



Microbial ecosystem in the oral cavity: Metabolic diversity in an ecological niche and its relationship with oral diseases

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Abstract. With the respect to microbial flora, the oral cavity is one of the most densely populated sites of the human body. The environmental diversity of the oral cavity promotes the establishment of distinct microbial communities, such as supragingival plaque, subgingival plaque and tongue coating. The properties of the environment determine which microorganisms can occupy a site, while the metabolic activities of those microbial communities subsequently modify the properties of the environment. Saccharolytic microorganisms in supragingival sites ferment carbohydrates into, principally, lactic acid and create a temporarily acidic environment. Conversely, in subgingival sites, asaccharolytic microorganisms metabolize nitrogenous compounds derived from gingival crevicular fluid (GCF) and create a neutral pH and anaerobic environment abundant in short-chain fatty acids and ammonia. In tongue coating, asaccharolytic activity toward cysteine and methionine produces sulfur compounds, the major components of oral malodor. Furthermore, changes in environmental factors can prompt the development of adaptive responses in individual microorganisms to new environmental conditions and introduce more pathogenic microorganisms into the microbial community. Non-mutans streptococci and *Actinomyces* are predominant in the supragingival ecosystem that cause acidification, resulting in both demineralization of tooth surface and introduction of more cariogenic microorganisms, mutans streptococci, to the ecosystem. *Fusobacteria* and *Prevotella* neutralize subgingival environment pH by nitrogenous metabolism and stimulate GCF efflux. The neutral pH and nitrogenous environment increases the proteolytic activity of *Prevotella* and facilitates the establishment of a more acid-intolerant, but periodontopathogenic bacterium, *Porphyromonas gingivalis*. An environment determined by microbial metabolic activity can characterize a microbial ecosystem, and environmental changes originated by metabolic activity can often modify microbial physiological activity, initiating a shift from healthy to pathogenic conditions in this microbial ecosystem. © 2005 Elsevier B.V. All rights reserved.

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1. Bacterial pathogenicity in a microbial ecosystem—ecological plaque hypothesis

Oral cavity is the entrance of the digestive tract, which is often regarded as the ‘inner outside’. The digestive tract is anatomically continuous and harbors approximately 1×10^{14} microorganisms, which is more than the approximately 6×10^{13} cells that constitute the entire human body. Of the various site of the body, the oral cavity is one of the most densely populated and in excess of 500 microorganism species have been isolated from the oral cavity using recently developed molecular biological methods. These microorganisms colonize oral surfaces where they form a microbial consortium referred to as dental plaque or oral biofilm.

Among the microorganisms that form such consortia in the oral cavity, mutans streptococci and *Porphyromonas gingivalis* have received considerable attention as the pathogens responsible for dental caries and adult periodontitis, respectively. However, these bacteria do not always comprise the major proportion of microflora in initial lesions of oral disease and increase in number as the disease lesion develops (Tables 1 and 2) [1–5]. These findings imply that they are not initiators of disease, but rather successors in the microbial consortium that are able of adapting to the disease environment where they promote disease through pathogenic factors. Furthermore, it is possible that members of ‘healthy’ microbial consortia (commensal microorganisms) have the potential to change the environment through physiological processes such as metabolic activities, subsequently facilitating the introduction of more pathogenic microorganisms, mutans streptococci and *P. gingivalis* to the consortium. In this context, the interaction between the microorganisms of microbial consortia and their environment is referred to as a microbial ecosystem. Changes in microbial and environmental dynamics in microbial ecosystems may increase the potential for pathogenicity within a microbial ecosystem and subsequently initiate and promote oral diseases. These successional changes have recently and tentatively been referred to by Marsh as the ‘ecological plaque hypothesis’ [6].

Oral diseases such as dental caries, periodontitis and oral malodor are always initiated at this interface between microbial ecosystem and host tissue, with continued infection sometimes causing infectious diseases of adjacent organs such as respiratory pneumonia.

Table 1
Proportion (%) of mutans streptococci in microflora from healthy sites and enamel caries

Investigators	Enamel caries lesion	Healthy site
van Houte et al. [1]	2.9 ± 3.4^a	0.3 ± 0.8
Sansone et al. [2]	11.6 ± 14.4^a	7.6 ± 9.6
van Houte et al. [3]	$13.9 (3.9)^b$	$0.3 (0.01)$

^a Mean \pm standard deviation.

