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# Contribution of Probiotics Streptococcus salivarius Strains K12 and M18 to Oral Health in Humans: A Review

Turner A. Stowik

*University of Connecticut*, [turner.stowik@uconn.edu](mailto:turner.stowik@uconn.edu)

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HONORS SCHOLAR THESIS

**Contribution of Probiotics *Streptococcus salivarius*  
Strains K12 and M18 to Oral Health in Humans:  
A Review**

PRESENTED BY

**TURNER A. STOWIK**

Honors Thesis Supervisor: Dr. Patricia Rossi, PhD, Molecular and Cell Biology,  
University of Connecticut

Honors Academic Advisor: Dr. Charles Giardina, PhD, Molecular and Cell Biology,  
University of Connecticut

DEPARTMENT OF MOLECULAR AND CELL BIOLOGY

THE UNIVERSITY OF CONNECTICUT

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## Abstract

The overgrowth and disequilibrium of pathogenic microorganism species both native and non-native to the oral cavity can manifest into a variety of different oral diseases, pathologies, and afflictions in humans, including dental caries, gingivitis, pharyngitis, halitosis, and oral candidiasis. Two bacterial strains with clinically-significant probiotic applications in curtailing the pathogenic bacterial growth involved in these conditions are *Streptococcus salivarius* strain K12 and *Streptococcus salivarius* strain M18. To summarize the most up-to-date *in vitro*, *in vivo*, and clinical research findings, administration of these *S. salivarius* strains typically in the form of probiotic lozenges results in colonization, reduction in inflammatory measures, and marked alterations to physical structure & gene expression of the oral epithelial cells of the pharynx, tongue, and buccal membrane. While K12 and M18's reduction of pharyngitis and halitosis has been largely attributed to bacteriocin production, the probiotic strains utilize different modes of action to reduce other oral pathologies. The prevention of dental caries, gingivitis and oral candidiasis appears to be ultimately influenced by K12 and M18's production of dextranase & urease, reduction of inflammatory cytokines, and direct physical attachment to pathogens, respectively. In addition to conferring several oral health benefits, these *S. salivarius* strains have been proven extremely safe for human consumption in clinical trials and have the potential for universal application as an alternative treatment to antibiotics in the aforesaid oral pathologies.

## Introduction

The well-defined interconnectivity between oral cavity health and systemic bodily health has earned the mouth the rightly-deserved title “the gateway to the body”. Oral health plays a variety of roles in maintaining overall systemic health and therefore, good oral health is essential for the well-being of humans. A significant contributor to oral health status is the vast microbiome of bacteria, fungi, and other organisms that reside within the oral cavity. At a given point in time, approximately 700 different taxa of bacterial species simultaneously inhabit the human mouth.<sup>1</sup> Some of these taxa are “good” bacteria that are capable of providing benefits to the oral cavity (and to the body as a whole), and some of these “bad” bacterial taxa exert detrimental effects. The “bad” bacteria can be problematic to humans by engendering dental caries, periodontal disease, strep throat, and a wide range of other diseases. The “good” bacteria have a host of beneficial effects on humans, including immunomodulation and prevention of pathogen colonization.<sup>1</sup> Certain “good” microorganisms can dampen the effects of “bad” microbes, while providing a host of positive effects on the oral cavity and body as a whole.

As a result of the beneficial qualities of certain “good” bacterial species, microbiologists conceived the notion of utilizing supplements of these species for human consumption. A discrete dose of viable “good” bacteria which confers benefits to the individual receiving the supplement is referred as a probiotic. One particularly important benefit of probiotics is the ability to treat inflammatory diseases and infections. Different probiotics can exert beneficial effects in specific targeted areas along the digestive tract, beginning at the oral cavity and ending at the colon. The majority of primary research

focuses on the gastrointestinal benefits of probiotics, but probiotics can indeed exert a wide array of benefits on other parts of the body, such as the oral cavity.<sup>2</sup> Compared to their gastrointestinal probiotic counterparts, these “oral probiotics” are relatively new probiotic formulations capable of combatting “bad” bacteria and disease in the oral region, which is extremely fundamental from a health perspective.

