Saliva as the Sole Nutritional Source in the Development of Multispecies Communities in Dental Plaque

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ABSTRACT Dental plaque is a polymicrobial biofilm that forms on the surfaces of teeth and, if inadequately controlled, can lead to dental caries or periodontitis. Nutrient availability is the fundamental limiting factor for the formation of dental plaque, and for its ability to generate acid and erode dental enamel. Nutrient availability is also critical for bacteria to grow in subgingival biofilms and to initiate periodontitis. Over the early stages of dental plaque formation, micro-organisms acquire nutrients by breaking down complex salivary substrates such as mucins and other glycoproteins. Once dental plaque matures, dietary carbohydrates become more important for supragingival dental plaque, and gingival crevicular fluid forms the major nutrient source for subgingival microorganisms. Many species of oral bacteria do not grow in laboratory monocultures when saliva is the sole nutrient source, and it is now clear that intermicrobial interactions are critical for the development of dental plaque. This chapter aims to provide an overview of the key metabolic requirements of some well-characterized oral bacteria, and the nutrient webs that promote the growth of multispecies communities and underpin the pathogenicity of dental plaque for both dental caries and periodontitis.

INTRODUCTION
Despite major improvements in oral hygiene over the last few decades, dental caries and periodontitis remain two of the most common diseases in industrialized countries. For example, in the most recent National Health and Nutrition Examination Survey conducted between 2009–2010 in the U.S., almost one in four children between 3–9 years living in poverty had untreated dental caries (1). In the U.K., almost 50% of children have obvious decay experience in permanent teeth by the age of 15 years (2). Periodontitis, an inflammatory condition that involves the loss of the supporting structures of teeth, is more common in older age groups. In the U.S., over one-third of adults ≥30 years have moderate to severe periodontitis (3). Both dental caries and periodontitis are caused by the growth of microbial biofilms on tooth surfaces, known as dental plaque.

The human mouth is an open environment that is exposed to microorganisms in the air and in foods and drinks that are consumed. Some of the bacteria that enter the mouth will remain only transiently as they are not adapted to thrive in this environment. It is currently estimated that around 1,000 species of bacteria are able to exist stably in the human mouth, and that individuals typically maintain between 50–200 species from this wider pool (4, 5). Although there are relatively large inter-individual variations in the oral microbiota, approximately 15 genera are almost universally found in supragingival dental plaque (above the gumline), including Actinomyces, Campylobacter, Capnocytophaga, Corynebacterium, Fusobacterium, Granulicatella, Neisseria,
Prevotella, Streptococcus, and Veillonella (5–7). Bacteria growing below the gumline in subgingival dental plaque are cut off from access to saliva and dietary nutrients and instead obtain nutrients from gingival crevicular fluid, a serum exudate. As a consequence, the subgingival microbiota tends to become enriched in anaerobic, proteolytic bacteria such as Filifactor, Fusobacterium, Parvimonas, Porphyromonas, Prevotella, Tannerella, and Treponema (8, 9).

Many of the proteolytic bacteria in subgingival dental plaque produce virulence factors that promote disease in animal models, and it is generally accepted that these organisms are involved in periodontitis in humans (10). Both dental caries and periodontitis are probably best explained by the ecological plaque hypothesis that was developed in the 1990s by Marsh and coworkers (11). This concept states that supragingival dental plaque develops naturally on tooth surfaces and reaches a state of microbial equilibrium, where many different microbial species coexist without causing harm to the host. Disease occurs when the equilibrium is perturbed by changes in the host, the microbiota, or the environment. In the case of dental caries, frequent intake of sugars imposes a selective pressure that promotes the growth of highly acidogenic species such as Streptococcus mutans or Lactobacillus species. Risk factors for periodontitis include smoking or the accumulation of plaque or calculus at the gum margins, which irritate the gingivae and lead to the formation of periodontal pockets.

