



REVIEW ARTICLE

Significance of Asaccharolytic *Eubacterium* and Closely Related Bacterial Species in the Human Oral Cavity

Futoshi Nakazawa*, Hiroshi Miyakawa, Mari Fujita, Arihide Kamaguchi

Department of Oral Microbiology, School of Dentistry, Health Sciences University of Hokkaido, Hokkaido, Japan

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Asaccharolytic *Eubacterium* species are obligate anaerobic, gram-positive rods frequently isolated from human oral specimens, particularly from infectious lesions, such as periodontal pockets and apical lesions. Many unknown bacterial strains have recently been isolated from infectious lesions. These strains were determined to be mainly asaccharolytic *Eubacterium* and/or phylogenetically closely related species. Many novel genera, such as *Slackia*, *Eggerthella*, *Cryptobacterium*, and *Mogibacterium*, have been established by molecular systematic techniques, including 16S rRNA gene sequencing analysis and DNA–DNA hybridization. Moreover, many species within the genus *Eubacterium* have been reclassified based on phylogenetic data. Many uncultured, undescribed, and unknown bacterial species thrive in the human oral cavity. However, to elucidate the etiology of oral infections and to develop novel diagnostic tools, a complete description of the microbial flora associated with these infections is required. This review focuses on recent findings on asaccharolytic *Eubacterium* and closely related bacterial species in the human oral cavity and on the etiological role of these bacterial species in oral infections in humans.

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1. Introduction

Asaccharolytic *Eubacterium* species, which are obligate anaerobic, pleomorphic non-spore-forming, gram-positive rods, have been isolated from human oral specimens, including those taken from periodontal pockets, infected pulp, and carious dentine. Some *Eubacterium* strains have also been isolated from subgingival areas associated with moderate or severe adult periodontitis.

Bergey's Manual of Systematic Bacteriology distinguishes the genus *Eubacterium* from other genera mainly on the basis of negative metabolic characteristics.¹ That is, the genera *Actinomyces*, *Bifidobacterium*, *Lactobacillus*, and *Propionibacterium* mainly produce succinic acid, acetic and lactic acids, lactic acid, and propionic acid, respectively, as major end products. Anaerobic, gram-positive rods that are not classified into any of the aforementioned four genera are automatically assigned to the genus *Eubacterium*, which results in an unclear classification of a collection of diverse organisms. Because of the nature of the definition, a considerable heterogeneity inevitably exists among the species assigned to the genus *Eubacterium*, which now contains many species and groups that are phenotypically and phylogenetically unrelated.

A previous report showed that 11 species of the typical oral *Eubacterium* had significant heterogeneity in whole-cell protein profiles using sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and in serological reactions using western immunoblotting.² Moreover, the DNA base compositions of these *Eubacterium* species varied widely from 38 to 62 mole % guanine+cytosine (G+C).³ Furthermore, DNA–DNA hybridization studies have indicated that the level of DNA relatedness among these *Eubacterium* species is from 1% to 16%. None of the species studied shared a high level of DNA homology with the type species of the genus *Eubacterium*.³ These data demonstrate that bacterial species included in the genus *Eubacterium* are not uniform. Therefore, a reclassification of these species is required.

It has been suggested for a long time that many unknown bacterial species, including viable but noncultivable, uncultured, unidentified, and undescribed strains, thrive in the human oral cavity. Many studies have indicated that unknown bacterial species from chronic infections, such as periodontal pockets and apical lesions, are mainly asaccharolytic, anaerobic, gram-positive rods.^{4–6} These species have been assigned to the genus *Eubacterium* according to the criteria of bacterial classification. Therefore, an applicable classification standard for *Eubacterium* and closely related bacterial strains should be established to elucidate the bacterial species involved in oral infections.

We have previously studied the morphological, biochemical, immunological, and genetic characteristics of many unknown bacterial strains isolated from oral infectious lesions. These studies

* Corresponding author. Department of Oral Microbiology, School of Dentistry, Health Sciences University of Hokkaido, 1757 Kanazawa, Ishikari-Tobetsu, Hokkaido 061-0293, Japan.