Of particular importance and the subject of extensive research, one “good” bacterial species that is utilized as a commercial oral probiotic is *Streptococcus salivarius*. This species is a spherical, gram positive, oxidase negative, and catalase negative bacterium.<sup>3</sup> *S. salivarius* is one of the earliest colonizers of the epithelial lining of the human mouth and nasopharynx.<sup>1</sup> The bacterium colonizes the tongue dorsum and pharyngeal mucosa of infants, who acquire the bacterium from their mother within two days after birth.<sup>4</sup> The two most well-studied strains of *S. salivarius* that are currently employed as probiotics are strains K12 and M18. *S. salivarius* K12 was first isolated from the saliva of a healthy child, and has been utilized as a commercial probiotic in New Zealand for over a decade.<sup>5</sup> K12 produces salivaricin A2 and salivaricin B, two bacteriocins that effectively inhibit phylogenetically related bacterial species.<sup>6</sup> In the human population, approximately 2% of children naturally possess *S. salivarius* strains that produce both salivaricin A and salivaricin B, which corresponds to the strain K12 bacteriocin profile.<sup>6</sup> Due to K12’s bacteriocin profile that effectively inhibited strep-throat-causing bacterial species in preliminary studies, BLIS K12™ was commercially developed and thus became the first *S. salivarius* K12 oral probiotic to specifically target oral health. Another well-studied strain of *S. salivarius* is strain M18. This strain exhibits a markedly different bacteriocin profile compared to strain K12 and effectively secretes the bacteriocins A2, 9, MPS and

M.<sup>7</sup> Due to M18's ability to inhibit dental caries-causing pathogens, BLIS M18™, which utilizes active strains of *S. salivarius* M18, was commercially developed to focus on dental health issues that K12 seemingly cannot address.<sup>7</sup>

When K12 and M18 are first introduced into the oral cavity typically in the form of a probiotic lozenge, they must colonize specific oral regions and be tolerated by the human host. Once the bacteria establish themselves, they can then confer their individually distinct oral health benefits to the human host. This primary research literature review will synthesize and analyze the most current research on these two commercially-utilized probiotic strains by discussing the colonization pattern and commensal relationship of K12 and oral epithelial cells, the clinical benefits that each strain confers to humans and the proposed mechanisms, oral probiotic safety and future implications.



## Colonization of the Oral Cavity by *S. salivarius* K12 & M18

Before *S. salivarius* K12 and M18 can exert their beneficial oral health effects, they must first effectively colonize the oral cavity. To begin their colonization of the oral cavity, individual *S. salivarius* K12 cells must adhere to oral epithelial cells and then rapidly reproduce to form colonies. *Orf166*, an open reading frame that is present on K12's megaplasmid, encodes a cell-surface protein, which ultimately enables this strain to attach to HEp-2 epithelial cells, along with the help of other adhesion factors.<sup>8</sup> Once *S. salivarius* K12 attaches, there is a remodeling of the host epithelial lining to facilitate the commensal interaction between the bacterial and host cell.<sup>1</sup> Specific epithelial cell cytoskeleton-related and adhesion-related genes are upregulated and downregulated, suggesting structural and adhesive changes in epithelial cells that can strengthen the cells' interactions with K12.<sup>1</sup> Once BLIS K12 has adhered to the epithelial lining, it colonizes areas along the upper respiratory tracts of children, including the oral cavity, nasopharynx, and adenoid tissues.<sup>9</sup> Within the oral cavity itself, the colonization sites of *S. salivarius* K12 are primarily the pharynx, tongue, and buccal membrane.<sup>4</sup> K12 colonizes the pharynx to a greater degree than the tongue and buccal membrane, reproducing to approximately  $1.24 \times 10^5$  CFU compared to  $1.3 \times 10^4$  CFU and  $4.6 \times 10^4$  CFU, respectively.<sup>4</sup> Even so, the colonies make up only less than 1% of the entire population of bacterial flora in these particular regions.<sup>4</sup> Following human consumption of K12 lozenges, the strain can last in these areas for as long as three weeks, but the CFUs tend to decrease dramatically after a week.<sup>4</sup> In the saliva, *S. salivarius* can be

present in concentrations as high as  $1 \times 10^7$  CFU/mL, which greatly surpasses the colonial concentrations in the other oral regions.<sup>7</sup>

Similar to the patterns of colonization by K12, the colonization of the oral cavity by *S. salivarius* M18 is dose-dependent.<sup>8</sup> When larger dosages are administered, an increased number of M18 bacteria are retained over a period of time.<sup>8</sup> However, when a persistent lower dosage is administered, there is no cumulative increase in the concentration of M18 in the oral cavity.<sup>8</sup> Upon M18 colonization of the oral cavity, there is little to no widespread perturbation of the oral microbiome.<sup>8</sup> Instead of large-scale shifts in bacterial composition, the proportions of bacterial species only shift slightly.<sup>8</sup> 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.