^b Mean (median).

Table 2
Proportion (%) of *P. gingivalis* in microflora from healthy and disease sites

Investigators	Periodontitis lesion	Gingivitis lesion	Healthy site
Moore et al. [4]	3.6 ± 1.2 ^a	0.0	0.0
Tanner et al. [5]	0.7 ± 0.7	2.8 ± 2.2	0.0

^a Mean ± standard deviation.

The author has investigated the metabolism of oral bacteria on the basis of the microbial ecosystem concept for a significant period of time and given that this metabolic activity is intrinsic to bacterial survival and environmental modification. The following is a review of how the metabolic activities of oral bacteria enable them to survive and become more pathogenic in the microbial ecosystem.

2. Diverse ecological niches in the oral cavity

The heterogeneity of tissue types in the oral cavity, such as teeth, tongue and mucosa, means that a variety of sites are available for colonization by oral microorganisms. Each site has unique characteristics and allows those microorganisms best suited to the environment to inhabit the site. The function or role of microorganisms in a habitat is referred as an ecological niche and a number of ecological niches exist in the oral cavity, including supragingival plaque, subgingival plaque and tongue coating. These ecological niches can be characterized by the environmental factors and the metabolic characteristics of the microbial flora occupying these sites (Table 3).

3. Supragingival plaque and dental caries—survival strategy of cariogenic bacteria

3.1. Acid production from carbohydrate metabolism and adaptation to acid stress in the supragingival area

The supragingival area consists of the stable environment of the tooth surface coated with salivary components such as proteins and glycoproteins. This continuous supply of

Table 3
Characteristics of supragingival plaque, subgingival plaque and tongue coating as ecological niches

	Supragingival plaque	Subgingival plaque	Tongue coating
Surface for microbial adhesion	Saliva-coated tooth	GCF-coated tooth GCF-coated epithelium	Saliva-coated epithelium
Nutrition	Saliva Carbohydrate	GCF Desquamated epithelium	Saliva Desquamated epithelium
pH	Neutral/acidic	Neutral	Neutral/acidic
Oxygen concentration	High/low	Low	High/low
Metabolic property of microbial ecosystem	Saccharolytic	Asaccharolytic/proteolytic	Saccharolytic/asaccharolytic/ Proteolytic

GCF, gingival crevicular fluid.

saliva acts as a nutrient supply for the microorganisms while carbohydrates derived from foods are also intermittently supplied (Table 2).

Streptococcus and *Actinomyces* species are predominant in the supragingival area. They can adhere the saliva-coated tooth surface by attachment between adhesins (located on bacterial cell surfaces) and receptors (contained mainly in the salivary coating on tooth surfaces) [7] and are known to utilize salivary components as nutrients. These bacteria are saccharolytic and degrade carbohydrates derived from foods through the Embden–Meyerhof–Parnas pathway to form lactic, formic, acetic, succinic and other organic acids, and concomitantly consume oxygen by NADH oxidase (Fig. 1). Taken together, these activities create acidic and anaerobic conditions. The acidification is rapid and the supragingival pH can reach around 4 within several minutes.

The acidification not only contributes to demineralization of the tooth surface, but also results in acid impairment of bacteria. As shown in Table 4, partial mortality occurs in non-mutans streptococci after an exposure to pH 4.0 for 60 min. However, these bacteria can respond to the acid stress and become more acid-tolerant, or aciduric, after transient and weak acidification (exposure to pH 5.5 for 30 to 60 min), indicating their ability to adapt to acidic conditions. These adaptive responses to acid stress are derived from the induction of proton-translocating ATPase (H^+ -ATPase), alkali production and stress proteins [8] and an increase in cell-membrane impermeability to protons may also be involved (Fig. 1). The increase in acidurance also enhances bacterial acid production at low pH (Table 4). Thus, once bacterial adaptation to acidic conditions begins, it

