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Review Article

REVIEW ARTICLE

Metabolites of the oral microbiome: important mediators of multikingdom interactions

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One sentence summary: More than 700 bacterial species live in the oral cavity and communicate through the production of unique small molecules to bring about diseases or to maintain mouth health.

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ABSTRACT

The oral cavity hosts over 700 different microbial species that produce a rich reservoir of bioactive metabolites critical to oral health maintenance. Over the last two decades, new insights into the oral microbiome and its importance in health and disease have emerged mainly due to the discovery of new oral microbial species using next-generation sequencing. This advancement has revolutionized the documentation of unique microbial profiles associated with different niches and health/disease states within the oral cavity and the relation of the oral bacteria to systemic diseases. However, less work has been done to identify and characterize the unique oral microbial metabolites that play critical roles in maintaining equilibrium between the various oral microbial species and their human hosts. This article discusses the most significant microbial metabolites produced by these diverse communities of oral bacteria that can either foster health or contribute to disease. Finally, we shed light on how advances in genomics and genome mining can provide a high-throughput platform for discovering novel bioactive metabolites derived from the human oral microbiome to tackle emerging infectious and systemic diseases.

Keywords: the oral microbiome; host–microbe interactions; microbial metabolites; bacteriocins; RiPPs

INTRODUCTION

Advances in molecular biology, including next-generation sequencing (NGS), metagenomics, transcriptomics, metabolomics and proteomic analysis (Integrative HMPRNC 2019), have revolutionized microbiology research and produced high-throughput genomic data to support microbiome-focused epidemiological, physiological and cellular studies (Shendure *et al.* 2017). This advancement has led to a more detailed characterization of the human gut microbiome (Brody 2020). While microbiota refers to the numerous living microorganisms, including bacteria, fungi, viruses and protozoa, which reside in a specific ecological niche, the microbiome includes not only the community of the microorganisms but also all their collective genomes and genetic material in a given environment, in addition to their produced metabolites (Berg *et al.* 2020). Thus, in addition to identifying the specific cohorts of microorganisms that populate the various mucosal niches of

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the host, researchers are beginning to develop a knowledge of how these microorganisms signal and interact in order to coexist and maintain host health (Fan and Pedersen, 2021) and impact disease states, including intestinal inflammation (Cao 2017), cancer (Whitmore and Lamont 2014) and cardiovascular diseases (Leishman, Do and Ford 2010).

Research involving the oral microbiome is predominantly focused on microbial species diversity and relative species abundance using either 16S rRNA gene sequencing or shotgun metagenomic sequencing of the oral specimens (Lamont, Koo and Hajishengallis 2018). These techniques produce sufficient data to identify the microbial communities inhabiting different oral sites (e.g. buccal mucosa, tongue, throat, sub- and supragingiva). However, less attention is devoted to the molecular substances produced by the oral microbiota and their encoding biosynthetic gene clusters and how these molecules can play a role in sustaining health or, conversely, fostering disease progression. The human oral microbiome is a rich source of metabolites of biotechnological merit, some of which have been commercialized as health-promoting applications (Barbour, Wescombe and Smith 2020). Natural metabolites of the human microbiome play significant roles as health and disease mediators and are utilized in medicine and biotechnology industries as antimicrobials, antifungals and anticancer agents (Garg *et al.* 2017). Thus, there is significant potential to make breakthroughs and discoveries of novel bioactive metabolites derived from the human microbiome (Milshteyn, Colosimo and Brady 2018).

An example of this is the newly discovered antibiotic lugdunin produced by *Staphylococcus lugdunensis*, a commensal organism residing in the nasal cavity. This novel thiazolidine-containing cyclic peptide was shown to possess a vigorous inhibitory activity against the health-threatening superbug methicillin-resistant *Staphylococcus aureus* (Zipperer *et al.* 2016). By contrast, some oral cavity species such as *Neisseria* spp. produce genotoxic bacterial metabolites (e.g. acetaldehyde), which have tumour-promoting functions by either generating mutations in the host cells or hindering normal antitumour immune responses. In this article, we discuss the current knowledge on microbial metabolites found in the oral microbiome of the human host, with a focus on their roles and functions in health and disease. Such metabolites include antimicrobials (bacteriocins), anticancer peptides, and organic and fatty acids. We also highlight the unique metabolites of putative oral pathogens and their roles in disease initiation and progression. Finally, we provide insights into genome mining as a successful approach to hunt for novel oral microbiome metabolites.

PRIMARY METABOLITES

Metabolites derived from fermentable carbohydrates

The breakdown of dietary macronutrients (carbohydrates, proteins and fats) starts in the oral cavity. The complex structure of the ingested biomolecules is primarily disrupted by digestive enzymes supplied by host and microbial sources (Inui *et al.* 2015; Pedersen *et al.* 2018). This degradation releases the interlinked building blocks of these complex molecules, which can then diffuse into the oral biofilm and undergo further metabolism. These sequential metabolic processes give rise to a wide array of metabolic end products that can impact the local ecology and biology of the oral microenvironment and also have implications further down the alimentary path into the intestines. The effects of such events are perhaps most evident concerning the well-studied microbial end-product metabolites, the short-chain fatty acids (SCFAs). Carbohydrate fermentation brings about SCFAs production; however, carbohydrates are not the only SCFAs-producing substrates, as other substances are utilized, to varying degrees, to produce SCFAs depending on their availability. The glycoconjugate structure of dietary carbohydrates, which broadly includes polysaccharides, oligosaccharides and resistant starches, is initially deconstructed by a set of enzymes known as the carbohydrate-active enzymes (CAZymes) into components transferrable to the bacterial cell (Inui *et al.* 2015). While it is not surprising that the gastrointestinal tract is the richest site of CAZymes, the primary site of carbohydrate metabolism, the fact that the oral cavity is the second most abundant source of these enzymes highlights the significance of oral digestion in carbohydrate fermentation (Cantarel, Lombard and Henrissat 2012). Besides dietary sources, oral bacteria can utilize the nine-carbon acidic sugar, sialic acid, derived from salivary mucins as a nutrient source after freeing it from the protein portion by sialidases (Lewis and Lewis 2012; Marsh *et al.* 2016). Following carbohydrate hydrolysis and mobilization of the five-carbon (C5) or the six-carbon (C6) monosaccharides, oral bacteria further process these molecules either by the pentose phosphate pathway (C5) or the glycolysis pathway (C6), producing pyruvate with subsequent ATP production (Takahashi 2015). Once produced, other branched pathways convert pyruvate into SCFAs, including lactate, acetate, butyrate, propionate and isobutyrate, as end products (Takahashi 2015). The antimicrobial potential of these SCFAs can dictate the nature of the relationship between oral coinhabitants, whether mutual or competitive. These SCFAs can shape the oral microbiome to favour the prevalence of certain bacterial species and is toxic to others. For instance, a high intake of dietary carbohydrates results in the expansion of the saccharolytic, acidogenic bacteria in the supragingival plaque at the expense of other strains (Georgios, Vassiliki and Sotirios 2015). This ultimately results in establishing an ecosystem with enhanced cariogenic potential, supragingivally, which fuels the demineralization of tooth surfaces resulting in cavitated carious lesions.

Like carbohydrate fermentation, oral bacteria also ferment amino acids to produce SCFAs (Norimatsu *et al.* 2015). This process is initiated when host and bacterial proteases and peptidases degrade proteins into peptides and amino acids, which are then deaminated and converted to SCFAs. These proteinaceous substrates are prominent in the subgingival bacterial environment, where nutrition is mainly dependent on glycoproteins, proteins, peptides and amino acids derived from the gingival crevicular fluid and desquamated epithelial cells (Tsuchida *et al.* 2018). However, the complex interconversion steps required to incorporate amino acids into biochemical pathways compared with monosaccharides limit the ability of oral bacteria to utilize them on a larger scale (Takahashi 2015). Moreover, the fermentative reactions of amino acids produce a wide range of compounds other than SCFAs that will be discussed later on (Fig. 1). SCFAs in the subgingival niche reconstitute the biofilm species, expanding the SCFAs producing population. The effect of SCFAs in the subgingival biofilm is very different from that of the supragingival, as they are known to exert cytotoxic effects and induce tissue inflammation and thus are strongly linked to periodontal inflammation (Magrin *et al.* 2020b)). This can have deleterious effects on oral epithelial cells, as milli-molar concentrations of SCFAs can promote apoptosis, autophagy and pyroptosis in these cells, affecting their viability (Magrin *et al.* 2020a).