Research shows that the form of probiotic bacteria that is consumed does indeed have an effect on rates of oral bacterial colonization. For example, BLIS K12 Throat Guard™, a lozenge containing strain K12, demonstrates an 80% effectiveness in colonizing the oral cavity of those who consume the probiotic.<sup>10</sup> However, when the consumers ingest the non-lozenge powdered formula of the probiotic, the effectiveness greatly diminishes to 33%.<sup>10</sup> The reduced efficacy of the powdered form can be attributed to a reduced oral cavity exposure time compared to when the lozenge form is administered.<sup>10</sup> Therefore, the apparent method for an efficacious administration of *S. salivarius* K12 appears to be the probiotic lozenge form, as opposed to the powdered variation.

# The Commensal Relationship of *S. salivarius* K12 & Oral Epithelial Cells

Once the strains of *S. salivarius* have colonized the oral cavity, the oral epithelial cell structure and gene regulation are influenced by strain K12 to facilitate a commensal relationship. *S. salivarius* K12's probiotic quality can be attributed to its unique interactions with oral epithelial cells that modulate physiological responses and innate defenses. Through its interactions with the host cells, *S. salivarius* promotes oral health, maintains homeostasis by reducing inflammation and pathogen-induced apoptosis, and allows itself to be tolerated by the human host.<sup>1</sup> *S. salivarius* K12 accomplishes these actions by inhibiting a pro-inflammatory response, stimulating an anti-inflammatory response, and modulating genes related to adhesion & homeostasis.<sup>1</sup>

*S. salivarius* K12 downregulates the inflammatory response by inhibiting the NF- $\kappa$ B pathway in human bronchial epithelial cells (16HBE14O-).<sup>1</sup> K12 does not initiate the synthesis of pro-inflammatory cytokines or chemokines. In fact, the strain actually decreases normal interleukin-8 (IL-8) secretion, as well as its secretion when epithelial cells are exposed to pathogenic *Pseudomonas aeruginosa*, *Salmonella* serovar Typhimurium flagellin, and the immunomodulatory host defense peptide LL-27.<sup>1</sup> Growth-related oncogene alpha (Gro $\alpha$ ), which is involved in leukocyte recruitment and proliferation, was similarly downregulated when exposed to flagellin in the presence of K12 cells.<sup>1</sup> Taken together, the downregulation of these processes illustrates the K12-induced reduction of the inflammatory response

In addition to inhibiting a pro-inflammatory response, K12 also stimulates an anti-inflammatory response. When human bronchial epithelial cells are exposed to *S. salivarius* K12 and gene expression in these epithelial cells is analyzed, NF- $\kappa$ B-stimulated binding sites in the promoter regions of K12-modulated genes are underrepresented.<sup>1</sup> This underrepresentation ultimately leads to a response that is anti-inflammatory. In addition, the nicotinic acetylcholine pathway was overrepresented.<sup>1</sup> This pathway has the potential for anti-inflammatory effects through its suppression of I $\kappa$ B phosphorylation and NF- $\kappa$ B-induced transcription.<sup>1</sup> Furthermore, the binding sites for CREB, a transcription factor that has been implicated in anti-inflammation, were significantly overrepresented in cells treated with K12.<sup>1</sup> Additional expression data demonstrates an underexpression of proapoptotic FAS signaling and transforming growth factor  $\beta$  pathways, both of which have anti-inflammatory consequences.<sup>1</sup>

Finally, K12 modulates homeostatic genes involved in transcription, translation, protein trafficking, exocytosis, nucleoside metabolism, and phosphate metabolism.<sup>1</sup> As previously discussed, the expression of genes involved with the cytoskeleton and adhesion are also modulated by strain K12. Based on the collective experimental data, the gene expression patterns suggest an attenuated epithelial cell inflammatory response in response to *S. salivarius*. In conclusion, K12 modulates genes involved in the innate response pathways and epithelial cell homeostasis to ensure that it is tolerated by the human host.