According to the ecological plaque hypothesis, low levels of supragingival dental plaque are not harmful. Indeed, oral healthcare relies largely on preventing dental plaque overgrowth through regular brushing and flossing. These procedures result in repetitive cycles of colonization and growth of microorganisms on the tooth, followed by removal of the maturing dental plaque. Evidence from animal studies indicates that dietary influences, such as fasting or ingesting high levels of simple sugars, have little effect on the initial accumulation of dental plaque (12). Instead, nascent dental plaque relies almost exclusively on salivary constituents for nutrition. This review will provide an overview of how bacterial consortia develop to obtain the maximum nutritional benefit from the catabolism of salivary substrates, and will explore the nutritional interactions between oral bacteria that contribute to dental plaque growth.

THE COMPOSITION OF SALIVA
Saliva is a complex biological fluid that originates from the major and minor salivary glands. Whole saliva also contains components from non-salivary origins such as gingival crevicular fluid, exfoliated epithelial cells, oral bacteria, and oral wounds. The relative contribution of each source varies between individuals and in an individual at different times. Stimulation of saliva production, for example by smell, taste, or mastication, increases flow from the parotid gland. Saliva flow is also affected by many other factors including age, hydration, physical exercise, drugs, circadian rhythms, nutrition, and systemic diseases (13). The contribution from gingival crevicular fluid is influenced by the presence and extent of periodontal disease (14). Therefore, the composition of saliva and, hence, the nutrients available to oral bacteria will vary greatly in different people and at different times. Nevertheless, there are many consistent characteristics of saliva, and a number of bacterial enzymes and scavenging systems have been identified that are critical for catabolism and uptake of salivary substrates.

On average, saliva is approximately 99% water, with the balance made up by organic and inorganic ions, peptides, proteins, and glycoproteins (Table 1). The major cations in saliva are sodium and potassium, whilst ammonium, calcium, and magnesium ions are present at lower concentrations (15). Chloride and phosphate are the most abundant anions, and nitrate, nitrite, sulfate, and thiosulfate tend to be present at sub-millimolar levels. In addition, organic anions including lactate, acetate, propionate, and formate have been detected in whole saliva, possibly as a consequence of the metabolism of dental-plaque bacteria (16). Trace metals are essential for microbial growth and are often a limiting factor within body fluids. The total concentration of trace metals can be measured directly by inductively coupled-plasma mass spectroscopy ICP-MS and it has been shown that the secretion rates of different trace metals vary dramatically day-to-day (17). Importantly, most trace metals are tightly bound to host proteins, and are not readily available to microorganisms.

The protein and glycoprotein content of human saliva has been studied in great detail. The total protein content of whole saliva is approximately 3,000 mg L⁻¹, whereas the concentration of stimulated saliva varies between approximately 500–1,000 mg L⁻¹. Free amino acids are present at low concentrations, and examples are given in Table 1. At least 400 different proteins have been detected in whole human saliva (18). Over 95% of salivary polypeptides belong to the major salivary protein families including acidic and basic proline-rich proteins, amylases, mucins MUC5B (MG1) and MUC7 (MG2), salivary agglutinin gp340, cystatin, histatins,
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TABLE 1 Concentrations of major components of saliva