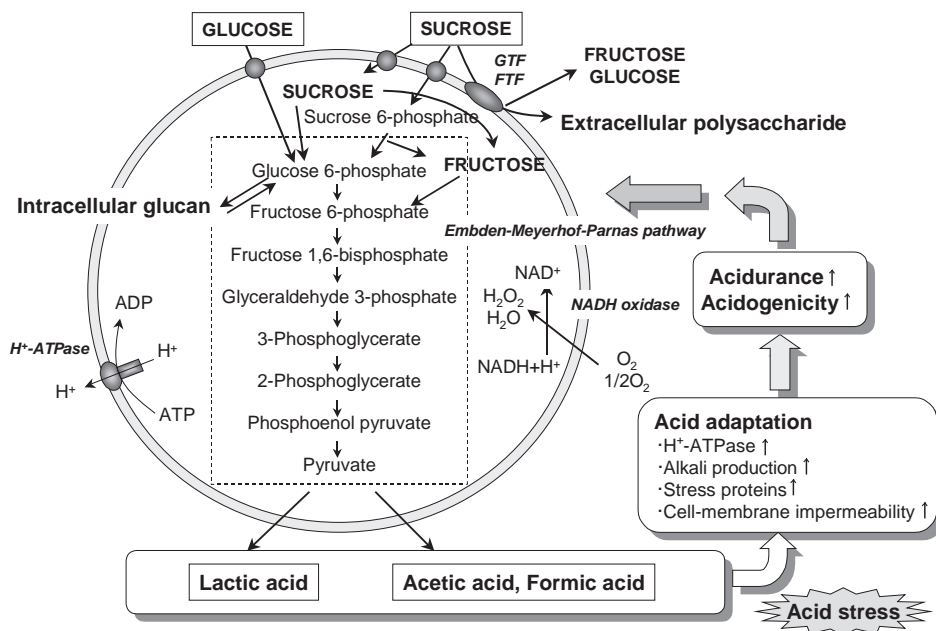


Fig. 1. Streptococcal carbohydrate metabolism and the associated ‘vicious cycle’ of acidification.

Table 4

Acidurance and acidogenicity of oral streptococci after transient acidification at pH 5.5 [8]

Bacterial species	Survival rate (%) at pH 4.0 for 60 min after an exposure to pH 5.5 for:			pH decrease in the presence of 90 mM glucose for 160 min after an exposure to pH 5.5 for:		
	0 min	30 min	60 min	0 min	30 min	60 min
<i>S. mutans</i> NCTC10449	93	88	103	3.70	3.68	3.62
<i>S. sanguinis</i> ATCC10556	3.6	29	15	4.33	4.15	4.12
<i>S. oralis</i> ATCC10557	0.75	12	15	4.10	4.00	3.96
<i>S. gordonii</i> Challis	0.0088	0.38	0.63	4.25	4.24	4.21
<i>S. mitis</i> SK142	0.011	0.58	0.37	4.15	4.10	4.10

precipitates a potentially dangerous cycle of enhancing bacterial acidurance and acidogenicity (Fig. 1).

3.2. Establishment of cariogenic microbial ecosystem in the supragingival area

In the supragingival area, non-mutans streptococci and *Actinomyces* species can acidify the environment, although they are not as acidogenic or aciduric as mutans streptococci. Further acquisition of acidurance and acidogenicity through acid adaptation can increase their cariogenic potential (Table 4). Some non-mutans streptococci such as *S. sanguinis* are known to convert oxygen into H₂O₂ (Fig. 1), which oxidizes the SCN⁻ in saliva to OSCN⁻ by the catalysis of salivary peroxidase. OSCN⁻ inhibits the glycolytic activity of mutans streptococci efficiently [9] and possibly represses their growth.

As supragingival plaque becomes thickened and mature, in addition to causing acidification, the oxygen concentration decreases and results in anaerobic conditions. These acidic and anaerobic conditions can then facilitate colonization of the supragingival plaque by more aciduric and oxygen-labile bacteria like mutans streptococci. In addition, mutans streptococci can produce water-insoluble extracellular glucan from sucrose by glucosyltransferase (GTF) (Fig. 1), further increasing their colonization potential. Mutans streptococci as well as non-mutans streptococci have the capacity to increase their acidurance and acidogenicity by acid adaptation (Table 4).