## Oral Health Benefits of *S. salivarius* K12 & M18 Probiotics

After becoming established in the oral cavity, *S. salivarius* K12 and M18 can exert their beneficial probiotic health effects on the human host. Both the K12 and M18 strains have demonstrated the potential to provide a wide array of health benefits to human hosts based on *in vitro*, *in vivo*, and clinical experimental investigations. One particularly significant benefit is the reduction of oral diseases, maladies, and afflictions including: (1) pharyngotonsillitis, pharyngitis & tonsillitis, (2) dental caries, (3) gingivitis & periodontitis, (4) halitosis, and (5) oral candidiasis.

### (1) Pharyngotonsillitis, Pharyngitis & Tonsillitis

Pharyngitis and tonsillitis both involve the symptoms of redness, pain, and inflammation of the throat and tonsils, respectively. Group A beta-hemolytic streptococci are typically the culprit of bacterial-induced pharyngitis.<sup>2</sup> The bacterium responsible for the large majority of bacterial pharyngitis cases is *Streptococcus pyogenes*, and if untreated, infection can result in rheumatic fever, deep space abscesses, and toxic shock.<sup>9</sup> Some cases of pharyngitis can become chronic, with recurrent episodes of infection.<sup>9</sup> When patients diagnosed with recurrent pharyngitis consume Bactoblis®, which contains *S. salivarius* K12, they exhibit an 80-96% reduction in the number of episodes of streptococcal pharyngitis and/or tonsillitis infections during a 90-day treatment regimen.<sup>2, 9, 11</sup> Following the 90 day regimen, they similarly exhibit a 60% reduction in the number of reports of pharyngitis in the six month period following the Bactoblis® regimen.<sup>9</sup> Retrospective studies of children with recurrent pharyngitis who were

treated with *S. salivarius* K12 Bactoblis® demonstrate the same trend of a significant reduction in pharyngitis cases throughout the course of treatment and for the following 9 months post-treatment.<sup>12</sup> In addition to preventing  $\beta$ -hemolytic streptococcal pharyngeal infections, one study reports an 80% reduction in viral pharyngeal infections.<sup>11</sup> This result should be further investigated to explore the potential for K12 in reducing non-bacterial infections.

The prevention of *S. pyogenes* infections can be attributed to the production of lantibiotics, a specific classification of antimicrobial peptides that contain lanthionine.<sup>13</sup> These lantibiotics are a subgroup of bacteriocins, which interfere with the growth of bacteria that are phylogenetically-similar to *S. salivarius*.<sup>12</sup> Strain K12 is capable of producing two lantibiotics: salivaricin A2 (SalA2) and salivaricin B (SboB).<sup>14</sup> Although salivaricin A2 and salivaricin B are simultaneously produced in this particular strain of *S. salivarius*, they are unrelated peptides.<sup>14</sup> SalA2 is a 2,368-Da peptide that has two amino acid substitutions at position 4 (S  $\rightarrow$  T) and at position 7 (I  $\rightarrow$  F) that differentiate this variant from salivaricin A.<sup>14</sup> SboB, a 2,740-Da peptide, is encoded by a cluster of eight genes that is flanked by large inverted repeat sequences.<sup>13</sup> Salivaricin B's mode of action in reducing *S. pyogenes* is bactericidal.<sup>15</sup> It is a broad-spectrum lantibiotic, capable of inhibiting the growth of *Streptococcus sanguinis*, *Streptococcus equisimilis*, *Streptococcus agalactiae*, *Streptococcus pneumoniae*, *Streptococcus sobrinus*, *Corynebacterium diphtheriae*, *Lactobacillus casei*, *Stomatococcus mucilaginosus*, and *Moraxella catarrhalis*.<sup>15</sup> The loci of the genes that encode salivaricin A2 and salivaricin B are located nearly adjacent to one

another on a 190-kb megaplasmid- the separation is only about 7.5-kb.<sup>13</sup> K12 can transfer its megaplasmid to different *S. salivarius* strains in subjects who are K12-colonized, indicating that the genes encoding salivaricin A2 and B are indeed transmissible.<sup>15</sup> When the megaplasmid is removed from a K12 bacterium, there is no longer an antagonistic influence on the growth of *S. pyogenes*.<sup>2</sup> Altogether, the data demonstrates that *S. salivarius* strain K12's secreted lantibiotics are capable of inhibiting this prominent pharyngitis-causing bacterial species.<sup>2</sup>

