

MINI-REVIEW

The rationale and potential for the reduction of oral malodour using *Streptococcus salivarius* probiotics

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The primary treatment for oral malodour is the reduction of bacterial populations, especially those present on the tongue, by use of a variety of antimicrobial agents or mechanical devices. However, shortly after treatment the problematic bacteria quickly repopulate the tongue and the malodour returns. In our studies, we have used a broadly-active antimicrobial (chlorhexidine) to effect temporary depletion of the oral microbiota and then have attempted to repopulate the tongue surface with *Streptococcus salivarius* K12, a benign commensal probiotic. The objective of this is to prevent re-establishment of non-desirable bacterial populations and thus help limit the re-occurrence of oral malodour over a prolonged period. In this paper, we discuss why contemporary probiotics are inadequate for treatment of oral malodour and examine the rationale for selection of particular bacterial species for future use in the treatment of this condition. In our preliminary trials of the use of a chlorhexidine rinse followed by strain K12 lozenges, the majority (8/13) of subjects with confirmed halitosis maintained reduced breath levels of volatile sulphur compounds for at least 2 weeks. We conclude that probiotic bacterial strains originally sourced from the indigenous oral microbiotas of healthy humans may have potential application as adjuncts for the prevention and treatment of halitosis.

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Introduction

Oral malodour is a problem which afflicts a large proportion of the adult population and it is one of the main reasons why many people are motivated to persist

with regular oral hygiene. The most common oral malodour compounds are by-products of the metabolism of certain species of oral bacteria, mainly those on the dorsum of the tongue. A diverse consortium of gram-negative and gram-positive bacteria have been found to contribute to the problem and by contrast, certain bacterial species that predominate in the mouths of 'healthy' subjects become noticeably absent in subjects with halitosis (Kazor *et al*, 2003). Given that oral microorganisms, especially those on the tongue, are the primary cause of halitosis, current treatments focus on the use of chemical or physical antibacterial regimes to reduce the numbers of these bacteria. However, these treatments are typically of only short-term benefit, as the offensive bacteria quickly recover to former numbers after treatment is stopped.

Probiotics, defined by the World Health Organization as 'live microorganisms which when administered in adequate amounts confer a health benefit on the host', may provide a supplementary treatment. Probiotics have been used to modulate bacterial populations in other regions of the human body either when it has been perceived that there is an 'imbalance' of the normal microbiota allowing unregulated growth of 'problematic' microorganisms or in order to replenish bacterial populations which play a specific role in a particular ecosystem. Some examples of successful applications of probiotics include the intestinal tract, where the occurrence of certain disease states has been reduced and the prevention of post antibiotic-associated diarrhoea (Reid *et al*, 2003), and in the vagina where they have been used to reduce the incidence of vaginosis and vaginitis (Reid *et al*, 2003).

Antimicrobial treatment indiscriminately depletes populations of both the problematic bacteria and those bacteria that are not thought to be implicated in halitosis, but which are likely to be important in the maintenance of a normal oral microenvironment. There are many factors that can influence the course of microbial succession and the ultimate restoration of a climax (balanced) ecosystem following exposure to broadly-active antimicrobials. Some bacteria are more resistant than others to the individual antimicrobial agents and these will repopulate the oral cavity more

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quickly when populations of their competitors have been depleted. The outcome of antimicrobial treatment is inevitably only a temporary reduction in malodour, until the halitosis-causing bacteria become re-established. Therefore, in order to achieve long-term reduction of malodour, either more targeted antimicrobials need to be applied that will minimize extermination of the beneficial microbiota, or specific replenishment of the microbiota with beneficial bacteria needs to take place shortly after antimicrobial therapy. The use of more specific antimicrobials is probably not feasible, given that there is a diverse array of microbial species already implicated in halitosis and undoubtedly more that are still requiring identification (Kazor *et al.*, 2003). Preventing the re-growth of odour-causing organisms by pre-emptive colonization of the oral cavity with non-virulent, commensal microorganisms seems like a reasonable alternative.