The microbial shift, or the increase in the population of aciduric bacteria such as mutans streptococci in supragingival plaque, has the effect of enhancing the cariogenic potential of supragingival plaque. The proportion of mutans streptococci is known to be higher at sites of white spot and enamel caries rather than healthy sites (Table 1), which could be due to the microbial shift caused by acidification in the microbial ecosystem. In this context, the predominant bacteria in supragingival plaque formation are non-mutans streptococci and *Actinomyces* species, which are regarded as initiators that are responsible for increasing cariogenicity of the site through acidification and subsequent acid adaptation upon frequent carbohydrate supply. Conversely, mutans streptococci are considered to be the successors that colonize sites where the acidic environment has already been established and then act as promoters that enhance cariogenicity of the sites through microbial shift as well as acid adaptation.

4. Subgingival plaque and periodontitis—survival strategy of periodontopathic bacteria

4.1. Protein, peptide and amino acid metabolisms in the subgingival area

Subgingival sites provide a stable tooth surface and an unstable epithelial surface, the latter of which continuously desquamates. Both surfaces are bathed with a continuous efflux of gingival crevicular fluid (GCF), derived from blood plasma and thus nutritionally rich in nitrogenous compounds such as amino acids, peptides and proteins. Desquamated epithelium can also be supplied as a nutrient (Table 3). As a gingival crevice deepens, the environmental factors in the subgingival site become more stable, i.e. neutral pH and anaerobic. Under these conditions, asaccharolytic and anaerobic and/or proteolytic bacteria such as *Fusobacterium*, *Eubacterium*, *Campylobacter*, *Prevotella* and *Porphyromonas* are found.

Proteolytic bacteria can degrade nitrogenous compounds into small peptides and amino acids by cell membrane-bound and/or extracellularly secreted proteases for subsequent use as metabolic substrates. For instance, *P. gingivalis* has gingipains (trypsin-like cysteine proteases) and dipeptidylpeptidases, while *P. intermedia* has several proteases that degrade albumin and immunoglobulins.

Unlike the saccharolytic bacteria that share a common glycolytic pathway, asaccharolytic bacteria exhibit diversity in their metabolic pathways. *P. gingivalis* and *Prevotella intermedia* have a series of metabolic enzymes responsible for the production of acetic and succinic acids from aspartic acid (acetic–succinic pathway) (Fig. 2) [10,11]. In addition, *P. gingivalis* has metabolic enzymes responsible for the production of propionic and butyric acids from glutamic acid (propionic–butyric pathway) and a succinyl-CoA synthase that bridges the acetic–succinic pathway to the propionic–butyric pathway [10]. Valine and leucine are also degraded to isobutyric and isovaleric acids, respectively. In general, ammonia is produced through amino acid deamination, and oxygen is consumed by coupling with a reducing power derived from amino acid metabolism, resulting in the establishment of anaerobic conditions. *Fusobacterium* species also utilize glutamic acid as a nutrient and produce acetic and butyric acids, but their metabolic pathway varies among species with at least 3 distinct pathways that have been reported [12]. *Eubacterium* species employ the ‘Stickland reaction’ to convert leucine into isocaproic or isovaleric acid [13]. Like *P. gingivalis* and *P. intermedia*, *Campylobacter rectus* metabolizes aspartic acid to succinic acid, but requires formic acid as a reducing agent [14].

P. gingivalis prefers small peptides as nutrients, particularly dipeptides rather than amino acids [15], while *P. intermedia* and *F. nucleatum* can utilize both but prefer amino acids to dipeptides [16]. This suggests that *P. gingivalis* is advantageous for the competition for peptides since the bacterium can degrade proteins to peptides by itself and immediately incorporate the resultant peptides. However, proteolytic bacteria can also supply peptides and amino acids to non-proteolytic, but asaccharolytic bacteria such as *Fusobacterium*. End products of amino acid metabolism are also essential nutrients for other bacteria. Oral treponema species are strictly dependent on isobutyric acid for growth. These findings suggest that metabolic competition and cooperation are both prevalent in the subgingival microbial ecosystem.