While K12 produces A2 and B, *S. salivarius* strain M18 produces salivaricin A2, 9, MPS, and M.<sup>7</sup> Salivaricin A2, MPS, and 9 are all megaplasmid-encoded and capable of inhibiting *S. pyogenes*.<sup>7</sup> While MPS, a 60-kDa peptide, has an inhibitory effect specific to *S. pyogenes*, A2 and 9 are both broader in their actions, capable of inhibiting additional respiratory tract pathogens.<sup>7</sup> Salivaricin M is responsible for inhibiting mutans streptococci and its expression is chromosomally-regulated.<sup>7</sup>

*S. salivarius* K12 was the first bacterium known to produce two distinct lantibiotics.<sup>13</sup> As production of these lantibiotics is energetically costly, producing multiple bacteriocins indicates the important role of *S. salivarius* in maintaining microbiological balance in the oral cavity.<sup>13</sup> The K12 bacteriocins have strong *in vitro* inhibitory activity against *S. pyogenes*, which provides a means of reducing the presence of this pharyngitis and tonsillitis-causing microbe in the oral cavity.<sup>7</sup> In addition to the bacteriocin-based mode of inhibition, K12 cells bind strongly to human epithelial cells (HEp-2 cells) and competitively interfere with *S. pyogenes*'s binding to this same cell line.<sup>7</sup>

## (2) Dental Caries

Dental caries is one of the most common childhood diseases and is characterized by the breakdown of tooth enamel and dentin due to “bad” bacteria.<sup>16</sup> 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 dentin.<sup>17</sup> Typically, a combination of mutans streptococci (particularly *Streptococcus mutans* and *Streptococcus sobrinus*) and individual factors (i.e. saliva composition, fluoride exposure, dietary & hygiene habits, etc.), can stimulate this decrease in the pH of the oral cavity.<sup>16</sup>

Treatment with *S. salivarius* 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 is assessed through 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.<sup>16</sup> When children who are at “high risk” for developing caries are treated with Carioblis®, an oral probiotic containing *S. salivarius* M18, they are less likely to develop dental caries based on the Cariogram outcome following a 90-day regimen.<sup>16</sup> In the untreated control group, on average, there was a 20% chance of avoiding cavities at day zero and this percentage only slightly increased to 37% after a period of 90 days.<sup>16</sup> In the M18-treated group, at day zero, subjects had an average of a 20% chance of avoiding new cavities (which mirrors that of the control group), while this



percentage significantly increased to 70% after the 90 day treatment.<sup>16</sup> Not only was the overall chance of developing dental caries significantly reduced by the M18 treatment regimen, but the amount of plaque and mutans streptococci both decreased by approximately 50% and 75%, respectively.<sup>16</sup> The untreated control group did not exhibit any differences in concentration of plaque or mutans streptococci.

The treatment of dental caries using *S. salivarius* M18 probiotics appears to yield greater benefit and effectiveness in a certain group of patients. Participants who have high plaque scores receive a greater degree of benefit from M18 treatment, as they exhibit superior levels of plaque reduction.<sup>18</sup> Additionally, patients who are colonized by *S. salivarius* M18 demonstrate greater plaque reduction compared to those subjects who were not colonized and were merely exposed to the bacterial probiotics.<sup>18</sup> Similarly, those patients who are M18-colonized exhibit a greater reduction of *S. mutans*.<sup>18</sup> Studies indicate that higher levels of colonization result in a greater reduction of this caries-causing bacterium in saliva, and thus an overall reduction in the development of dental caries itself.<sup>18</sup>