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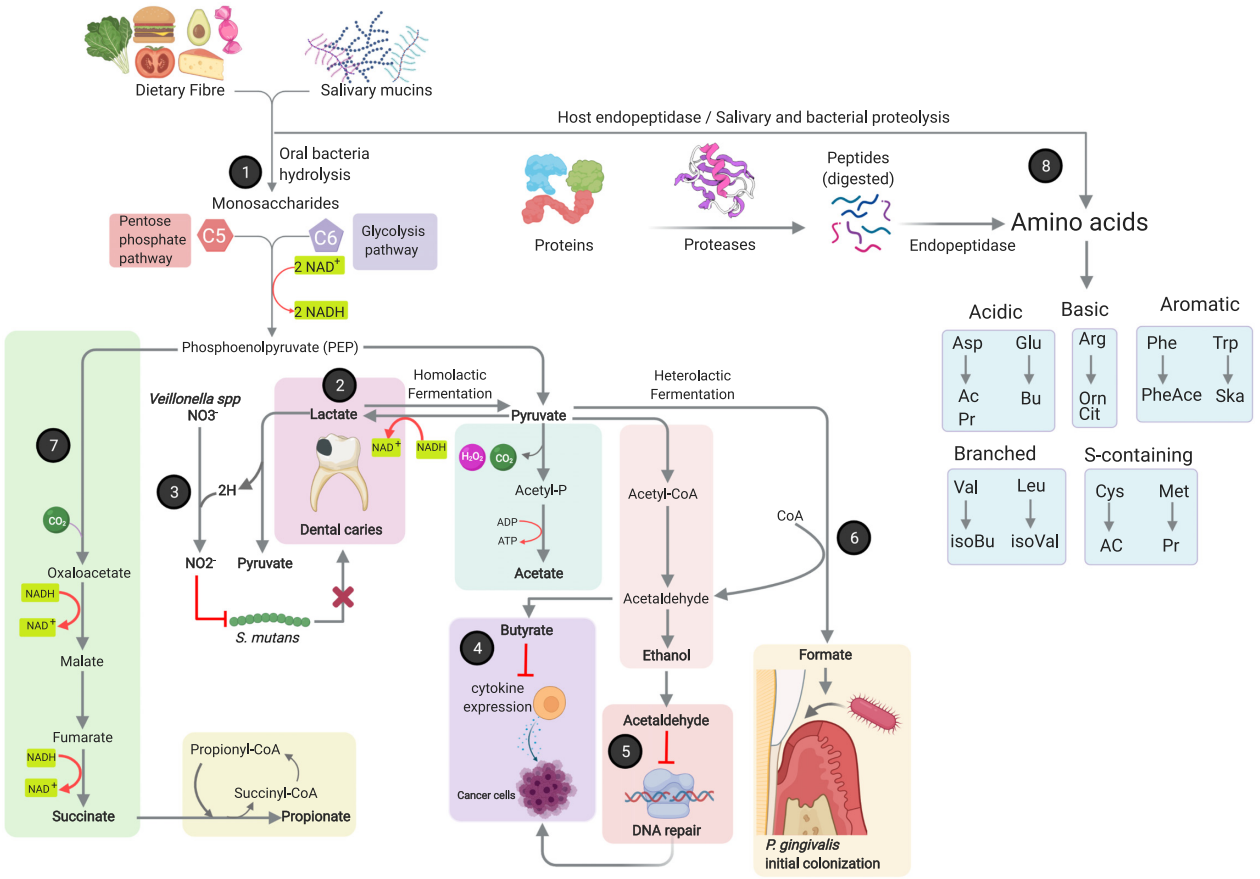


Figure 1. Primary metabolites of oral microbial fermentation of accessible carbohydrates and proteins. Carbohydrates entering the oral cavity are hydrolysed into oligosaccharides, disaccharides and monosaccharides by multiple host and bacterial glycosidase and polysaccharidase enzymes. Two types of carbohydrate transport systems are utilized by oral microbiota—the phosphoenolpyruvate (PEP)-dependent phosphotransferase system and the binding-protein transport system. The generated monosaccharide substrates are catabolized either via pentose-phosphate or glycolysis pathways to form PEP (1). This process produces pyruvate and generates two NADH. The SCFAs, acetate, propionate and butyrate can be derived from PEP or pyruvate via several pathways. Lactate production from pyruvate via homolactic fermentation results in lactic acid production, the major end product of glycolysis by acidogenic *S. mutans* (a suspected causative agent of dental caries) under conditions of sugar excess (2). *Veillonella* species, one of the significant anaerobes in the oral cavity, were recently found to utilize lactate and convert nitrate (NO_3^-) into nitrite (NO_2^-), which results in growth inhibition of *S. mutans* (3). Pyruvate fermentation by oral microbiota can also yield other metabolites such as butyrate (which was found to inhibit cytokine expression) (4) and acetaldehyde (which interferes with DNA repair) (5). Heterolactic fermentation of pyruvate can result in formate production, which is consumed by periodontitis-associated bacteria to establish colonization (6). Succinate can be derived from PEP via the fumarate reductase pathway and can be further converted to propionate by some oral bacteria (7). Proteins derived from food and salivary mucins are digested by host and oral bacterial proteases and endopeptidases to yield various amino acids (8). Oral bacteria can utilize these smaller molecules via degradative pathways to produce acetate (Ac), propionate (Pr) and butyrate (Bu). Arginine can be converted to citrulline (Cit) by arginine deiminase and is subsequently degraded to ornithine (Orn). This pathway is shared by many *Streptococcus* and *Actinomyces* species. The aromatic amino acids phenylalanine and tryptophan are metabolized to form phenylacetate (PheAce) and skatole (Ska), respectively. The branched-chain amino acids valine and leucine are metabolized to form the branched-chain fatty acids isobutyrate (isoBu) and isovalerate (isoVal), respectively. The sulfur-containing amino acids Cystine (Cys) and methionine (Met) are metabolized to form acetate and propionate, respectively. This figure was created with biorender.com.

The role of SCFAs in disease

The development of many oral diseases is driven mainly by the metabolic activity of the bacteria constituting the oral biofilm and can occur due to the overgrowth of commensal microbes. Extrinsic factors, mainly those affecting substrate availability, alter the oral environment allowing pathogenic bacteria to outcompete those maintaining health, thus contributing to disease development (Lu, Xuan and Wang 2019). As mentioned earlier, oral bacteria involvement in tooth decay depends on metabolic pathways that involve the uptake and metabolism of carbohydrates and SCFAs production, including lactic and acetic acids, which lower the pH, allowing more acidogenic bacteria to thrive (Nyvad and Takahashi, 2020). The resultant acidic conditions shift the average balance of the demineralization and remineralization processes towards the former, resulting in carious lesions (Pitts *et al.* 2017). Lactic acid represents one of the primary acids involved in this process. The most studied cariogenic oral bacterial genera, *Streptococcus* and *Lactobacillus*, are well-known lactic acid producers (Moye, Zeng and Burne 2014; Caufield *et al.* 2015). In a humanized mouse model of monozygotic twins, lactic acid was detected in the saliva-derived biofilm (*in vitro*) at higher levels in the group with active caries, suggesting its importance in the cariogenic process (Wu *et al.* 2018). On the other hand, bacteria such as *Bifidobacterium* and *Scardovia wiggsiae* depend on acetic acid production in mediating their pathogenicity. The two species are abundant acetic acid producers, and they were both found to avert fluoride-induced glycolysis inhibition. Though fluoride inhibits the glycolytic enzyme

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enolase, important for carbohydrate metabolism, its inhibitory impact on normal glycolysis in these species was weaker than that of other cariogenic bacteria such as *Streptococcus mutans* (Manome *et al*. 2019; Kameda *et al*. 2020).