Given the large number of internet sites dedicated to the sale of probiotic products to people with halitosis, one would anticipate that there are many well-substantiated scientific claims of the efficacy of these products. In reality, however, there are no properly-conducted clinical trials of probiotics in halitosis prevention. Unfortunately, many probiotic products either do not contain the microorganisms stipulated on the label or contain a microbial preparation of low viability (Huff, 2004). Our understanding of the mechanisms involved in probiotic action is in its infancy, but, it is already becoming apparent that replenishment is most efficacious with use of bacteria derived from a similar ecosystem to the proposed site of treatment (Colodner *et al.*, 2003; Reid *et al.*, 2003). Previous attempts to alleviate population imbalances at different sites using a single microorganism have not always been successful (Colodner *et al.*, 2003). The phenotypic profile of probiotic bacteria is very important and validation of the key characteristics of probiotic strains must be established in properly controlled clinical trials conducted according to strict scientific criteria. To date, the principal applications of oral probiotics have been for the prevention of dental caries (Nase *et al.*, 2001; Ahola *et al.*, 2002; Comelli *et al.*, 2002; Montalto *et al.*, 2004). Dairy strains such as *Streptococcus thermophilus* and *Lactococcus lactis* have been investigated for their ability to weaken dental plaque biofilms (Comelli *et al.*, 2002). Lactobacilli exist as commensals in the oral cavity, although they have a strong association with caries (Byun *et al.*, 2004). Probiotic lactobacilli of intestinal origin have achieved only variable results in the reduction of *S. mutans* levels (Nase *et al.*, 2001; Montalto *et al.*, 2004). There is little if any literature on the use of probiotic microorganisms specifically isolated from the mouth for the maintenance of oral health. Given that all of the previously applied oral probiotics have been derived from the intestinal tract or elsewhere it seems that they may not be the most appropriate choice for halitosis prevention.

Indeed, a bacterium isolated from the oral cavity intuitively appears more likely to persist and to have characteristics important for establishing a stable oral

microenvironment, especially if this bacterium is naturally predominant within the microbiotas of 'healthy' subjects. Given that the dorsum of the tongue is the origin of most malodour problems, a candidate probiotic to counter this condition should be able to persist within this particular ecosystem. Bacterial strains usually have affinity for particular tissues because of the presence of specific adhesive molecules on their cell surfaces (Reid and Burton, 2002). The production of anti-competitor molecules such as bacteriocins (protein antibiotics) also appears to confer an ecological advantage to some bacteria. When present in low concentrations they typically are signalling or inducer molecules, yet in higher concentrations they can exert antibiotic (killing) activity against their bacterial competitors. Therefore, production of bacteriocins by a bacterium can give it a useful advantage in highly competitive ecosystems. De Boever and Loesche (1995) suggested that the proteolytic microbiota colonizing the tongue makes the major contribution to halitosis through its action in degrading dietary and host proteins. Therefore, a probiotic strain that efficiently colonizes the tongue surface and does not produce odorous metabolic by-products would be highly advantageous.

Although there are very few in depth studies of the oral microbial populations of subjects with and without halitosis, some significant associations appear to be emerging. Previous culture-based studies have indicated that there is an increase in the ratio of aerobic to anaerobic bacteria after successful antimicrobial treatment and also an increase in *S. salivarius* cell counts (De Boever and Loesche, 1995). Also, a recent comprehensive 16S rDNA clone library study by Kazor *et al.* (2003) showed that certain species were found specifically in individuals who were 'healthy' or in subjects having a 'diseased' state. *Streptococcus salivarius*, *Rothia mucilaginosa* and certain *Eubacterium* species were more frequently detected in 'healthy' subjects.