E-mail: nakazawa@hoku-iryo-u.ac.jp (F. Nakazawa).

demonstrated that all these strains were asaccharolytic, anaerobic gram-positive rods and were classified into the genus *Eubacterium* or as strains closely related to *Eubacterium*.^{2,3} Furthermore, a few were proposed as novel genera and/or species according to their polyphasic characteristics, including morphological and biochemical characteristics, whole-cell protein profiles, results of western immunoblotting and DNA–DNA hybridization, and sequencing of the 16S rRNA gene.^{4–6}

In this review, we summarize our previous studies and focus on recent findings on the asaccharolytic *Eubacterium* species and its closely related bacterial species. This review also focuses on the significance of these bacteria in oral infections.

2. Unknown Bacterial Strains

More than 350 bacterial species are known to inhabit in the human oral cavity that make up the complex oral microflora ecosystem. Almost all of these bacterial species are specific to the human mouth and play important roles in maintaining a healthy oral environment. However, some of these bacterial species may also induce oral infections, such as periodontal disease and caries.

Paster et al⁷ and Kazar et al⁸ reported the existence of 700 phylotype clones in the human oral cavity; half of these clones were derived from previously uncultured and/or unknown bacterial species using 16S rRNA gene cloning. Moreover, polymerase chain reaction (PCR) with specific primer sets derived from the 16S rRNA gene sequences was used to estimate that approximately 50% of the oral microflora have not yet been cultured.⁹ Surprisingly, Keijser et al¹⁰ detected more than 19,000 species-level phylotypes in the oral microflora of healthy adults. These data indicate that most of the bacterial strains in the human oral cavity are unknown species that may cause infectious diseases.

Some previously studied bacterial species are causative microorganisms of specific oral infections. For example, *Streptococcus mutans* causes caries and *Porphyromonas gingivalis* causes periodontitis. However, it is possible that some unknown species may play a more serious role in the development of oral infection. Therefore, studies of unknown bacterial strains are important in clarifying the mechanisms of oral infection.

3. Anaerobic Bacteria

The predominant bacterial species of individual sites in the oral cavity are influenced by many complex conditions, such as pH, moisture content, and nutritional availability. Because bacteria in the oral cavity are mainly anaerobic, including obligate and facultative strains, the degree of anaerobicity is considered to be the most crucial factor for microflora formation in the oral cavity.

The level of the anaerobic condition is denoted in terms of oxidation–reduction potential (ORP; mV). For example, the ORP of tap water is approximately +600 mV, and the ORP of body fluids and internal organs ranges from +300 to –500 mV.^{11,12} The crown of the tooth has an ORP of +200 mV immediately after brushing, but this value decreases rapidly to –200 mV as the amount of dental plaque increases over 4 days. Moreover, it is known that the deep portion of a periodontal pocket with severe periodontitis has an ORP of –400 mV, which is an intensively anaerobic environment. These data indicate that the human oral cavity is generally anaerobic and provides a suitable environment for the growth of anaerobic oral bacterial strains. Many studies have proven that obligate anaerobic strains predominantly inhabit infectious lesions with extremely low ORP values.^{13,14}

Active oxygen derivatives, such as superoxide radicals, hydroxyl radicals, and hydrogen peroxide, are produced from oxygen and cause serious damage to the DNA and cytoplasmic membranes of bacterial cells. Aerobes produce superoxide dismutase, catalase,

and peroxidase to escape the cellular damage caused by these active oxygen derivatives under aerobic conditions. However, because anaerobic bacteria generally do not produce these enzymes, they have difficulty surviving in an oxygen-rich environment. Based on their level of oxygen tolerance, anaerobes are divided into two groups: obligate anaerobes (less tolerant) and facultative anaerobes (more tolerant).

As described earlier, most of the bacterial strains in the oral cavity are either obligate or facultative anaerobes. There are very few culturable obligate aerobic strains in the human oral cavity. Thus, bacterial strains in clinical specimens have to be cultured and maintained in an anaerobic glove box to avoid the loss of anaerobic bacteria. In general, the sealed box contains 80% N₂, 10% H₂, and 10% CO₂, and the ORP inside the box is kept at –400 mV or lower, which are appropriate growth conditions for obligate and facultative anaerobes. Dispersion, dilution, and inoculation of the clinical samples can also be performed inside the box.

4. Transition of Bacterial Classification

Bacteria have been generally classified based on their morphological and biochemical characteristics, such as gram stain reaction, fermentation of carbohydrates, and enzymatic profiles. Moreover, the chemical structure of their cell wall, whole-cell protein profiles, and serological reactions are traditionally used as taxonomic tools.