Lactate may also play a role in reprogramming immune cells in a manner that supports the persistence, rather than the limitation, of biofilm-induced inflammation. This recent observation suggests that lactate produced by *S. aureus* in prosthetic joint infections inhibits histone deacetylase 11 (HDAC11), causing epigenetic changes (Heim *et al*. 2020). These changes augment IL-10 transcription in macrophages prompting them to adopt anti-inflammatory functions, allowing inflammation to persist. Thus, some evidence suggests that lactate-producing *S. aureus* might be among the earliest colonizers and a common pathogen involved in dental peri-implant inflammation (Salvi *et al*. 2008; Thurnheer and Belibasakis 2016). If so, similar lactate-associated mechanisms are possibly implicated in *S. aureus*-driven dental implant pathologies. Indeed, accumulating evidence suggests that there might be similar ties to those observed in prosthetic joint infections since leukocytes in patients with dental implants exhibit higher IL-10 production (Thomas *et al*. 2013).

Furthermore, expression of mir-27a, a negative regulator of IL-10, was found to be downregulated in gingival tissue samples at peri-implantitis sites compared with healthy implant sites (Xie *et al*. 2014; Wu *et al*. 2017). Moreover, the positive IL-10 regulator, miR-145, which represses the *Il10* gene silencer, HDAC11, was found to be significantly upregulated (Lin *et al*. 2013; Wu *et al*. 2017). Since this mechanism is similar to that described above, it can be implied that lactate produced by the prevalent peri-implantitis pathogen, *S. aureus*, may adopt the same IL-10 promoting capacities as those observed in prosthetic joint infections; however, further studies are warranted to clarify the nature of this relationship.

SCFAs, including butyrate, have also been linked to many aspects of the pathogenesis of the periodontal disease. From the initial steps of biofilm formation, butyrate stimulates fimbrillin-dependent colonization of the early colonizer *Actinomyces oris* and contributes to the formation of significant biofilm growth (Suzuki, Shimizu and Senpuku 2018). As other bacteria continue to populate the biofilm during the pathogenic process, butyrate producers such as *Porphyromonas gingivalis*, *Tannerella forsythia*, *Prevotella intermedia*, *Fusobacterium nucleatum* and *Treponema denticola* continue to expand (Pham *et al*. 2010; Lu, Xuan and Wang 2014). Furthermore, biofilm-forming bacteria exhibit higher tendencies of SCFAs production, as evident in the putative periodontal pathogen *F. nucleatum*. Differential expression of six out of the seven enzymes involved in butyrate production was found in this bacterium when forming a biofilm compared with when growing in a planktonic state, indicating the importance of butyrate as a virulence factor for this bacterium during plaque buildup (Llama-Palacios *et al*. 2020). In addition to its detrimental effects on the integrity of the epithelial barrier, butyrate can also affect the mounting of a proper immune response against periodontitis-associated bacteria. Butyrate can reduce the adhesion protein intercellular adhesion molecule-1 (ICAM-1) expression, suppressing the recruitment and transmigration of immune cells (Magrin *et al*. 2020a). Furthermore, it was found to attenuate the effector functions of neutrophils, a significant host immune cell type within the oral cavity, by modifying neutrophil production of inflammatory mediators and suppressing their phagocytic ability (Correa *et al*. 2017). Altogether, these effects prevent the containment of pathogenic bacteria, favouring disease development.

The previously mentioned mechanism come together to support the development and progression of cancer. The acidogenic and aciduric species can support the invasion and metastasis of malignant cells by contributing to a tumour favouring an acidic environment via acid production (Karpinski 2019). Several *in vitro* studies have confirmed the importance of these acidic conditions in enhancing the metastatic ability of tumour cells (Schlappack, Zimmermann and Hill 1991; Jang and Hill 1997; Mazzio, Smith and Soliman 2010). Hooper and coworkers characterized the bacterial species isolated from oral squamous cell carcinoma and found that the vast majority of these microorganisms were saccharolytic and aciduric (Hooper *et al*. 2007). Furthermore, SCFAs interfere with mounting a proper immune response against tumour cells by attracting myeloid-derived suppressive cells, expanding this immunosuppressive population of cells within the tumour microenvironment (Kostic *et al*. 2013). As previously mentioned, butyrate, on the other hand, was found to suppress the cytokine-induced expression of ICAM-1 on oral squamous carcinoma cells (Magrin *et al*. 2020a). The importance of this adhesion molecule lies in its ability to induce the transmigration of leukocytes to the pathologically affected sites and suppress macrophage polarization towards the tumour promoting M2 phenotype. Hence, butyrate can alter the immune response against cancer cells and promote a tumour permissive environment (Fig. 2).

Perhaps another mechanism by which the increased production of SCFAs in oral pathologies can support tumour development is by reactivating latent viruses that have been linked to malignancies. For instance, butyric acid, propionic acid, isovaleric acid and isobutyric acid produced by periodontitis-associated bacteria have been found to reactivate Kaposi's sarcoma-associated herpesvirus (KSHV) (Gruffaz *et al*. 2020). This effect is mediated by the inhibition of histone deacetylase (HDAC) and the downregulation of several epigenetic modulators (EZH2, SUV39H1), which represses histone trimethylation (Yu *et al*. 2014). As a consequence, KSHV infected cells exposed to SCFAs exhibit histone hyperacetylation, transactivating the viral chromatin. Hence, higher tendencies of KSHV lytic reactivation can be expected in individuals with periodontal disease, with a higher potential of developing oral Kaposi's sarcoma. Similarly, butyrate also activates the Epstein-Barr virus, a well-known risk factor of developing lymphomas (Astakhova *et al*. 2016) (Fig. 2). Therefore, sustaining viral latency by controlling SCFAs production by oral bacteria could yield beneficial effects in controlling the development of virally induced malignancies.

Metabolites derived from amino acid metabolism

The oral cavity is continuously bathed with biofluids that are nutritionally high in nitrogenous compounds, including proteins, peptides and amino acids (Norimatsu *et al*. 2015). Proteomic analysis of salivary secretions alone yielded over a thousand proteins and peptides, while hundreds were detected in the gingival crevicular fluid (GCF) (Denny *et al*. 2008; Bostanci and Belibasakis 2018). Progress has been made towards understanding how oral proteolytic bacteria utilize these compounds to produce a diverse number of derivatives. The complexity of the biochemical pathways involved in breaking down these molecules renders them unsuitable substrates, limiting the bacterial utilization of numerous amino acids (Takahashi and Sato 2001). However, those substrates that can be processed generate a range of bioactive by-products, many of which possess significant toxicity.