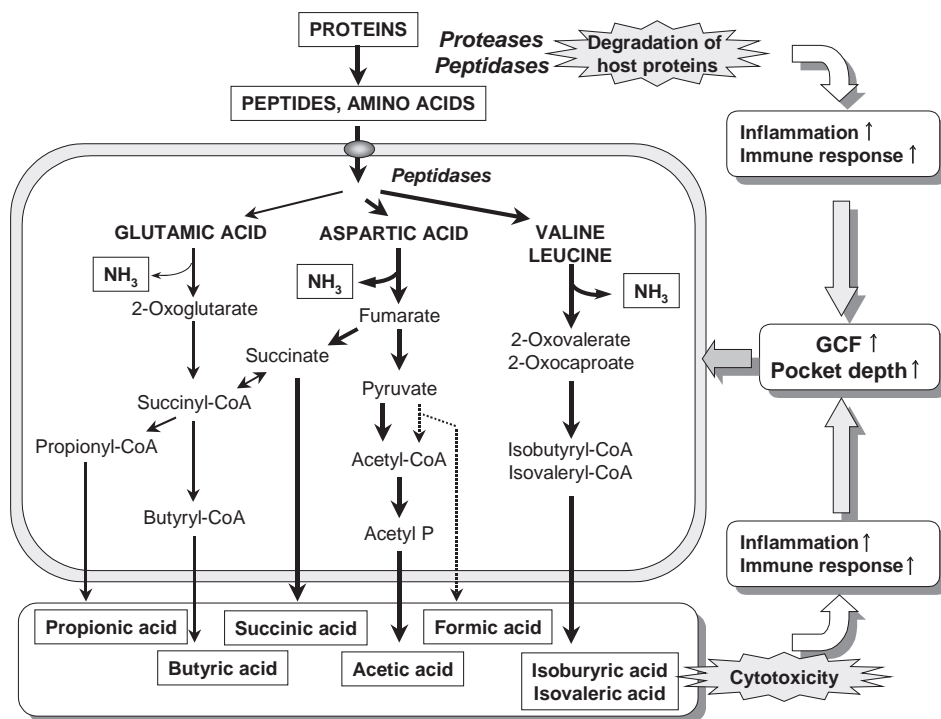


Fig. 2. Amino acid metabolism by *P. gingivalis* [10] and *P. intermedia* [11] and the effects of these metabolic activities on the host responses. Metabolic pathways for both bacteria are indicated with thick lines. Additional pathways for *P. gingivalis* are indicated in thin lines, while additional pathways for *P. intermedia* are indicated in broken lines.

4.2. Establishment of periodontopathogenic microbial ecosystem in the subgingival area

Bacterial proteases and metabolic end products, as well as bacterial cell components like lipopolysaccharide, have the potential to induce host responses such as inflammation and immunoreactions (Fig. 2). Proteases degrade host tissues directly, and disturb the host defense indirectly through the degradation of host defense proteins such as complements, immunoglobulins and the blood coagulation system. Metabolic end products such as short chain fatty acids (propionic, butyric, isobutyric and isovaleric acids), ammonia and sulfur compounds (hydrogen sulfide and methyl mercaptan) impair host cell functions and subsequently disturb the host defense [17].

Microorganisms living in subgingival plaque prefer neutral pH and anaerobic conditions (Table 5). This is particularly true of *P. gingivalis* which prefers neutral pH [18,19] and is thus rarely detected in supragingival areas where the environmental pH has become acidic by saccharolytic bacteria. Conversely, *P. intermedia* and *F. nucleatum* are capable of growth at acidic and neutral pH, and are frequently found in supragingival plaque [20]. In addition, *P. intermedia* and *F. nucleatum* are capable of neutralizing the acidic environmental pH by changing the acid–base balance through amino acid metabolism [19,21]. Furthermore, *P. intermedia* can utilize glucose as well as nitrogenous

Table 5

Effect of pH on the growth of *P. gingivalis*, *P. intermedia*, *P. nigrescens* and *F. nucleatum* [18,19]

	Range of pH enables bacteria to grow
<i>P. gingivalis</i> ATCC 33277/ <i>P. gingivalis</i> W83	7.0 ^a –6.5
<i>P. intermedia</i> ATCC 25611/ <i>P. nigrescens</i> ATCC 25261	7.0 ^a –5.0
<i>F. nucleatum</i> ATCC 25586/ <i>F. nucleatum</i> ATCC 10953	7.0 ^a –5.5

^a Growth at pH > 7.0 was not tested.

compounds, and alter its metabolic characteristics depending upon the nutrients available. In the absence of glucose, this bacterium increases its proteolytic activity and formation of cytotoxic metabolic end products [22].