<table>
<thead>
<tr>
<th>Component</th>
<th>Approximate concentration (mg L⁻¹)</th>
<th>Comments</th>
<th>Reference(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total protein</td>
<td>500–3,000</td>
<td>Higher concentrations in whole saliva than parotid; concentration decreases with higher stimulated flow rates</td>
<td>(88–91)</td>
</tr>
<tr>
<td>Urea</td>
<td>120–200</td>
<td></td>
<td>(89)</td>
</tr>
<tr>
<td>Glucose</td>
<td>5–14</td>
<td>Approximately 2-fold lower in stimulated vs resting saliva</td>
<td>(20, 88)</td>
</tr>
<tr>
<td>Cations</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sodium</td>
<td>0–800</td>
<td>Approximately 4-fold higher in stimulated saliva</td>
<td>(15, 89)</td>
</tr>
<tr>
<td>Potassium</td>
<td>430–1,000</td>
<td></td>
<td>(15, 89)</td>
</tr>
<tr>
<td>Ammonium</td>
<td>18–72</td>
<td></td>
<td>(15)</td>
</tr>
<tr>
<td>Calcium</td>
<td>20–110</td>
<td></td>
<td>(15, 89)</td>
</tr>
<tr>
<td>Magnesium</td>
<td>0.17–7.2</td>
<td></td>
<td>(15, 17, 92)</td>
</tr>
<tr>
<td>Anions</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chloride</td>
<td>280–690</td>
<td>Unstimulated saliva</td>
<td>(16)</td>
</tr>
<tr>
<td>Phosphate</td>
<td>140–330</td>
<td>Unstimulated saliva</td>
<td>(16)</td>
</tr>
<tr>
<td>Bicarbonate</td>
<td>0–1,220</td>
<td>Concentration increases with saliva flow rate</td>
<td>(88, 90)</td>
</tr>
<tr>
<td>Nitrate</td>
<td>4.3–100</td>
<td>Unstimulated saliva</td>
<td>(16)</td>
</tr>
<tr>
<td>Nitrite</td>
<td>0–18</td>
<td>Unstimulated saliva</td>
<td>(16)</td>
</tr>
<tr>
<td>Sulfate</td>
<td>6.7–21</td>
<td>Unstimulated saliva</td>
<td>(16)</td>
</tr>
<tr>
<td>Thiocyanate</td>
<td>7.6–34</td>
<td>Unstimulated saliva</td>
<td>(16)</td>
</tr>
<tr>
<td>Fluoride</td>
<td>0.01–0.048</td>
<td>Increases transiently following fluoride mouthrinse/dentifrice</td>
<td>(88, 91)</td>
</tr>
<tr>
<td>Lactate</td>
<td>0–5.4</td>
<td>Unstimulated saliva</td>
<td>(16)</td>
</tr>
<tr>
<td>Acetate</td>
<td>1.8–48</td>
<td>Unstimulated saliva</td>
<td>(16)</td>
</tr>
<tr>
<td>Propionate</td>
<td>0–10</td>
<td>Unstimulated saliva</td>
<td>(16)</td>
</tr>
<tr>
<td>Formate</td>
<td>0–1.4</td>
<td>Unstimulated saliva</td>
<td>(16)</td>
</tr>
<tr>
<td>Trace metals</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Aluminium</td>
<td>0.0014</td>
<td></td>
<td>(17)</td>
</tr>
<tr>
<td>Copper</td>
<td>0.0015–0.034</td>
<td></td>
<td>(17, 92)</td>
</tr>
<tr>
<td>Iron</td>
<td>0.12</td>
<td></td>
<td>(92)</td>
</tr>
<tr>
<td>Manganese</td>
<td>0.0029–0.0045</td>
<td></td>
<td>(17, 92)</td>
</tr>
<tr>
<td>Rubidium</td>
<td>0.064</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Strontium</td>
<td>0.0022</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Zinc</td>
<td>0.014–0.08</td>
<td></td>
<td>(17, 92)</td>
</tr>
</tbody>
</table>

*Only amino acids that were detected in both of the referenced studies are shown.

and statherin (19). By weight, the majority of poly-peptides in saliva are glycosylated, by N-linked or O-linked glycosylation. To release carbohydrate moieties from these glycoproteins, bacteria must produce a wide array of glycosidases that together can cleave many different glycosidic linkages. Free sugar levels in saliva are low, typically <100 μM glucose in non-diabetics for example (20), and therefore glycoproteins are an important source of energy for bacteria in the oral cavity.