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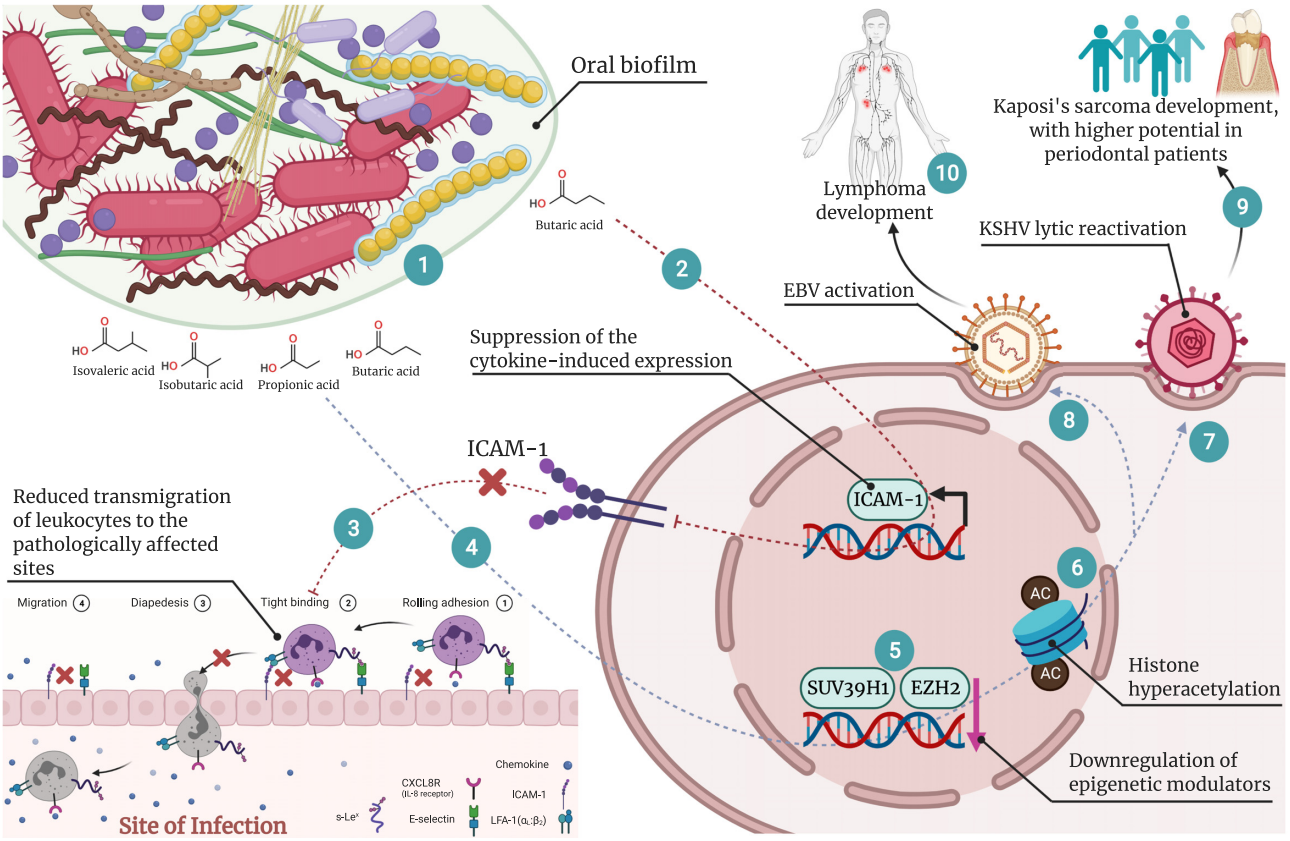


Figure 2. The role of SCFAs in disease. (1) In dysbiotic conditions, the oral biofilm populations shift in favour of acidogenic bacteria, which produce SCFAs as end products of carbohydrates metabolism. The resulting acidic and low pH conditions support the colonization of cariogenic bacteria and are linked to various aspects of the pathogenesis of the periodontal disease. (2) Butyric acid (butyrate) suppresses the cytokine-induced expression of intercellular adhesion molecule-1 (ICAM-1) on oral squamous carcinoma cells. (3) As a result of ICAM-1 suppression, transmigration of leukocytes to the pathologically affected sites is reduced, suppressing the immune response to cancer cells. (4) SCFAs produced by periodontitis-associated bacteria, including butyric acid, propionic acid, isovaleric acid and isobutyric acid, downregulate epigenetic modulators (EZH2, SUV39H1) (5) and inhibit histone deacetylase (HDAC), which results in histone hyperacetylation (6). This results in the activation of KSHV (7) and EBV (8). This virus activation can lead to the development of Kaposi's sarcoma (9) and lymphoma (10), respectively. This figure was created with biorender.com.

Proteases' initial protein degradation into peptides and amino acids permits their subsequent cellular uptake by oral bacteria (Feng *et al.* 2019). Specific bacterial proteases have been linked to pathologies both locally and systemically. For example, the cysteine proteases gingipains released by *P. gingivalis* play a role in *P. gingivalis*-supported atherosclerotic diseases. Once these disease-associated oral bacteria gain access into the circulation, they can release these proteases, raising the local levels of the bioactive anaphylatoxin complement fragment 5a (C5a) (Wingrove *et al.* 1992). Moreover, they modulate the lipoprotein levels in the blood by degrading the key plasma lipid regulator, apolipoprotein E (ApoE), thereby contributing to lipoprotein accumulation in the vasculature (Lonn *et al.* 2018).

Unlike carbohydrate metabolism, the utilization of amino acids is more prevalent in the subgingival bacterial niches, where the environmental factors support such processing (Takahashi 2005). With the progressive deepening of gingival crevices and the formation of pockets in periodontal pathologies, and restricted access to dietary sugars, the conditions become more suitable for proteolysis. In these deep crevices, compounds produced by amino acid-degrading bacteria, such as *Fusobacterium*, raise the pH to almost neutral or even alkaline levels (Chew *et al.* 2012). An alkaline environment permits the colonization of acid-sensitive species, including *P. gingivalis*, that also depends on their proteolytic activities as virulence factors to trigger inflammatory responses (Baek *et al.* 2017). In addition, the acidic, basic, aromatic and sulfur-containing amino acid metabolites may negatively affect the host, exerting pro-inflammatory and cytotoxic effects as discussed further below.

Acidic amino acids

Oral bacteria vary in the metabolic pathways that utilize different amino acids. For example, glutamic acid, the most abundant amino acid in the dental biofilm, is readily metabolized into the SCFA, butyric acid, by several oral bacteria (Singer and Kleinberg 1983). Meanwhile, species such as *P. gingivalis* and *P. intermedia* process aspartic acid to produce acetic and propionic acids (Takahashi 2003). Increased metabolism of these molecules can lead to the development of periodontal pathologies. In pregnancy, subgingival microbial metabolic signatures revealed that the metabolism of both of these amino acids was enhanced (Balan *et al.* 2020), contributing to the exaggerated periodontal responses observed in pregnancy, as the excess production of these cytotoxic end products can induce tissue inflammation.

Basic amino acids

Other amino acids such as arginine can either be supplied from dietary components or produced in the body from citrulline

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through *de novo* synthesis (Morris 2016). Arginolytic bacteria utilize this amino acid mainly by a series of reactions termed the arginine deiminase system (ADS), yielding ornithine and citrulline and releasing other products such as CO_2, ATP and ammonia (Liu, Nascimento and Burne 2012). The alkalinity produced by this pathway can neutralize the sugar metabolism derived acidification. Indeed, monitoring ADS activity in children who develop dental caries revealed that the formation of carious lesions is linked to lower arginine degrading capacity (Nascimento *et al*. 2019). Such event can be explained by the fact that ammonia production by ADS neutralizes glycolytic demineralizing acids and promotes the establishment of dental health maintaining microbiota. Hence, the use of ADS+ strains as probiotics and incorporation of arginine in the formulations of oral healthcare products could be a promising approach for the prevention and management of dental caries (Nascimento 2018). Such application was tested by evaluating the arginolytic and saccharolytic effects of an 8% arginine toothpaste (Koopman *et al*. 2017). As expected, the significant levels of arginine in this toothpaste boosted the arginolytic capacity in the saliva of its users, while a significant decline appeared in their salivary sucrose metabolism.

In oral cancer, enriching the oral milieu with oral alkali-producing species, such as *Streptococcus gordonii* and *Actinomyces* spp., with potent arginine metabolic activities can counteract the acidity and limit the expansion of acidogenic bacteria, thus blocking their tumour promoting activities (Chattopadhyay, Verma and Panda 2019). Such a mechanism has been suggested as part of a multimodal therapeutic approach to impede or disrupt the pathogenic oral biofilm. While the effects of arginine metabolism are mainly beneficial to the oral microbiome, this is not the case for respiratory tract pathogens. The oral bacteria-generated metabolite ornithine can undergo further decarboxylation to form polyamines, increasing virulence and supporting the survival of respiratory pathogens (Banerji *et al*. 2021). A recent study reported that oral amino acid metabolism products, including ornithine, may support the pathogenicity of the opportunistic respiratory pathogen *Pseudomonas aeruginosa* (Phan *et al*. 2018).