These findings suggest the following succession in the establishment of a pathogenic microbial ecosystem in the subgingival area. Firstly, *F. nucleatum* and *P. intermedia* colonize a shallow gingival pocket (where the pH is variable and sometimes becomes acidic) and then promote the establishment of a neutral pH environment. This bacterial colonization induces the host defense, such as inflammation of the subgingival area, and results in the increase of GCF rich in host proteins (Fig. 2). This situation may induce the colonization of a more proteolytic, but acid-intolerant bacterium, *P. gingivalis*, and enhance the pathogenicity of *P. intermedia* though the increase in proteolytic activity and cytotoxic end products. The increase of GCF efflux is obviously effective against the bacterial attack since GCF is rich in various host defense proteins. However, it should be noted that it might supply metabolic substrates to proteolytic and asaccharolytic bacteria living in the periodontal pocket. The activity of subgingival bacteria to degrade host defense proteins, as well as direct destruction of host periodontal tissue, is central to their metabolic adaptation for survival in the subgingival area.

5. Tongue coating and oral malodor

Tongue coating consists of desquamated epithelium from the tongue, saliva and microorganisms, and as supragingival sites, food supply is intermittent (Table 3). The papillary structure of the dorsum of the tongue provides a suitable habitat for many microorganisms, and the environment can also be characterized by anaerobic conditions that are conducive for the growth of obligatory anaerobes. A wide range of bacteria including *Actinomyces*, *Streptococci*, *Veillonella*, *Fusobacterium* and *Prevotella* are found and the tongue may act as a reservoir or cradle of oral bacteria.

Oral malodor is a relatively common condition in adults and approximately 90% of oral malodor is believed to originate from foul-smelling gases such as volatile sulfur compounds (VSC) produced through oral bacterial metabolism of sulfur amino acids. The major components of VSC are hydrogen sulfide (H₂S), methyl mercaptan and dimethyl sulfide. High-odor subjects usually have a higher total bacterial load on the rear dorsum of the tongue [23]. Periodontal disease-related bacteria including *Porphyromonas* and *Prevotella* are responsible for VSC production in malodor subjects with periodontal diseases [24], while commensal bacteria such as *Actinomyces* and *Veillonella* are for H₂S production in malodor subjects without periodontal disease [23].

H₂S is produced mainly from L-cysteine by L-cysteine desulfhydrase [25], while methyl mercaptan is mainly from L-methionine by methionase (L-methionine- α -deamino- γ -mercaptomethane-lyase) [26]. These amino acids are supplied as forms of amino acids or small peptides containing the corresponding amino acids. Proteolytic bacteria are involved in the supply of metabolic substrates from proteins, but the predominant proteolytic bacteria in the tongue coating have not yet been identified. *Fusobacterium* and *Peptostreptococcus* can produce H₂S from glutathione, which is found in most tissue cells and the periodontal pocket. Both H₂S and methyl mercaptan are not only malodorous but also toxic to host tissue. Generally, however, VSC metabolism and its regulation by oral bacteria in the microbial ecosystem of the tongue coating have not been fully investigated.

6. Significance of metabolic activity in the microbial ecosystem

Recent developments of molecular biological techniques for microbial identification have clarified that oral microflora form a microbial complex, or biofilm, consisting of in excess of 500 microbial species. In addition, studies of interactions between bacterial cells and between bacteria and host cells are in the process of revealing that this microbial complex forms a community—a ‘microbial ecosystem’—in which microorganisms interact competitively and cooperatively. We are relatively familiar with the answer to the question ‘Who are they?’, and have now progressed to the question ‘What are they doing there?’ The physiological characteristics of the oral flora, from energy production for survival to pathogenicity are determined by the metabolic activity they exhibit. Understanding the range of metabolic characteristics of the microbial ecosystem could be an answer to this question, and would also provide us with an answer to the question of how we could control the microbial ecosystem; in other words, how we could control oral diseases such as dental caries, periodontal diseases and oral malodor.

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