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 discussed, M18 releases salivaricin M, which limits the growth of the caries-causing bacterial species, *S. mutans* and *S. sobrinus*.<sup>16</sup> Moreover, the strain secretes dextranase and urease. While dextranase catalyzes the breakdown of dextran, urease facilitates the hydrolysis of urea.<sup>16</sup> As dental plaques are rich in dextran, dextranase can aid in solubilizing the plaques that

contribute to the breakdown of tooth enamel and dentin. 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.<sup>16</sup>

### **(3) Gingivitis & Periodontitis**

Gingivitis is characterized by the 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*.<sup>3</sup> These bacteria implicated in gingivitis and periodontitis induce inflammation of the gums by releasing multiple cytokines, including IL-6 and IL-8.<sup>3</sup> Another contribution to the development of gingivitis and periodontitis is the accumulation of plaque on the surfaces of human dentition, especially near the gingiva.<sup>18</sup>

When BLIS M18 lozenges are administered to subjects, measures of gingivitis including supragingival 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.<sup>19</sup> After a cease in the regimen, there is an increase in all these parameters, such as an increased number of bleeding sites.<sup>19</sup> Both strains K12 and M18 are 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.<sup>3</sup> The previously described ability of K12 to reduce the production of cytokines when it is commensally associated with oral epithelial cells, coupled with its ability to reduce cytokine levels induced by gingivitis-causing bacterial species, further supports K12's anti-inflammatory role in the oral cavity. Moreover, as *S. salivarius* M18 confers plaque reduction to human hosts, this can be an additional means of reducing the gingival inflammation involved in gingivitis.<sup>18</sup> In summary, the probiotics M18 and K12 reduce levels of gingival inflammation and plaque, which in turn leads to a reduction in severity measures of gingivitis and periodontitis.

#### **(4) Halitosis**

Although halitosis, commonly known as oral malodor or “bad breath”, is not a medical problem, it is a generally undesirable oral hygiene concern.<sup>20</sup> Halitosis is prominently treated by using antimicrobial agents or mechanical devices to reduce bacterial populations that particularly inhabit the lingual region of the oral cavity.<sup>21</sup> Halitosis is primarily caused by the presence of volatile sulfur compounds (VSC), sulfur-containing metabolic by-products of anaerobic bacteria.<sup>20</sup> *S. salivarius* itself does not produce large quantities of VSCs, and therefore has an extremely minimal contribution to oral malodor.<sup>20</sup> When lozenges containing *S. salivarius* K12 are consumed for a week by human subjects with high VSC levels, their VSC levels were significantly reduced in 85% of test subjects, indicating a reduction in this important marker of halitosis.<sup>20</sup> Moreover, there was the greatest bacterial composition change in the group that received *S. salivarius* lozenges, and K12 was detected in the saliva samples of

subjects after 7 and 14 days.<sup>20</sup> The subjects that had larger quantities of K12 present in saliva samples exhibited the greatest reduction in VSC levels.

Even if the host is not colonized by K12, the bacteria are still capable of increasing the lantibiotic concentrations in saliva, which can be effective in reducing halitosis-causing bacterial species. *In vitro*, K12 reduces the growth of black-pigmented bacteria and several Gram-positive bacterial species implicated in halitosis by producing salivaricin A and salivaricin B.<sup>20</sup> The Gram-positive bacteria that are implicated in halitosis that *S. salivarius* inhibits include: *Streptococcus anginosus* T29, *Eubacterium saburreum*, *Micromonas micros*, *Atopobium parvulum*, *Eubacterium sulci*, *Parvimonas micra*, and *Solobacterium moorei*.<sup>22</sup> Although K12 antagonizes numerous Gram-positive bacteria that cause halitosis, there are no known Gram-negative bacteria that are effectively inhibited.<sup>22</sup> When halitosis-causing bacterial species are reduced and replaced with non-halitosis-causing bacterial species (such as *S. salivarius* K12), this serves as a viable means to reduce halitosis.

As current treatments for halitosis tend to have short-term results, with VSC-producing bacteria quickly repopulating the oral cavity, there is a present need for a longer-term solution. Since a majority of subjects maintained reduced VSC levels for two-weeks following consumption of K12 lozenges, there is a potential role for K12 as a longer-term treatment solution for halitosis.<sup>21</sup> As the literature supporting the role of *S. salivarius* K12 in long-term reduction of halitosis is relatively minimal, more research will be needed to determine if K12 is a viable treatment measure.