Aromatic amino acids

Putrefactive degradation of the aromatic amino acid tryptophan by anaerobic Gram-negative bacteria, including *Porphyromonas intermedia*, *F. nucleatum* and *P. gingivalis*, can produce a range of toxic indolic and phenolic compounds. The derivative indole is synthesized from tryptophan by the catabolic action of the enzyme tryptophanase. On the other hand, skatole is synthesized by a series of reactions in which indolepyruvate and indoleacetate are intermediates (Codipilly and Kleinberg, 2008). Both indole and skatole have substantial implications in disease development, and the production of these metabolites by bacteria has been linked to several conditions (Roager and Licht 2018). Furthermore, these compounds, especially skatole, are odoriferous, having a solid fecal matter-like smell (Mogilnicka, Bogucki and Ufnal 2020). Hence, they may contribute to oral malodor (also known as halitosis). Though not a severe health concern, many of the bacteria associated with halitosis are also associated with periodontal disease and oral cancers (Hampelska *et al*. 2020).

Aromatic amino acid metabolites can contribute to cancer. Their pro-cancerous activities are derived by activating the ligand-activated transcription factor, aryl hydrocarbon receptor (AHR) (Murray, Patterson and Perdew 2014). This transcription factor is activated under normal conditions by ligands derived from environmental or microbial sources via the tryptophan or arachidonic acid metabolic pathways. Uncontrolled AHR activation can have detrimental effects on host cells and has been reported to support tumourigenesis. In this respect, oral microbial samples collected from sites of OSCC and their adjacent mucosa displayed hyperactivity of tryptophanase (Yost *et al*. 2018). This can catalyse tryptophan degradation, giving rise to its derivates indole, pyruvate and ammonium, which can all be further metabolized into agonists of AHR. Indeed, AHR ligands derived from *P. gingivalis* have been found to regulate the AHR transcription factor in OSCC cells (Yost *et al*. 2018). It was shown that they expanded the ALDH expressing cell population and a notable increase in their migration ability. Similarly, cultivating the early oral colonizer *Streptococcus mitis* with oral epithelial cells can lead to the activation of AHR (Engen *et al*. 2017), this was confirmed based on a 3-fold increase in the CYP1A1 mRNA levels, considered as the hallmark gene for AHR activation.

It is also noteworthy that the signaling cascades associated with AHR can alter the development and function of local immune cells. AHR activation promotes T-cell differentiation into preferentially Foxp3+ T_{reg} and Th17 cells and act as an anti-inflammatory factor in macrophages (Gutierrez-Vazquez and Quintana 2018). Thus, AHR signaling can act as a double-edged sword, with potential pro-inflammatory activities and anti-inflammatory effects. In atopic dermatitis (AD), which is autoimmune in nature, alteration in the diversity of the oral microbiome is observed, specifically a reduction in the abundance of the genera known to induce pro-inflammatory immune responses. In concordance, enrichment of tryptophan metabolism has been recognized in these AD-modified bacterial communities (Li *et al*. 2019), which suggests that metabolites associated with oral microbiota dysbiosis can suppress inflammation in the skin to produce manifestations of AD.

Other than tryptophan, catabolism of phenylalanine via redox reactions can result in its conversion to the alkaline compound phenylacetate. By regulating the pH of the oral biofilm, phenylalanine metabolism can help limit the formation of dental caries (Shi *et al*. 2020). Conversely, periodontitis-associated bacteria require phenylalanine to grow and produce phenylacetate, which is robustly associated with the clinical signs of periodontal disease (Liebsch *et al*. 2019). Higher concentrations of these phenolic compounds have also been proposed to interfere with typical taste perception, yet in order to confirm, additional investigation is required (Gardner, So and Carpenter 2020).

Sulfur-containing amino acids

Following the pattern of aromatic amino acids, metabolites stemming from sulfur-containing amino acids produce a foul smell in the intraoral niche. Hydrogen sulfide (H_2S), a volatile sulfur compound, stands out as a prominent metabolic product of cysteine and methionine proteolysis (Hampelska *et al*. 2020). While anaerobic Gram-negative bacteria produce this halitosis-forming gas, Gram-positive microorganisms may also produce H_2S indirectly by cleaving the carbohydrate side-chains of salivary glycoproteins (Suzuki *et al*. 2019). The commensal bacteria responsible for the production of H_2S are usually maintained at low levels; however, the elevated oral microbial load associated with oral disease results in elevated overall levels (Dilek *et al*. 2020). The excess H_2S generated can amplify inflammation, contributing to the pathogenesis of the periodontal disease. One possible explanation of how this effect can be mediated is by the ability of H_2S to trigger the release of the inflammatory cytokines IL-1β and IL-18 from leukocytes, exacerbating the inflammatory response (Basic *et al*. 2019).

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The immunomodulatory functions of H_2S suggest that it may be linked to a plethora of oral diseases. It can manipulate the T-cell mediated immune response by regulating the expression of TET-family methylcytosine dioxygenases. These trigger the convergence of 5-methylcytosine to 5-hydroxymethylcytosine, resulting in a T_{reg} cell-specific hypomethylation pattern and stabilizing Foxp3 expression (Yang *et al.* 2015). This heightened T_{reg} inclined immune response may potentially affect the anticancer immune response, as this subset of cells is known to suppress antitumour immunity (Togashi, Shitara and Nishikawa 2019). H_2S was also found to activate the COX2, AKT and ERK1/2 pathways in oral cancer cells (Zhang *et al.* 2016), and the activation degree depended on the H_2S dose, prompting the proliferation of the oral cancer cells. In light of this, H_2S can be a potential therapeutic target in cancer to boost the antitumour immune responses and suppress the H_2S-mediated proliferative capacity on oral cancer cells.

Metabolites derived from lipid metabolism

A fat-rich diet induces alterations in the oral microbiota, preferentially favouring an increase in periodontitis-associated bacteria such as *F. nucleatum* and *P. intermedia* (Blasco-Baque *et al.* 2012). In turn, these microorganisms are involved in the production of lipases, contributing to primary lipid digestion (Brignot and Feron 2019). However, lipases from specific oral pathogens can have alternate functions in addition to lipid lysis. For example, the glycerol ester hydrolase (Geh) secreted by *S. aureus* is an important virulence factor of this microbe and has immunomodulatory functions in addition to its conventional enzymatic action. By inactivating the *S. aureus* lipoproteins, this pathogen can avert Toll-like receptor two mediated immune recognition, thereby allowing it to colonize and trigger oral infections (Chen and Alonzo 2019).

Once metabolized, some lipid metabolites can have significant pathogenic potential, as in the choline-derived *Streptococcus sanguinis* metabolite, trimethylamine (TMA), which is successively converted in the liver to form trimethylamine N-oxide (TMAO). As the only TMA-forming oral bacterium, *S. sanguinis* can augment the role of the gut microbiome in TMA accelerated atheroma formation (Chao and Zeisel 1990). Increased serum levels of TMAO can direct disruption of the reverse cholesterol transport process and increase the formation of foam cells (Wang *et al.* 2015; Warrier *et al.* 2015). In the meantime, microbiota-induced periodontal disease negatively affects serum lipid metabolic control, causing dyslipidaemia (Nepomuceno *et al.* 2017) mediated partially by the *P. gingivalis* virulence factor gingipains. As previously mentioned, gingipains degrades the key plasma lipid regulator, ApoE, responsible for promoting the clearance of triglyceride-rich lipoproteins and promoting cellular cholesterol efflux (Hara and Yokoyama 1991; Yan *et al.* 2005; Lonn *et al.* 2018). This gingipain-mediated fragmentation of ApoE results in conformational changes that can impede its receptor interaction, thereby contributing to lipoprotein accumulation in the vasculature. Jointly, these events can alter the lipid profiles of periodontitis patients.

Specific members of the oral microbiota have been found to possess certain features that allow them to translocate and colonize the foetal–placental unit, subsequently contributing to infections (Ikegami, Chung and Han 2009; Ye *et al.* 2020). Mapping the metabolic pathways of the placental microbial community revealed that subjects with chorioamnionitis, bacterial infections in the chorion and amnion membranes surrounding the fetus that suffered from preterm birth had a significant increase in glycerophospholipid metabolism (Prince *et al.* 2016). It is not surprising that this metabolic shift is similar to that observed in dysbiotic oral communities, owing to the resemblance between both the oral and placental bacterial communities (Barnes *et al.* 2011; Ye *et al.* 2020). The significance of these findings is based on the fact that metabolic products of glycerophospholipid metabolism, such as arachidonic acid, are well-known inducers of inflammation and promote the synthesis of the labour inductive lipid compounds, prostanoids (Menon *et al.* 2011). Although this metabolic alteration has not been attributed to specific bacterial strains, this finding suggests that the presence of multiple disease-associated bacteria in the placenta could reshape this environment and generate an environment more susceptible to premature birth.