Recent advances in molecular biology have allowed the study of microbial communities, including the undescribed, uncultured, and unculturable species. Direct amplification by PCR of housekeeping genes from a mixed culture biomass, followed by purification and sequencing, has allowed the analysis of complex microbial communities.⁹ The gene encoding the small subunit rRNA has been particularly useful for this purpose.¹⁵

Sequencing of bacterial 16S rRNA genes has made it possible to use phylogenetic relationships for bacterial classifications and has significantly changed previous bacterial taxonomies. For example, the genus *Bacteroides* previously had more than 50 registered species. However, phylogenetic analysis using 16S rRNA gene sequences has reclassified many of these species to other genera, including novel genera, such as *Porphyromonas*, *Prevotella*, and *Tannerella*.¹⁶ Currently, the type species of the genus *Bacteroides* is *Bacteroides fragilis*, and only 14 species have remained in the genus. Moreover, the genus *Streptococcus* has been reclassified based on 16S rRNA gene sequence similarities. Species within the genus *Streptococcus* are divided into six genetic groups based on their phylogenetic characteristics: pyogenic, anginosus, mutans, mitis, salivarius, and bovis.¹⁷

All bacteria have the 16S rRNA gene, which is composed of approximately 1500 bases. Large databases of 16S rRNA sequences, such as that made available by the Ribosomal Database Project, now exist.¹⁸

At present, the *Bergey's Manual of Systematic Bacteriology* has been revised for the first time in 20 years. The Editor-in-Chief, Dr G.M. Garrity, clearly states that phylogenetic characterization using gene sequence library data is the most important determinant of bacterial classification.

5. Novel *Eubacterium* Species

Spratt et al¹⁹ studied more than 50 PCR products of asaccharolytic gram-positive rods phylogenetically related to *Eubacterium* in the human oral cavity. They concluded that these rods may be divided roughly into four phylogenetic groups and that many bacterial species inhabiting the disease sites have not been reported yet.

Until recently, *Eubacterium brachy*, *Eubacterium lentum*, *Eubacterium nodatum*, and *Eubacterium timidum* were registered as the only asaccharolytic *Eubacterium* species. Uematsu et al²⁰ then

proposed *Eubacterium saphenum* as a novel species of *Eubacterium* for asaccharolytic strains isolated from the periodontal pocket. This species resembles *E. nodatum* but does not hydrolyze arginine. It differs from previous asaccharolytic *Eubacterium* species in whole-cell protein profiles, G + C contents, and DNA homology obtained from DNA–DNA hybridization studies.

Poco et al²¹ have proposed *Eubacterium minutum*, isolated from periodontal pockets, and *Eubacterium exiguum*, isolated from necrotic pulps and periapical lesions, as novel asaccharolytic *Eubacterium* species.²² They are strict anaerobic, gram-positive rods that grow slowly and form very tiny colonies on brain heart infusion (BHI) blood agar. They show different characteristics in terms of structural cell protein profiles of Rapid ID 32A API ZYM code and serological reaction patterns. They exhibit low levels of DNA homology with previously established *Eubacterium* species. *Eubacterium minutum* produces small amounts of butyric acid as the metabolic end product and does not hydrolyze arginine. In contrast, *E. exiguum* produces no end product in peptone–yeast extract–glucose broth, dose-hydrolyzed arginine, and does not reduce nitrate. *Eubacterium exiguum* is similar to *E. lentum* in terms of the G + C content.

Cheeseman et al²³ proposed two novel species, *Eubacterium infirmum* and *Eubacterium tardum*, which created original clusters in the phylogenetic tree constructed by 16S rRNA gene sequence data. *Eubacterium infirmum* produces small amounts of acetic and butyric acids, but *E. tardum* produces only butyric acid as the metabolic end product. Neither species hydrolyzes arginine. However, Wade et al²⁴ reported that *E. minutum* and *E. tardum* are synonymous species based on data in the phenotypic and genetic analyses of type strains of the two species.