Streptococcus salivarius strains appear to be excellent candidates for an oral probiotic, since they are early colonizers of oral surfaces and are amongst the most numerically-predominant members of the tongue microbiota of 'healthy' individuals (Carlsson *et al.*, 1970; Kazor *et al.*, 2003). This species also has only a limited ability to produce volatile sulphur compounds (Yoshida *et al.*, 2003) and is unlikely to contribute significantly to oral odour. *Streptococcus salivarius* has not been implicated either in caries or in other infectious diseases of humans and is most closely related to *S. thermophilus*, a bacterium widely used in the dairy food industry (formerly *S. salivarius* ssp. *thermophilus*). We have tested *S. salivarius* strains for production of a variety of characteristics relevant to their potential application as oral probiotics including: bacteriocins, persistence in the oral cavity, adhesion to various oral cells and viability on freeze drying and storage. *Streptococcus salivarius* strain K12 is considered to be a particularly good candidate, since it produces at least two lantibiotic-type bacteriocins which are especially potent against gram-positive bacteria. *Streptococcus salivarius* K12 (BLIS Technologies Ltd, Dunedin, New

Zealand) is currently marketed in New Zealand for the prevention of streptococcal sore throats.

In order to determine whether strain K12 might also be useful in the treatment of halitosis, a series of bacterial strains representative of species implicated in halitosis were tested to see if they were inhibited by the two bacteriocins produced by strain K12. Inhibition was observed of *S. anginosus*, *Eubacterium saburreum* and *Micromonas micros*, but not of *Porphyromonas gingivalis* and *Prevotella intermedia* (Burton et al, 2004). On the other hand, when fresh saliva was inoculated onto agar medium impregnated with the bacteriocins produced by strain K12, inhibition of black-pigmented bacteria identified as *Prevotella* species was observed (J.P. Burton, C.N. Chilcott, C.J. Moore, unpublished data). In a preliminary study, a course of lozenges containing strain K12 were taken by 13 subjects with confirmed halitosis following mouth-rinsing with chlorhexidine. When measured after 1 week of using the K12 lozenges (4 days after ceasing use of the chlorhexidine), 11 of the subjects had volatile sulphur readings that were reduced by at least 100 ppb when compared with pretreatment levels. Eight subjects maintained substantially reduced VSC levels for at least 14 days (J.P. Burton, C.N. Chilcott, C.J. Moore, unpublished data). All subjects showed an increase in the levels of *S. salivarius* as a proportion of their total salivary populations and other measures of halitosis such as BANA reactivity and organoleptic scores were reduced (Burton et al, 2004).

Whilst the majority of the subjects tested in this pilot study had a favourable outcome, the mechanism(s) of volatile sulphur compound reduction was not clearly established. *Streptococcus salivarius* strain K12 does not appear to exhibit *in vitro* inhibition of all species implicated in halitosis and, therefore, other mechanisms of competition, such as saturation of attachment sites may have been influential. Antimicrobial treatments of polymicrobial diseases rarely target all of the bacteria implicated in the disease process, but the killing of some of the critical members of the consortia can cause disruption of the disease-associated population. Thus, the bacteriocins produced by strain K12 may have prevented re-growth of key microbial participants in the halitosis-associated ecosystem. Recent data from our laboratory also indicates that the bacteriocins produced by strain K12 are auto-inducible and that they can cross-stimulate bacteriocin production by other indigenous *S. salivarius* and related species. Thus, the administration of strain K12 may also have boosted the production levels of these antibacterial compounds by the subjects' existing oral microbiota.

In this article, we have briefly explored the rationale for use of probiotics in the prevention of halitosis. Consideration has been given to the nature of currently available oral probiotics and to the importance of strain selection and careful phenotypic characterization. We believe that competitive *S. salivarius* strains have great potential for the control of halitosis and for the prevention of a variety of oral bacterial infections. Whilst further double-blind, placebo-controlled studies are clearly necessary, the outcome of our pilot study encourages us to further investigate *S. salivarius* strains as a novel adjunct for the treatment of halitosis.

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