SECONDARY AND SPECIALIZED METABOLITES

A healthy microbiome is in a symbiotic relationship with the human host, providing a defense mechanism against pathogen colonization. Oral chronic infectious diseases are caused by a localized dysbiosis of the oral microbiome. Small, specialized molecules produced by oral microbes, including antimicrobial peptides, are crucial for communication within the oral microbial world. Current advances in molecular biology have allowed the exploration of biosynthetic gene clusters (BGCs) responsible for producing many small secondary metabolites by the oral microbiome. Through sampling of oral microbes, almost 5000 BGCs were identified across genomes representing a vast array of taxonomic diversity (Aleti *et al.* 2019). The most frequent secondary metabolites produced by the oral microbiota are the ribosomally synthesized and posttranslationally modified peptides (RiPPs). Figure 3 depicts an overview of the generic RiPPs biosynthetic pathway.

In most cases, RiPPs production involves the ribosomal synthesis of the peptide precursor attached to its leader peptide. This precursor is then modified by enzymes such as cyclase, dehydratase, oxidoreductase and others to produce a polycyclic structure. The modified peptide is then separated from its leader by protease activity to be secreted outside the producing cells as a bioactive molecule. The RiPPs group includes small molecules such as bacteriocins, lantipeptides (or lantibiotics) and sactipeptides. Additionally, recent studies showed that some bacterial residents of the oral cavity could also produce hybrid polyketide nonribosomal peptides (PKS-NRPs).

Bacteriocins

Many oral microorganisms, including commensals and opportunistic pathogens, can produce a tandem array of antimicrobials belonging to different classes of metabolites. The most well-characterized examples of such molecules are bacteriocins produced by Gram-positive and Gram-negative bacteria (Jack, Tagg and Ray 1995; Rea *et al.* 2011). Bacteriocins are generally defined as ribosomally synthesized antimicrobial peptides or proteins produced by certain bacterial strains with the capacity to either eliminate or inhibit the growth of specific colonizing competitors (Heilbronner *et al.* 2021). There are distinct classes of bacteriocins produced in the oral cavity: class I, lantibiotics; class II, small (<10 kDa), relatively heat-stable, nonlantibiotic peptides; class III, large (>30 kDa), heat-labile proteins; and class IV, complex bacteriocins that contain essential lipid or carbohydrate moieties in addition to protein (Barbour, Wescombe and Smith 2020).

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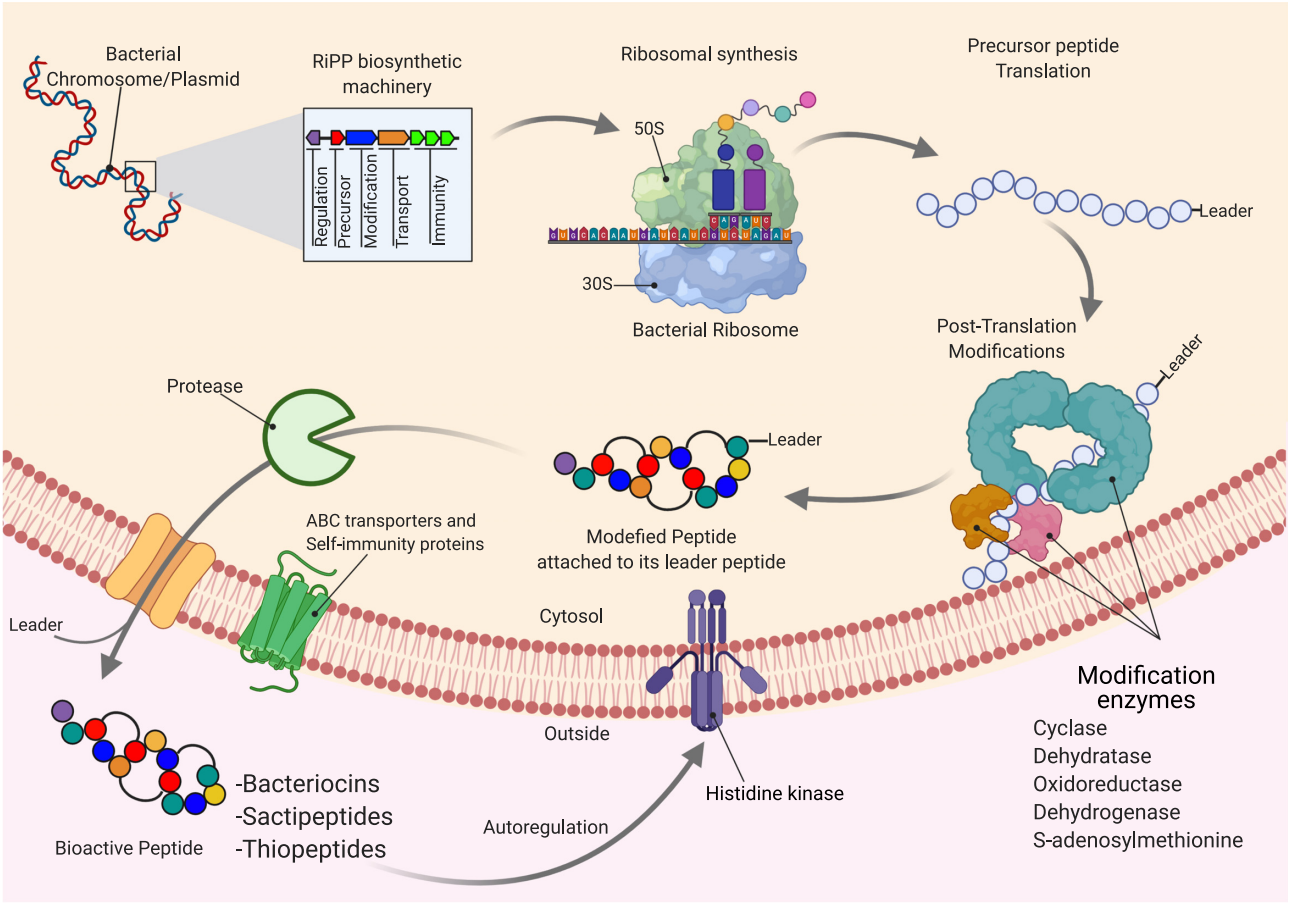


Figure 3. Overview of a generic RiPP operon showing the general path for biosynthesis. After being ribosomally synthesized, oral RiPPs are enzymatically modified, resulting in a unique polycyclic structure. However, this structure is not bioactive yet and requires protease cleavage of the leader peptide. The secreted modified peptides can sensitize the producer cell through a positive feedback loop by activating a transmembrane histidine kinase receptor. Many of the producing bacteria are also equipped with transporter proteins encoded in the RiPP biosynthetic loci to expel the active metabolites from the cell membrane, therefore preventing self-killing. This figure was created with biorender.com.

The perceived principal function of bacteriocins is to limit the growth of competitor bacteria in complex microbial communities. They are the product of an ongoing evolutionary cold war between competing bacterial species. Their abundance in different host microbiomes suggests their contribution to maintaining specific microbial communities' long-term stability (Garcia-Bayona and Comstock 2018). Many bacterial members of the human oral microbiota, including *Streptococcus* spp., *Lactobacillus* spp., *Fusobacterium* spp. and *Prevotella* spp., produce one or more bacteriocin-like inhibitory substances. The most well-characterized oral microbial group producing bacteriocin belongs to the genus *Streptococcus*. One of the very first attempts to isolate bacteriocin-like metabolites from oral streptococci was carried out on *Streptococcus pyogenes*. This species is associated with pharyngitis (DeWyer *et al.* 2020), pharyngotonsillitis (Ubukata *et al.* 2020) and other diseases and produces bacteriocin-like inhibitory substances (later referred to as BLIS) able to interfere with the growth of other oral streptococci (Tagg, Read and McGiven 1973).