6. Genera Closely Related to *Eubacterium*

After the publication of the novel *Eubacterium* species mentioned earlier, *E. exiguum* and *E. lentum* were determined to have very similar descriptions. On the other hand, *Peptostreptococcus heliotrinreducens*, isolated frequently from oral abscesses by Murdoch and Mitchellmore²⁵ was shown to be dissimilar to all other *Peptostreptococcus* species by a phylogenetic study.²⁶ Furthermore, Goodacre et al²⁷ demonstrated that the type strain of *P. heliotrinreducens*, which was identified on the basis of enzyme profiles, was in fact *E. exiguum*.

Based on 16S rRNA sequence similarity data, Wade et al²⁸ proposed that *E. exiguum* and *P. heliotrinreducens* should be placed into a new genus, *Slackia*, as *Slackia exigua* and *Slackia heliotrinreducens*, respectively. They also proposed a second new genus, *Eggerthella*, to include *E. lentum* as *Eggerthella lenta*, which share 89.8% and 90.8% 16S rRNA sequence similarity with *S. heliotrinreducens* and *S. exigua*, respectively.^{28,29} These species were found to constitute a branch of *Coriobacteriaceae* in a phylogenetic tree with genera *Coriobacterium* and *Atopobium*.

In the same year, Kageyama et al³⁰ demonstrated that *E. lentum* should be reclassified into another genus related to *Coriobacterium glomerans* and genus *Atopobium*. They also proposed to reclassify *Eubacterium aerofaciens* into the novel genus *Collinsella* as *Collinsella aerofaciens*, on the basis of phenotypic and phylogenetic characterizations of 212 strains of *E. aerofaciens*.³¹ The validity of the reclassification demonstrated by Wade et al and Kageyama et al was confirmed by the results of G + C contents and DNA–DNA hybridization studies.³²

We have also analyzed 10 novel strains isolated from periodontal pockets, necrotic pulp, infected root canals, and tongue plaque. These were typical strains closely related to *Eubacterium* and inert in biochemical tests; they could not be assigned to any previously established species. After comparisons of the profiles of structural proteins and serological reaction patterns, G + C content, DNA–DNA

homology, and 16S rRNA sequence similarities with established bacterial species, we proposed two novel genera, *Cryptobacterium* and *Mogibacterium*.^{4–6} *Cryptobacterium curtum* did not produce any metabolic end products. *Mogibacterium*, which produced only phenyl acetic acid, was divided into four novel species: *Mogibacterium pumilum*, *Mogibacterium vescum*, *Mogibacterium diversum*, and *Mogibacterium neglectum*. We also proposed that the strains previously classified as *E. timidum* should be reclassified into the genus *Mogibacterium* as *Mogibacterium timidum*.

Although 55 species were previously registered under the genus *Eubacterium*, many species have been reclassified using phylogenetic characterization. Furthermore, many novel genera and species, mainly asaccharolytic *Eubacterium* and species related to *Eubacterium*, were established from isolates of human oral infections. Table 1 shows the new classification of *Eubacterium* species frequently isolated from the human oral cavity and genera and species closely related to asaccharolytic *Eubacterium*. Table 2 indicates the specific characterization of asaccharolytic *Eubacterium* and novel genera closely related to *Eubacterium* at the genus level.

7. Pathogenesis

It has been well established that asaccharolytic *Eubacterium* species are significantly associated with many kinds of oral infections, such as periodontitis, periapical infections, and oral abscesses.^{33,34} In particular, many studies have reported that asaccharolytic *Eubacterium* might play important roles in periodontitis. For example, antibody titers against *Eubacterium* species in the serum of patients with periodontitis are higher than those in healthy persons, suggesting that these bacterial species cause immunological reactions in periodontal lesions.^{35–37}

Smith et al investigated the serum antibody response to *E. brachy*, *E. nodatum*, *S. exigua*, and *M. timidum*.³⁸ They demonstrated that *S. exigua* is among the predominant bacteria found in moderately and severely affected periodontal sites, with a significant association with diseased as opposed to healthy sites. Serum immunoglobulin A (IgA) levels against *M. timidum* and *S. exigua* were also shown to be elevated in case of refractory periodontitis. The presence of an elevated systemic antibody response suggests that these asaccharolytic *Eubacterium* and closely related species have sufficiently breached the host defenses to stimulate an immune response.