## (5) Oral Candidiasis

The overgrowth of *Candida albicans*, a common yeast species, can result in oral pathologies such as oral candidiasis, which is more commonly referred to as thrush. Thrush causes severe inflammation as well as the potential for infection, especially in populations of individuals who are immunosuppressed.<sup>23</sup> *In vitro*, K12 prevents the adherence of *C. albicans* to a plastic petri dish substratum.<sup>23</sup> *In vivo* administration of *S. salivarius* K12 in a mouse model demonstrates a dose-dependent reduction in oral candidiasis symptomology. Although administered dosages of K12 do not completely eradicate populations of *C. albicans*, the mice tongues have fewer lesions, less fungal growth, and reduced pathogenicity (decreased fungal mycelial invasion of tongue epithelium).<sup>23</sup> Therefore, the overall effect of K12 is the inhibition of *C. albicans* colonization.

The reduction of oral candidiasis by *S. salivarius* K12 can be attributed not to a direct fungicidal effect, but to the bacterium's ability to directly bind to hyphae of *C. albicans*.<sup>23</sup> The probiotic microbe surrounds and attaches to *C. albicans* during fungal germ tube formation and mycelial expansion, which reduces the pathogenic yeast's ability to adhere to substrates.<sup>23</sup> K12 preferentially binds to the fungal mycelia but not to individual yeast cells themselves. As many of the antimicrobial effects of strain K12 are due to its bacteriocin production, the direct yeast cell-to-bacterial cell contact is seemingly a unique mode of inhibition. *S. salivarius* K12 thus acts by preventing adhesion of *C. albicans* to epithelial surfaces in the oral cavity, which causes the yeast to be ingested down the esophagus to eventually be excreted by the human host.<sup>23</sup>

## Safety Assessment of BLIS K12 Oral Probiotics

Based on its performance in clinical trials and *in vitro* tests, *S. salivarius* appears to be a highly safe bacterium for human consumption as a probiotic. When an antibiogram test for the species was performed using the antibiotic disk sensitivity method, strain K12 demonstrated sensitivity to many commonly used antibiotics, such as penicillin and amoxicillin.<sup>1</sup> However, the strain exhibited moderate levels of resistance to gentamicin and ofloxacin, with zones of inhibition in the range of 14-18 mm.<sup>1</sup> Overall, the bacterium lacks resistance to any clinically-significant antibiotics, indicating that even in obscure cases of *S. salivarius* infection, it can be easily combatted by common antibiotic treatments. Using two commercially-available microorganism identification tests (the API 20 Strep and API 50CH tests), which can help map metabolic profiles of different microbes, none of the fermentation or enzymatic reactions that are investigated in these commercial tests indicate that strain K12 would have a hazardous effect on humans.<sup>1</sup> The organism does not produce any hazardous metabolic by-products, such as D-lactate.<sup>10</sup> When this bacterium underwent blood cell lysis testing on human blood agar, sheep blood agar, and buffered CNA-P agar, there was no hemolytic activity observed in any of these mediums.<sup>1</sup> In testing the presence of virulence factors in strain K12 using PCR and Southern hybridization, there were no streptococcal virulence determinants or factor genes detected in K12.<sup>1</sup> At this point in time, there is no genome sequence of *S. salivarius* available. A genome sequence could provide a more in-depth analysis of virulence factors in this bacterial species and is one limitation that yields some

uncertainty in definitively stating that *S. salivarius* K12 lacks streptococcal virulence factors altogether.