Further research showed that this antimicrobial metabolite belongs to the lantibiotic class of bacteriocins that disrupt membrane potential as a mode of action against targeted cells (Jack *et al.* 1994a). Research on other oral streptococci led to the isolation and characterization of salivaricin A, the first lantibiotic bacteriocin produced by *Streptococcus salivarius*, a commensal microbe within the oral cavity (Ross, Ronson and Tagg 1993). The focus on *S. salivarius* increased as a potential candidate for oral probiotic development due to its bacteriocinogenic nature, biosafety profile and its close relation to *Streptococcus thermophilus* (Wescombe *et al.* 2012). Both *S. thermophilus* and *S. salivarius* harbour *sbcD* gene(s) and its related product(s) are considered to have a role in repairing recombinogenic double-stranded DNA breaks, thus dampening the efficiency of DNA recombination and stabilize the genome. This gene is missing in pathogenic streptococci genomes but present in the dairy streptococci (Hols *et al.* 2005). The common constituent of dental plaque, *S. mutans*, is one of the most prolific bacteriocin producers (Biswas 2020). This microorganism can resist bacteriocin-mediated killing by other commensals by producing mutacins, lantibiotic and nonlantibiotic bacteriocins of its own. Mutacins production is one of *S. mutans* strategies to effectively interfere with the growth of other microbial competitors and improve colonization and establishment in oral biofilms (Merritt and Qi 2012; Bowen *et al.* 2018). While significant characterization of bacteriocins produced by Gram-positive oral bacteria has already been done, especially for the streptococci isolates, minimal data is available regarding bacteriocins produced by Gram-negative oral bacteria Table 1. Understanding the complete antagonistic signaling of oral bacteriocins and other antimicrobial substances will provide

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Table 1. Examples of bacteriocins produced by oral bacteria.

Bacteriocin(s)	Class	Mw (Da)	Producer	References
By Gram-positive bacteria				
Salivaricins[a]	I, III	2315–3566	*Streptococcus salivarius*	(Barbour, Wescombe and Smith 2020)
Mutacins	I, II	2263–5195	*Streptococcus mutans*	(Nicolas and Lavoie 2007)
Streptococcin	I	2794	*Streptococcus pyogenes*	(Jack *et al.* 1994b)
Streptins	I	2424–2821	*Streptococcus pyogenes*	(Wescombe and Tagg 2003)
BHT	I, II	2802–5195	*Streptococcus rattus*	(Hyink, Balakrishnan and Tagg 2005)
Pneumocins	II	NA	*Streptococcus pneumoniae*	(Wholey *et al.* 2019)
Pneumolancidins	I	NA	*Streptococcus pneumoniae*	(Maricic *et al.* 2016)
Streptocin STH	II	NA	*Streptococcus gordonii*	(Heng, Tagg and Tompkins 2007)
Sanguinicin K11	II	5070	*Streptococcus sanguinis*	(Wescombe *et al.* 2009)
Stellalysin	III	27 000	*Streptococcus constellatus*	(Heng *et al.* 2006b)
Dysgalacticin	III	21 493	*Streptococcus dysgalactiae*	(Heng *et al.* 2006a)
Paracasin SD1	III	24 028	*Lactobacillus paracasei*	(Wannun, Piwat and Teanpaisan 2014)
Fermencin SD11	III	33 593	*Lactobacillus fermentum*	(Wannun, Piwat and Teanpaisan 2016)
Salivaricin LS1	II	10 000	*Lactobacillus salivarius*	(Busarcevic *et al.* 2008)
Salivaricin LS2	II	4115	*Lactobacillus salivarius*	(Busarcevic and Dalgalarrondo 2012)
Acnecin	III	12 000	*Cutibacterium acnes*	(Fujimura and Nakamura 1978)
Bacteriocin-like	Unknown	NA	*Corynebacterium matruchotii*	(Nakamura *et al.* 1984)
By Gram-negative bacteria				
P12.2 bacteriocin	Unknown	27 296	*Fusobacterium nucleatum*	(Ribeiro-Ribas *et al.* 2009)
Melaninocin	III	105 000	*B. melaninogenicus*	(Nakamura *et al.* 1981)
Corrodecin	III	23 625	*E. corrodens*	(Apolonio *et al.* 2008)
Bacteriocin-like	Unknown	NA	*A. actinomycetemcomitans*	(Lima *et al.* 2002)
Nigrescin	Unknown	41 900	*Prevotella nigrescens*	(Teanpaisan, Narawatthana and Utarabhand 2009)

[a]Most salivaricins belong to class I bacteriocins (lantibiotics); however, salivaricin MPS produced by some *S. salivarius* strains belongs to class III. Mw, molecular weight; NA, data is not available.

vital information to design and develop therapeutic solutions to fight against oral diseases. For example, the lantibiotic mutacin 1140 is a peptide antibiotic produced by *S. mutans* with potent broad-spectrum inhibitory activity towards many significant bacterial pathogens (Pokhrel *et al.* 2019).

Sactipeptides

Sactipeptides are RiPPs containing a sactionine thioether bridge installed posttranslationally and required for their antimicrobial activity (Grove *et al.* 2017). Although they have yet to be isolated from oral microorganisms, sactipeptides such as streptosactin produced by the dairy bacterium *S. thermophilus* were found to be encoded in the genomes of members of the oral microbiome such as *S. constellatus*, *S. gordonii*, *S. oralis* and *S. parasanguinis* (Bushin *et al.* 2020).

Signaling and potential anticancer peptides

The primary mode of action of antimicrobial peptides is integrating into the targeted cell membrane and inducing membrane permeability (Lazzaro, Zasloff and Rolff 2020). This cell-killing activity suggested that these peptides could be used in cancer treatment (Felício *et al.* 2017). The oral and upper-respiratory-tract microorganism *Streptococcus pneumoniae* was found previously to produce a secreted peptide Pep27 that was found to facilitate cell death in *S. pneumoniae*. Researchers have found that this peptide also possesses anticancer properties. Lee and coworkers reported that Pep27 could penetrate the plasma membrane and then induce apoptosis in both caspase and cytochrome c-independent manners.

Interestingly, an analogue created from the authentic peptide Pep27anal2 was even more potent since it has a slightly longer stable helical conformation than Pep27 (Lee *et al.* 2005). In addition, Pep27anal2 inhibited proliferation of leukemia (AML-2, HL-60, Jurkat), gastric (SNU-601) and breast (MCF-7) cancer cell lines. Thus, this example encourages and suggests that other similar metabolites of the oral microbiome could be identified and developed for anticancer therapeutics.

Polyketide nonribosomal metabolites (PKS-NRPS)

Polyketides and nonribosomal metabolites are secondary metabolites synthesized by polyketide synthase (PKS) and large nonribosomal peptide synthetase enzyme complexes (NRPS). *Proteobacteria*, *Actinobacteria*, *Firmicutes* and *Cyanobacteria* are the bacterial phyla containing a higher number of the gene loci responsible for producing a wide variety of nonribosomal peptides and polyketide metabolites (Wang *et al.* 2014). A recent study showed that a cariogenic strain of *S. mutans* produces a novel metabolite that belongs to the (PKS/NRPS) family called reutericyclin and a novel unacylated tetramic acid (mutanocyclin). Furthermore, it was shown that *S. mutans* use reutericyclin to interfere with the growth of neighbouring oral commensals to persist in the oral biofilm and cause dysbiosis (Tang *et al.* 2020). In a separate study, *S. mutans* strain UA159 was shown to produce mutanobactin A, a hybrid PKS-NRPS metabolite that regulates a range of stress tolerance factors and influences the yeast-mycelium transition of *Candida albicans* (Joyner *et al.* 2010).