The Acquired Enamel Pellicle

Salivary proteins and glycoproteins adsorb to the tooth surface and form a conditioning layer, termed the acquired enamel pellicle (AEP), which acts as a substrate for bacterial attachment. Some constituents of saliva adsorb to enamel more readily than others and therefore the AEP is enriched in certain components compared with whole saliva. The AEP is formed when teeth erupt during childhood and is never fully removed even by professional dental hygiene. However, the AEP is continually remodelled and replenished by salivary polypeptides (21). During the formation of AEP, proteins with affinity for calcium and phosphate, such as histatins and statherin, tend to accumulate early (22). Subsequently, histatins, but not statherin, decrease in abundance, probably due to proteolysis. Proteins that
interact with other proteins accumulate in the AEP over time, including mucin MUC5B, amylase, lysozyme, and lactoperoxidase (22). Some of these enzymes remain stable within the AEP and potentially may influence bacterial colonization (21). For example, amylase in the AEP may help to degrade starch into shorter maltosaccharides that can be utilized by dental-plaque bacteria.

**NUTRIENT REQUIREMENTS OF ORAL BACTERIA**

Broadly, oral bacteria can be divided into saccharolytic strains, which include many of the early colonizers and cariogenic bacteria, such as *Streptococcus* spp., *Neisseria* spp., *Lactobacillus* spp., *Scardovia* spp., *Actinomyces* spp., and asaccharolytic, strictly anaerobic proteolytic bacteria commonly associated with subgingival dental plaque, such as *Porphyromonas* spp., *Prevotella* spp., and *Parvimonas* species. It is important to note, however, that our understanding of oral bacterial metabolism comes exclusively from analysis of the bacteria that have been cultured in the laboratory, and approximately 50% of oral bacteria have not been cultured to date (4). Most oral bacteria have relatively complex nutritional requirements, and the development of chemically defined media capable of supporting growth has been instrumental in defining their nutrient requirements. There are now chemically defined media available that support the growth of at least some strains of several different oral bacterial genera including *Streptococcus*, *Actinomyces*, *Fusobacterium*, *Treponema*, and *Porphyromonas* (23–26). Some of the key nutrients required for the growth of bacteria in isolation are discussed below. It is likely that many of the key insights into oral bacterial metabolism in the future will begin from *in silico* predictions of metabolic pathways and potentials.

**Carbohydrates**

The production of organic acids from simple carbohydrates by microorganisms is central to the dental caries process. *Streptococcus mutans* and *Lactobacillus* spp. are able to metabolize a wide range of carbohydrates and to survive and grow at the low pH levels generated by carbohydrate metabolism. These bacteria are frequently elevated in carious lesions (27). Sucrose is thought to be particularly important as it is converted to high molecular weight exopolysaccharides by *Streptococcus* spp. and other oral microorganisms, and these polysaccharides provide protection and attachment sites for dental-plaque bacteria (28). However, the concentrations of simple sugars such as glucose are very low in saliva, and it is only once acidogenic bacteria are established within dental plaque that they are able to metabolize sufficient quantities of dietary carbohydrates to drive the progression of dental caries. The integration of highly acidogenic bacteria into dental plaque may be facilitated by the production of low levels of organic acids by moderately acidogenic early colonizers such as *Streptococcus* spp. (for example, *S. mitis*, *S. oralis*, *S. sanguinis*, and *S. gordonii*) and *Actinomyces* spp. (29). *In vitro*, these early colonizers can metabolize a relatively broad range of simple sugars (30, 31). However, simple sugars are present only at low concentrations in saliva and during the development of dental plaque it is likely that salivary glycoproteins are the most important source of carbohydrates.

The degradation of salivary glycoproteins requires a battery of enzyme activities to release the carbohydrate moieties and cleave the peptide backbone. The complete degradation of glycoproteins requires a consortium of microorganisms that each contribute different enzyme activities (32). Glycosidases produced by oral *Streptococcus* spp. and *Actinomyces* spp. include N-acetyl-β-D-glucosaminidase, β-D-galactosidase, α-L-fucosidase, α- and β-mannosidase, and sialidase (neuraminidase) (32–35). Gram-negative periodontal pathogens, such as *Porphyromonas gingivalis* and *Tannerella forsythia*, also produce a range of glycosidases including α- and β-mannosidases, sialidases, and β-hexosaminidases (36, 37). Together these enzymes can release oligosaccharides from complex host substrates such as MUC5B or IgA1 (32, 38).

