr Lewis Wannamaker (who provided postdoctoral mentorship at the University of Minnesota) fuelled Tagg's conviction that a competitive commensal streptococcus could be found to counter infection by *S. pyogenes*.

Although several Scandinavian studies reported some success from clinical applications of mixtures of various oral streptococcus species having either uncharacterised or non-specific inhibitory activity, the data concerning colonisation efficacy, strain characterisation and safety studies appeared rather sparse[6,7]. Early studies by Sanders *et al.* reported that protection against *S. pyogenes* might be provided by *Streptococcus salivarius* producing enocin, a putative antagonist of pantothenate utilisation, but purification of the active agent was not achieved and no follow-up studies have been reported[8].

Figure 1. From an (unidentified) newspaper cutting *circa* 1970. Note: Dr Mushin was Organiser of the Inaugural National Scientific Meeting (1959) and elected Honorary Life Member of the Australian Society for Microbiology in 1970.

Our own longitudinal studies of the ecology of the oral microbiotas of Dunedin schoolchildren led to the recognition that *S. salivarius* was the commensal streptococcal species that was capable of expressing the strongest and widest variety of bacteriocin-like activities (termed bacteriocin-like inhibitory substances or BLIS) directed against *S. pyogenes*[9]. Enhancing its credentials as a candidate probiotic, *S. salivarius* is the streptococcal species which is present in highest numbers in the human oral cavity and it also has an exemplary safety profile (being most closely related to the yoghurt species, *Streptococcus thermophilus*). One *S. salivarius* (named K12), isolated from a healthy child, was inhibitory *in vitro* to all tested *S. pyogenes* and was shown to produce two novel lantibiotics, salivaricin A and salivaricin B. Following this discovery, a prospective study of 282 schoolchildren showed that children harbouring populations of *S. salivarius* expressing salivaricin A and/or salivaricin B had *circa* 47% fewer acquisitions of *S. pyogenes*[10].

BLIS-producing *S. salivarius:* the new-age probiotics applicable to all ages

S. salivarius K12, the prototype of the BLIS-producing oral probiotics, was launched in New Zealand in the year 2002 as the product Throat Guard, for maintenance of oral health. Subsequently it has been shown to be effective for the control of halitosis and also (as Travel Guard) for the stimulation of anti-viral defences. Subsequently, a second probiotic *S. salivarius* (strain M18), differing from K12 in also having BLIS activity against certain dental caries-associated species has been launched with a market focus on the promotion of tooth and gum health[11].

These BLIS-producing *S. salivarius* differ from previous probiotics applied to the oral cavity in that they: (a) have well-characterised inhibitory activity against a variety of oral pathogens; (b) exhibit adhesion specificity and avidity for oral tissues, thereby increasing their colonisation efficacy and oral retention; and (c) exhibit immune reactivity due to their interaction with immunocytes or immunologically responsive oral tissues, thereby resulting in enhanced immune defence against virus infection[12].

Figure 2. John Tagg MSc (Melb) graduation in 1969 with Dr Rose Mushin.

The large variety of potential and actual applications for *S. salivarius* probiotics has led to the concept of BLIS-producing *S. salivarius* being the oral probiotics for all ages (Figure 3). At birth, *S. salivarius* is an early coloniser, with the mother typically being the natural donor. Interestingly, strain K12 has been shown to be inhibitory *in vitro* to group B streptococci, the major bacterial pathogen for newborn infants. It is known that during the first years of life *S. salivarius* establishes within the nasopharynx and our preliminary studies indicate that BLIS-producing *S. salivarius* when present at this site can reduce the occurrence of otitis media. School-aged children are particularly susceptible to acute *S. pyogenes* infection and its serious sequelae such as rheumatic fever, the original target for *S. salivarius* probiotic intervention. Dental caries also presents as a significant public health issue for young children and it is probiotic strain M18 that displays the greatest potential for modulation of the caries potential of dental plaque. Adults afflicted by halitosis or early-onset periodontal disease (associated with a surfeit of odiferous and proteolytic anaerobes) have derived benefit from use of *S. salivarius* probiotics due to the ability of these probiotics to help restore a streptococcus-orientated healthy balance to the tongue and other oral soft tissue microbiotas[13]. The elderly are often susceptible to oral candidosis, either due to prolonged antibiotic exposure or to accumulated deficiencies in their immune defences. Recent mouse model studies have indicated a direct influence of strain K12 on candida disease progression, an observation further supportive of the contention that BLIS-producing *S. salivarius* may indeed prove to be "the probiotics for all ages"[11,14].

Biographies

John Tagg is a Professor Emeritus in the Department of Microbiology and Immunology at the University of Otago, Dunedin, New Zealand. His research focus is on the detection and characterisation of anti-competitor molecules (termed BLIS) by streptococci and on the commercial development of BLIS-producing probiotics. Now a research consultant to the Dunedin-based company BLIS Technologies Ltd, he is a Past President and Honorary Member of the New Zealand Microbiological Society.

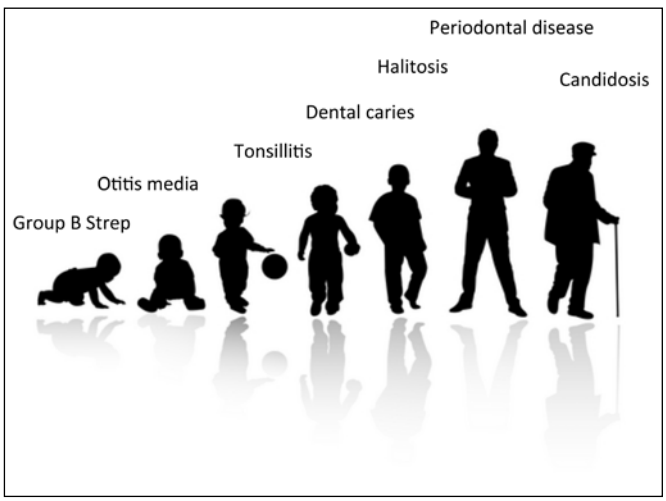

Figure 3. Lifelong applications of BLIS-producing *S. salivarius*

John Hale is a scientist at BLIS Technologies Ltd. Following his PhD studies at the University of Otago he completed postdoctoral studies at the University of British Columbia (Vancouver) and Monash University (Melbourne). His research interests include understanding the mechanisms of action of antimicrobial peptides.

Philip Wescombe obtained his PhD from the University of Otago and since then has been employed as a scientist at BLIS Technologies Ltd. His research focus is on the characterisation of new probiotic strains with an emphasis on bringing them to market after effecting improvements to their fermentation yield and shelf life stability and establishing their efficacy using both *in vivo* and *in vitro* testing programs.

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