In clinical-based safety assessment studies, human subjects consumed lozenges containing *S. salivarius* K12 at regular intervals and reported no adverse symptoms over the course of 28 days. There was also no significant change in the levels of *S. salivarius* or facultative anaerobic bacteria in saliva samples of subjects before and after the probiotic regimen.<sup>1</sup> Additionally, BLIS K12™ was tested to identify its potential to induce point mutations in microorganisms. A bacterial reverse mutation test was performed at varied bacterial concentrations, and the bacterium appeared to lack any mutagenicity. In the short-term, even when administered orally to rats at high dosages of K12 (5 g/kg), there are no apparent adverse effects caused by the strain. There was no sign of cancer, tissue abnormality, or infection acutely after 14 days.<sup>10</sup> As the typical dose of *S. salivarius* K12 is 7.5 mg/kg, there is little possibility of toxic effects in humans.<sup>10</sup> Using several health indicators, including gross pathology, biochemical tests, hematology, body & organ weight, histopathology, and urinalysis, in sub-acute testing (28 days), there similarly was no toxic effect in rats, even at the highest doses.<sup>10</sup> In other clinical safety assessment studies, 100 healthy adults consumed lozenges with K12 concentrations of  $1 \times 10^9$  CFU and it was determined that after colonization, the bacteria's persistence in the oral cavity was not at levels higher than subjects who naturally possess strain K12.<sup>10</sup> A final validation of the virtually non-existent toxicity and side-effects is the fact that hundreds of thousands of doses have been administered in New Zealand (mainly in children) in the past fifteen years and there have been no apparent side-effects.<sup>6</sup> There have been no reports of infections caused by *S. salivarius* strain K12 or

M18, and reports of infection by other *S. salivarius* strains are generally quite uncommon. Between the *in vitro* and clinical investigations, the data indicates that strain K12 has a low pathogenic potential and a minimal chance of causing disease in humans. There is essentially no indication that BLIS K12 probiotics have a negative impact on humans, and therefore, they appear to be extremely safe for human consumption.



## **Future of *S. salivarius* Strain K12 & M18 Oral Probiotics**

In the field of probiotic research, there is a present need for more information about the relatively novel branch of oral probiotics. As there still is a rather small pool of research about *S. salivarius* strain K12 and M18, future clinical testing will be necessary to confirm the roles of these probiotics in terms of practical clinical application. While the majority of the oral probiotic research focuses on K12, more research should be conducted on M18 and other *S. salivarius* strains that provide oral health benefits. Similarly, much of the current research focuses on the overall effects of K12 and M18 on oral health measures, rather than the mechanisms that are the underlying cause of these effects; this is one area that particularly needs to be investigated in further detail. Of the studies presented in this review, the majority have a small sample size. To improve the validity of the data and the accuracy of conclusions that can be drawn from the research, more extensive studies should be conducted that are double-blinded, placebo-controlled, and have large pools of participants who are randomly assigned to an experimental or control group.

Despite the limitations of the present studies on the subject, there are several socioeconomic implications in the treatment of the described infections, maladies, and afflictions through the use of *S. salivarius* K12 and M18 probiotics. In one study, the number of days that recurrent pharyngotonsillitis-prone children who consume Bactoblis® K12 were on antibiotics (amoxicillin and clavulanic acid) was 30 times lower than those who did not take the probiotic lozenges.<sup>8</sup> Similarly, the number of days this same cohort of children were on antipyretics (acetaminophen and ibuprofen) was 14

times lower.<sup>8</sup> In addition to an overall reduction in the need for drug treatments, there were lifestyle impacts as well. Both children and parents respectively missed 93% less school/preschool and work when children followed the K12 regimen compared to those who were not treated by the probiotic.<sup>8</sup> As antibiotic regimes can be quite costly and missed work days can pose a financial burden to families, K12 probiotic treatment could be financially advantageous for at least some pools of patients.

The oral probiotics discussed in this primary research literature review have the potential to be universally utilized as an alternative to antibiotics and/or other treatments used to combat and prevent a variety of oral pathologies. As described previously, several oral afflictions can be significantly reduced in a clinical setting. Of interest, patients presenting with pharyngitis and/or tonsillitis appear to have a particularly promising K12 treatment outlook based on the data from current literature. Given the present concern about the development of antibiotic resistance in bacterial species, to help combat this issue, these probiotics could be used as an alternative treatment to antibiotics in milder pathogenic infections. Furthermore, since much of the developed world is inextricably tied to modern medicine practices, implementing the use of probiotics in clinical practice may prove extremely difficult, but not impossible. For example, many dentists in New Zealand routinely prescribe K12 and M18 probiotic formulations to prevent dental caries and to combat mild gingivitis. In recent years, there has been an increase in probiotic usage within the United States and abroad, which, coupled with the excellent clinical efficacy demonstrated in a variety of research investigations, bodes well for the future of the oral probiotics *S. salivarius* K12 and M18.

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