The catabolism of sugars results in organic acids that, in turn, may be utilized as substrates by certain oral bacteria. For example, many strains of *Actinomyces* spp. can grow on lactate as the sole nutrient source (39). The periodontal species *Aggregatibacter actinomycetemcomitans* grows preferentially on lactate even when glucose and fructose are available (40), and lactate is a key nutrient for oral *Veillonella* spp., *Neisseria* spp., and *Haemophilus* spp. (41, 42). Some of the potential metabolic exchanges between oral bacteria are discussed in more detail below.

**Proteins and Amino Acids**

Subgingival dental plaque is isolated from the salivary environment of the oral cavity, and instead is fed by the serum exudate gingival crevicular fluid (GCF). In comparison to saliva, GCF is a relatively rich source of proteins; the total protein concentration in GCF is approximately 20,000–130,000 mg L⁻¹ (43). Proteins provide a critical source of nutrients for subgingival dental plaque bacteria. The bacterial species most
strongly associated with periodontitis, *P. gingivalis*, *Treponema denticola*, and *T. forsythia*, each produce extracellular proteases that degrade the synthetic trypsin analogue N-benzoyl-DL-arginine-2-naphthylamide (BANA), and BANA has been employed (with limited success) as a test for the presence of these organisms (44). Free amino acids may also be an important source of nutrients for oral bacteria. For example, *F. nucleatum* has been shown to ferment glutamate, lysine, and histidine (45), glycine is catabolized by *T. denticola* (46), and proline is metabolized by *Neisseria* spp. (42). In supragingival dental plaque, the catabolism of arginine by oral streptococci releases ammonia, which elevates the pH in the local environment and may help to protect against dental caries (47).

In addition to energy production, amino acids are essential for bacterial growth processes. Most oral bacteria are auxotrophic for multiple amino acids and chemically defined growth media typically contain all 20 natural amino acids. The development of a chemically defined medium for oral streptococci in the 1970s provided a resource for determining amino acid requirements through ‘leave-one-out’ experiments (48, 49). These studies identified a number of auxotrophies that were shared between species, as well as some that were specific to individual strains of a species. For example, cysteine was universally required by streptococci, and arginine was needed by all *S. sanguinis* strains, whereas glutamate, lysine, and histidine were required by only some Mutans Streptococcus strains (48, 49). Interestingly, certain amino acids were only required by Mutans Streptococci in aerobic conditions, and particularly in the absence of sodium carbonate. More recently, it has been shown that *S. gordonii* requires arginine for aerobic growth but not in the absence of oxygen (50). In *S. sanguinis* SK36, a comprehensive knockout mutagenesis has confirmed the activity of enzymes encoded by genes in each of the amino acid biosynthetic pathways that are present in this strain (51).

**Nucleic Acids**

Extracellular nucleic acids, and extracellular DNA (eDNA) in particular, are a major source of phosphorous for microorganisms in some environments, such as deep-sea sediments (52). Evidence is accumulating that oral bacteria release DNA into the surrounding biofilm environment (53). In periodontal pockets, eDNA accumulates from the release of neutrophil extracellular traps (54). At the same time, many oral bacteria produce extracellular deoxyribonuclease (DNase) enzymes. It is possible that eDNA provides a nutrient store for oral bacteria and that the production of DNase enzymes allows the mobilization of this store. However, at present there is little evidence regarding the nutritional importance of eDNA for oral bacteria.