Uematsu and Hoshino³⁹ isolated and identified 422 strains from the periodontal pockets of seven patients with chronic periodontitis. They showed that 42% of these isolates were asaccharolytic *Eubacterium* species or closely related strains.³⁹ Hoshino⁴⁰ reported that asaccharolytic *Eubacterium* species are the predominant bacteria of infectious lesions in smooth surface decay and of

Table 1 Reclassification of *Eubacterium* and the related species isolated from human oral cavity

Current name	Remarks
<i>Atopobium fossor</i>	Novel genus <i>Eubacterium fossor</i>
<i>Collinsella aerofaciens</i>	Novel genus <i>Eubacterium aerofaciens</i>
<i>Cryptobacterium curtum</i>	Novel genus and species
<i>Eggerthella lenta</i>	Novel genus <i>Eubacterium lentum</i>
<i>Eubacterium combesii</i>	Novel species
<i>Eubacterium infirmum</i>	Novel species
<i>Eubacterium minutum</i>	Novel species
<i>Eubacterium saphenum</i>	Novel species
<i>Mogibacterium diversum</i>	Novel genus and species
<i>Mogibacterium neglectum</i>	Novel genus and species
<i>Mogibacterium pumilum</i>	Novel genus and species
<i>Mogibacterium vescum</i>	Novel genus and species
<i>Mogibacterium timidum</i>	Novel genus <i>Eubacterium timidum</i>
<i>Pseudoramibacter alactolyticus</i>	Novel genus <i>Eubacterium alactolyticum</i>
<i>Slackia exigua</i>	Novel genus <i>Eubacterium exiguum</i>

Table 2 Characteristics of genus *Eubacterium* and the related genera

Taxon	G + C %	End products*	Arginine hydrolysis	Nitrate reduction
<i>Eubacterium</i> [†]	38	Butyrate	Negative	Negative
<i>Eggerthella</i>	62	None	Positive	Positive
<i>Cryptobacterium</i>	51	None	Positive	Negative
<i>Mogibacterium</i>	46	Phenyl acetate	Negative	Negative
<i>Slackia</i>	60	None	Positive	Negative

* End products produced in peptone–yeast extract–glucose broth.; † Data of *Eubacterium minutum* as typical saccharolytic *Eubacterium* species.

the infected layers of dentin, suggesting that these bacterial species are involved in the progression of dental caries.

It is generally believed that infectious lesions, such as those of periodontal pockets and infected root canals, are rich in proteins and peptides derived from tissues and blood. Asaccharolytic strains use these proteins and peptides as an energy source, which promote their growth in infectious lesions. Asaccharolytic *Eubacterium* and closely related strains are the major bacterial groups in oral lesions and play important roles in these infections.

Furthermore, some asaccharolytic *Eubacterium* and closely related strains produce butyric acid from arginine through ornithine, catalyzed by ornithine carbamoyltransferase. Butyric acid produces breath odor and has pathogenic activity, such as inhibition of proliferation of human gingival fibroblasts and induction of apoptosis in splenic T cells.⁴¹ These activities of butyric acid may be involved in the exacerbation of infectious lesions.

Although it is necessary to further elucidate potential pathogenic mechanisms, previous studies support the hypothesis that asaccharolytic *Eubacterium* species and closely related strains may play an important role in many oral infections.

8. Future Prospects

The role of specific bacterial species in the etiology of oral infections (e.g., the role of *Streptococcus mutans* in dental caries and *Porphyromonas gingivalis* in periodontitis) have been well demonstrated over the past 50 years. However, most oral infections are polymicrobial and may not adapt to Koch's postulate. In polymicrobial infections, the pathogenicity of each species and its interaction with other species play an important role in the progression of infections.

Wilson et al.⁹ reported that 50% of the bacterial strains inhabiting sites of chronic oral infection have not been identified. Djais et al.⁴² recently reported several unknown strains from endodontic lesions and periodontal pockets, which were asaccharolytic, anaerobic, gram-negative coccoids phylogenetically related to the genus *Dialister*; however, their species classification is yet to be determined. Taken together, current data indicate that bacterial composition in lesions is not entirely clarified and that little is known about the role of bacterial species in the spread of oral infections.

Identification of new bacterial species using improved molecular techniques would mean that mixed microflora associated with oral polymicrobial infections can be investigated in their entirety without the inherent biases of culture. The ability to identify novel organisms associated with oral disease will improve our knowledge of the etiology of oral infections and will allow the discovery of additional marker organisms valuable in disease diagnosis and treatment monitoring.

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