GENOME MINING AS A POWERFUL TOOL FOR THE DISCOVERY OF NOVEL METABOLITES PRODUCED BY COMMENSAL ORAL MICROBIOTA

Genome mining is a relatively new bioinformatics tool that involves the characterization and identification of novel natural products and their biosynthetic genetic loci harboured on genomes of sequenced microorganisms. As NGS and genomics

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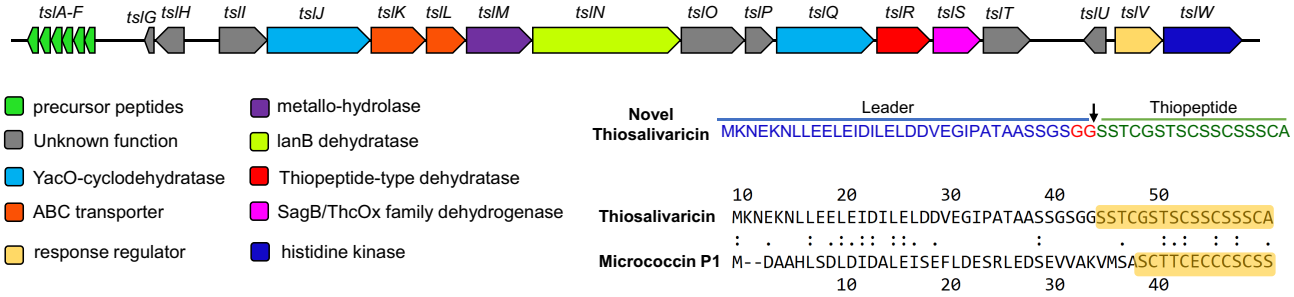


Figure 4. Discovery of novel thiosalivaricin biosynthetic gene cluster through *in silico* genome mining and predicted precursor peptide. The thiosalivaricin locus harbours ~23 genes involved in the ribosomal synthesis of the precursor peptides, posttranslational modifications (such as cyclodehydratase, hydrolase, lanthionine dehydratase, thiopeptide dehydratase and dehydrogenase), transportation, regulation and other accessory elements that are believed to play a role in self-immunity. Consistent with its novelty, thiosalivaricin from oral streptococci was found to have a unique sequence compared with the micrococcin P1 peptide.

are advancing, more detailed information can be acquired through genome mining, including predicting the putative functions of enzymes and proteins involved in the biosynthesis of microbial-derived natural metabolites (Ziemert, Alanjary and Weber 2016). Thousands of microbial genomes and DNA databases are available publicly, with further info on the putative functions and biosynthetic pathways of encoded metabolites. The classical approach for genome mining of novel metabolites is the mining for genes encoding the putative enzymes involved in the biosynthesis and maturation of the secondary metabolites. The advantage of this principle is that the biosynthetic machinery for many of the microbial metabolites is usually conserved. Hence, it is much easier than an unsupervised search of the highly diverse core genes encoding the metabolites precursors. Using the genome mining approach (via *AntiSMASH ver* 5.1.2), on selected oral microbial genomes that are known to secrete antimicrobial secondary metabolites, we found a novel biosynthetic cluster encoding for the production of novel streptococcal thiopeptides, which we have termed thiosalivaricin (Fig. 4). The biosynthesis locus was detected in genomes of the streptococcal species *S. salivarius*, *S. downei*, *S. sobrinus* and *S. equinus* with GenBank accession numbers QEM31461, WP_002998585, WP_019782216 and WP_039697794, respectively. Generally, thiopeptides are isolated from soil and marine microorganisms. Therefore, identifying them as potential products of oral commensals is surprising. The newly discovered thiosalivaricin has a leader sequence that is likely cleaved after a 'GG' motif to produce a putative thiopeptide sequence S-S-T-C-G-S-T-S-C-S-S-C-S-S-S-C-A. Blast analysis was performed against thiopeptide databases (ThioFinder) with no significant matching. The antimicrobial peptide database (APD) showed very low homology to the micrococcin P1 thiopeptide (Bennallack *et al.* 2014), suggesting that thiosalivaricin is novel.

Thiopeptides are a class of posttranslationally modified antibiotics with complex structures, including thiazoles, dehydroamino acids and a substituted nitrogen heterocycle chain (Vinogradov and Suga 2020). They are known for their inhibitory activity against antibiotic resistant bacteria (Chellat, Raguz and Riedl 2016; Vinogradov and Suga 2020). In addition, the biological activity of certain thiopeptides was shown previously to extend to anticancer and anti-inflammatory properties (Zheng *et al.* 2015).

The status of research on streptococcal thiopeptides derived from the oral cavity is still at the very early stages. However, selective genomes available through databases as mentioned above suggest that streptococcal thiopeptides might be successfully produced as bioactive metabolites. The more complete genome sequences of oral bacteria becoming available, the better estimation can be drawn regarding the distribution and abundance of thiopeptides loci within the oral cavity. Moreover, advanced genome mining enables the prediction of the molecular weight and structure of thiopeptides, making hunting for these molecules easier through mass spectrometry and other proteomics approaches. The discovery of thiopeptide biosynthetic loci in streptococcal microbes derived from the oral microbiome sheds light on the importance of genome mining and bioinformatics to further research on the oral microbes secreted metabolites.

CONCLUDING REMARKS

The balance of oral commensal microbes and disease-associated microbes, and how this contributes to oral health and disease and influences the host oral immune response, are central to understanding and treating common human oral health problems. Secreted bacterial metabolites can directly impact oral health status and impact the immune interface's nature with the oral microbial ecosystem. Thus, molecules identified from commensal microbes or produced as by-products of microbial metabolism that function to restrict the flourishing of other potentially harmful strains have tremendous potential value as therapeutic agents for maintaining a healthy oral cavity.

Further work is imperative to determine the effects of microbial-derived factors on the immune response and immune cell function. A number of recently identified microbial factors likely have pleiotropic effects, including immunomodulatory effects that have not been well characterized. In addition to antibiotic function (Zipperer *et al.* 2016), microbial metabolites can promote neutrophil and monocyte recruitment through a TLR-dependent mechanism (Bitschar *et al.* 2019). Similarly, the microbially produced metabolite, H_2S, stimulates oral leukocytes to promote further leukocyte recruitment (Basic *et al.* 2019). Alternatively, microbial-derived molecules, including butyrate, can potentially be immunomodulatory agents to limit hyperinflammatory responses in oral diseases (Magrin *et al.* 2020b). Neutrophils are the primary immune cell in the oral cavity, which are constitutively recruited in health and disease (Fine *et al.* 2016, 2021) and can respond to oral microbial species in a strain-specific manner (Oveisi *et al.* 2019). Furthermore, oral inflammation can impact neutrophil mobilization and priming/activation systemically (Fine *et al.* 2020). Significant research is required to determine how microbial metabolic factors influence the activation state of neutrophils in circulation and once they have been recruited into the oral cavity.

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Oral microbial metabolites, including RiPPs, have an excellent potential for future development in direct therapeutic applications. Understanding their role as mediators within microbial ecosystems and exploring the precise mechanisms of these metabolites in DNA competence, quorum sensing, biofilm formation and intra- and interspecies signaling within the oral cavity will open the doors for new oral health applications. For instance, the DNA competence regulatory network in *S. pneumoniae* was linked to the interbacterial competition system through the production of bacteriocins (Veening and Blokesch 2017). These pneumococcal bacteriocins contribute to neighbour predation and pathogen colonization, and targeting the expression of these molecules by creating new genetic or enzymatic tools is a feasible option to treat oral and upper respiratory tract infectious diseases. The commensal biofilm is considered an essential aspect of protection against invading disease-associated species, since they fill a niche that might otherwise be colonized. The metabolites produced by oral commensals that function to help limit pathogen invasion and colonization would be considered 'beneficial' to the host in this sense, and they can be developed into metabolite-producing microbes. However, metabolites produced by disease-associated bacteria are also of interest to either be developed as stand-alone therapeutics or targeted when overexpressed by opportunistic pathogens colonizing the oral cavity. Both of these avenues may lead to novel treatments to combat opportunistic infections in this era of emerging resistance to conventional antimicrobials.

AUTHOR CONTRIBUTIONS

AB designed the manuscript and the figures. AB and OE reviewed the literature and wrote the manuscript. MO helped with the literature review. NF and MG edited the manuscript and helped with writing. All authors proofread the manuscript.

Competing interests. No competing interests.

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