**Vitamins and Cofactors**

Requirements for vitamins among different streptococci are relatively uniform, and pantothenate and nicotinic acid were essential or growth stimulatory for all 18 strains tested in one study (55). Culture media for anaerobic oral bacteria typically include menadione (vitamin K) and hemin to facilitate the growth of strict anaerobes such as *P. gingivalis*. Hemin is critical for the growth of black-pigmented strains (*Porphyromonas* spp. and *Prevotella* spp.); however, requirements for menadione are strain-dependent (26). Oral treponemes are dependent on volatile fatty acids and require either isobutyric acid or 2-methylbutyric acid for growth (56). The growth of periodontal bacteria may be enhanced by hormones from the host. For example, cortisol, which is elevated in saliva under stressful conditions, has been shown to promote the growth of *P. gingivalis* (57). Steroid hormones have also been shown to promote growth of *A. actinomycetemcomitans* (58). Stress, obesity, and systemic diseases such as diabetes affect the host’s hormonal status and each of these is considered a risk factor for periodontitis (59). It is possible that the concentrations of specific hormones in the oral environment may impact on the growth of subgingival bacteria, and the pathogenicity of dental plaque.

**Trace Metals**

Sequestration of trace metals is a common strategy for restricting the growth of invading pathogens. Iron, in particular, is tightly bound by host proteins such as transferrin and lactoferrin. The acquisition of heme iron is essential for the growth of black-pigmented anaerobes, and the mechanisms of iron acquisition from the host by *P. gingivalis* have been studied in detail (60). A combination of hemagglutinin and proteases (gingipains) are deployed for binding to host erythrocytes and releasing hemoglobin. The hemin is then imported into *P. gingivalis* cells by the iron-heme transport system encoded by the *htrABCD* genes, a TonB-linked receptor with an ATP-binding cassette (ABC) uptake system encoded by hemin-transport genes *btrABCD*, or a heme-uptake system encoded by the *hmu* locus (60).

In *S. mutans* the uptake of iron is mediated by an ABC transporter designated SloABC (61). In addition to iron (Fe3+), SloABC also transports Mn2+. Homologous
ABC transporters are present in other oral streptococci including S. gordonii (ScaCBA), S. parasanguinis (FimCBA), and S. sanguinis (SsaACB), although the ScaCBA system in S. gordonii appears to be more selective for Mn^{2+} ions than Fe^{3+} (62–64). In each case, the expression of the ABC transporter is repressed by a DtxR family regulator primarily in the presence of Mn^{2+} (63, 65–67). In the case of S. gordonii, the ABC transporter is strongly up-regulated in serum or saliva, indicating that bioavailable Mn^{2+} is scarce in these body fluids (67). The precise intracellular functions of Mn^{2+} within streptococcal cells are not yet fully clear, but one important role for this metal is in protection against oxidative stress (63, 68). It is noteworthy that the P. gingivalis Mn^{2+} transporter FeoB2 is upregulated following a switch from anaerobic to microaerophilic conditions, which is also consistent with a role for Mn^{2+} in protection against oxidative stress in this organism (69).

Many different metal ions are essential cofactors for bacterial enzymes, including cobalt (Co^{2+}), copper (Cu^{2+}), and zinc (Zn^{2+}). Nickel (Ni^{2+}) is rarely required by bacteria, but in Streptococcus salivarius and Actinomyces johnsonii (previously Actinomyces WVA 693) Ni^{2+} ions are required for the activity of urease (70). A high-affinity Ni^{2+} transporter has been identified in S. salivarius, encoded by the genes ureM/Q/O (71).

**METABOLIC CO-OPERATION BETWEEN ORAL BACTERIA**

Overall, the nutrient content of saliva is sufficient to provide all essential nutrients and lacks only a strong source of energy for strains of Streptococcus spp. and Actinomyces spp., and these bacteria grow well when an exogenous energy source such as glucose is added (72). It is estimated that humans produce 500–600 mL of saliva per day (73), and the continual replenishment of nutrients may enable growth even when the overall concentration of glucose is low. Nevertheless, there is strong evidence that interspecies interactions play a key role in growth on saliva within the oral cavity and that the derivation of energy from host salivary substrates is maximised by the co-operative degradation of macromolecules and the recycling of waste products between oral bacteria.

**Food Webs**

Many of the waste products of oral bacterial species are important nutrients for other oral bacteria and several synergistic interactions have been demonstrated in vitro (Fig. 1A). Early studies on P. gingivalis and T. denticola identified a synergy based on the exchange of isobutyric acid, produced by P. gingivalis, for T. denticola-secreted succinate. More recently, interactions between this pair have been further investigated using DNA microarrays to assess global gene expression in mixed-species chemostat cultures (46). Here, it was found that P. gingivalis also produces thiamine pyrophosphate and free glycine, which are utilized by T. denticola. Thiamine pyrophosphate is upregulated by P. gingivalis in response to interactions with T. denticola. By contrast, glycine is liberated from glycine-containing peptides by P. gingivalis proteases, and is rapidly catabolized by T. denticola into lactate and acetate. In turn, P. gingivalis is able to utilize lactate for growth, particularly under strictly anaerobic conditions (69). Short-chain fatty acids, including butyric acid, are also produced by F. nucleatum and it is possible that T. denticola benefits from the presence of F. nucleatum (74). Organic acids, and lactate in particular, are central to many of the metabolic interactions between oral bacteria (Fig. 1A).

Following the widespread introduction of genomic and metagenomic sequencing, attempts have been made to develop *in silico* models to predict the key metabolic interactions between bacteria in communities such as dental plaque and to link them to co-occurrence patterns. Network-interaction models have been developed to assess co-occurrence relationships within different body environments, and it is interesting to note that the genera Streptococcus., Porphyromonas, and Veillonella, which have all been shown to undergo metabolic interactions with partner oral bacteria (Fig. 1A), form network ‘hubs’ with large numbers of co-occurrence relationships (75). On the other hand, Streptococcus (facultatively anaerobic, saccharolytic) has a very different metabolism from Tannerella (obligate anaerobe, proteolytic) and these genera display a negative co-occurrence relationship (75). Co-occurrence patterns were also identified that involve oral microorganisms that are not well-characterized metabolically. For example, TM7 phylum co-occurred with Moreyella on the tongue dorsum and a positive relationship was observed between Synergistetes phylum and Treponema in subgingival dental plaque. One member of the Synergistetes phylum, Fretibacterium fastidiosum, has been cultured in the laboratory and shown to be asaccharolytic and to produce acetate, propionate, and hydrogen sulfide (76). However, the full nutrient requirements of this species are not yet understood and growth requires extracts from other oral bacteria.
Recently, a representative of the TM7 phylum was grown in axenic laboratory culture on Fastidious Anaerobe Agar supplemented with 5% defibrinated horse blood (77). Whilst the metabolic potential of this strain has not yet been characterized in detail, synergistic biofilm growth was observed in co-cultures with *S. gordonii*, *Parvimonas micra*, or *F. nucleatum* compared with monocultures.
Metabolic Exchange as a Driver for Spatial Organization

Many oral bacteria bind to phylogenetically distinct cells through specific adhesin-receptor interactions in a process known as coaggregation (78). One function of coaggregation may be to bring cells into close proximity where metabolic co-operation is maximized. S. gordonii and Veillonella spp. coaggregate in vitro, and are often found in close proximity in early dental plaque [Fig. 1B; (79)]. Veillonella spp. benefit from lactate produced by streptococcal metabolism of carbohydrates. In turn, Veillonella produces a signal or nutrient, possibly maltose, that induces the up-regulation of S. gordonii amyB gene expression in neighboring cells (80). A more complex spatial relationship has been identified between S. gordonii and A. actinomycetemcomitans (81). In this case, A. actinomycetemcomitans obtains lactate from S. gordonii. However, H2O2 from S. gordonii is inhibitory towards A. actinomycetemcomitans. It appears that the production of the biofilm matrix-degrading glycosidase Dispersin B by A. actinomycetemcomitans is fine-tuned to facilitate the positioning of A. actinomycetemcomitans at a safe distance (≥4 μm) from S. gordonii, but still close enough to scavenge lactate.

Dental plaque typically contains 50–200 phylogenetically distinct microorganisms, and the potential for pairwise interactions is vast. However, dental plaque develops in a relatively reproducible spatiotemporal pattern and certain microorganisms, primarily Streptococcus spp., are almost always found in high numbers at the first stages of plaque development. Using a simplified model of dental biofilm development, it was found that metabolically similar bacteria with overlapping enzyme activities tend to be found in association with one another at different stages in biofilm formation and that gradients of metabolic functions extend across the different layers of the biofilm (82). At the same time, there appears to be a trade-off between the presence of similar enzymes in close proximity and the potential for synergistic interactions, which require complementary enzyme functions. Overall, the analysis is consistent with a major role for metabolism in driving the spatiotemporal organization of dental plaque.

**METABOLISM OF ORAL BACTERIA IN VIVO**

The ‘-omics’ technologies, and particularly those applied to whole communities (metagenomics, metaproteomics, metatranscriptomics, metabolomics, and others) are already beginning to revolutionize our understanding of oral microbial metabolism in the host. Proteomics has been applied in a variety of different approaches, such as to analyse changes in the metabolic potential of P. gingivalis following uptake into host cells (83). In the absence of significant growth, pathways for transcription and protein production were generally elevated in internalized P. gingivalis, indicating that the host cytoplasm provides a relatively nutrient-rich environment. In mixed-species cultures the S. gordonii proteome responds in a partner-specific manner (84). Thus, with F. nucleatum, the pathway for S. gordonii acetate production was decreased, whereas in interactions with P. gingivalis, the pathway for conversion of pyruvate to acetate was increased. With both species, lactate-production pathways were increased compared with monocultures. P. gingivalis has been shown to utilize lactate (69), and therefore interactions with S. gordonii may provide a source of nutrients. Similar analyses have recently been reported that center on the F. nucleatum proteome in two- or three-species communities (85). Overall, interspecies interactions led to reductions in amino acid fermentation and increases in butanoate production. It is possible that P. gingivalis competes with F. nucleatum for amino acids, although it is less clear why amino acid fermentation should be decreased in co-culture with S. gordonii.

Metabolomics technologies have been applied to investigate microbial metabolism in supragingival dental plaque, and largely appear to reflect the presence of Streptococcus spp. and Actinomyces spp. in this environment (86). Metatranscriptomics provides a very sensitive, although somewhat indirect, measure of metabolic potential in the mouth. The metatranscriptomic analysis of subgingival dental plaque showed differences in metabolism between healthy and periodontally affected sites (87). Specifically, diseased sites were enriched in pathways for lysine fermentation to butyrate, catabolism of histidine, nucleotide biosynthesis, and pyruvate fermentation. These changes were concordant with shifts in the microbial population. In the case of butyrate production, for example, F. nucleatum was the only organism found to have a pathway for the conversion of lysine to butyrate (87).

**SUMMARY**

Oral biofilms represent highly complex microbial communities that can exist in harmony with the host, but are also prone to cause disease following shifts in the microbial balance within the community. Metabolism appears to be a central driver for intermicrobial interactions that lead to the establishment of dental plaque...
and, in some instances, lead to changes in the plaque composition that result in dental caries or periodontitis. Computational-modelling methods and analysis of genes, proteins, transcripts, or metabolites at the microbiome level are starting to yield exciting new insights about the key metabolic pathways within oral microbial communities. At the same time, further studies of more simplified two- or three-species interactions are required to elucidate the many different synergistic and competitive interactions between bacteria and to place them in the context of oral health or disease. Ultimately, it is anticipated that modulating microbial metabolism in the oral cavity may prove an effective means for controlling oral disease.

REFERENCES


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Saliva as the Sole Nutritional Source in the Development of Multispecies Communities in Dental Plaque


