表1　肺炎および発熱日数との関連性が認められた診査項目

診査項目	肺炎（343名）		発熱日数（299名）	
	発症	なし	10日以上	9日以内
	35名	308名	46名	253名
舌の湿潤度，人数（%）				
5.0 mm以上	7 (20.0)	147 (47.7) [a]**	12 (26.1)	125 (49.4) [a]**
1.0～4.9 mm	14 (40.0)	123 (39.9)	18 (39.1)	103 (40.7)
1.0 mm未満	14 (40.0)	38 (12.3)	16 (34.8)	25 (9.9)
義歯の使用，人数（%）				
なし	28 (80.0)	185 (60.1) [b]*	37 (80.4)	146 (57.7) [b]**
あり	7 (20.0)	123 (39.9)	9 (19.6)	107 (42.3)
身体活動レベル，人数（%）				
歩行可	3 (8.6)	98 (31.8) [a]**	2 (4.3)	85 (33.6) [a]**
車椅子使用	10 (28.6)	101 (32.8)	9 (19.6)	92 (36.4)
寝たきり	22 (62.9)	109 (35.4)	35 (76.1)	76 (30.0)
認知症，人数（%）				
軽度	12 (34.3)	194 (63.0) [b]**	12 (26.1)	166 (65.6) [b]**
重度	23 (65.7)	114 (37.0)	34 (73.9)	87 (34.4)
嚥下障害，人数（%）				
なし	9 (25.7)	178 (57.8) [a]**	9 (19.6)	152 (60.1) [a]**
軽度	9 (25.7)	103 (33.4)	14 (30.4)	90 (35.6)
重度	17 (48.6)	27 (8.8)	23 (50.0)	11 (4.3)
抗菌薬の使用，人数（%）				
0	23 (65.7)	269 (87.3) [a]**	33 (71.7)	223 (88.1) [a]*
1～4日	5 (14.3)	24 (7.8)	7 (15.2)	18 (7.1)
5日以上	7 (20.0)	15 (4.9)	6 (13.0)	12 (4.7)

[a]有意差検定にはMantel-Haenszelのカイ2乗検定を用いた。[b]有意差検定にはPearsonのカイ2乗検定を用いた。* $P < 0.05$, ** $P < 0.01$

（筆者作成）

研究にこだわることは，被験者に不必要なX線写真の撮影を強要することになりかねず，昨今の倫理審査制度を考えると，研究の範囲を著しく狭める結果になりかねない。

いずれにしても，本研究において発熱日数と肺炎発症率が各クラスターで同様の傾向を示したことは肺炎の臨床診断の妥当性を裏づけていると思われる。さらに，クラスターA，B，C，Dにおける肺炎による死亡率はそれぞれ，17%，60%，50%および100%であり，明らかにクラスターAの肺炎による死亡率が他のクラスターに比べて低いという結果とも矛盾しない。死亡率については，調査期間中の全死亡数が少ないため統計的有意差を示すことはできなかったが，これらの結果は肺炎発症の頻度がクラスターAの細菌叢を有する高齢者に少ないことを示していると言えそうである。

それでは実際にどのような細菌がこのような健康にかかわっているのであろうか。そこで，各クラスターの特徴となるTRFsに割り振られる細菌

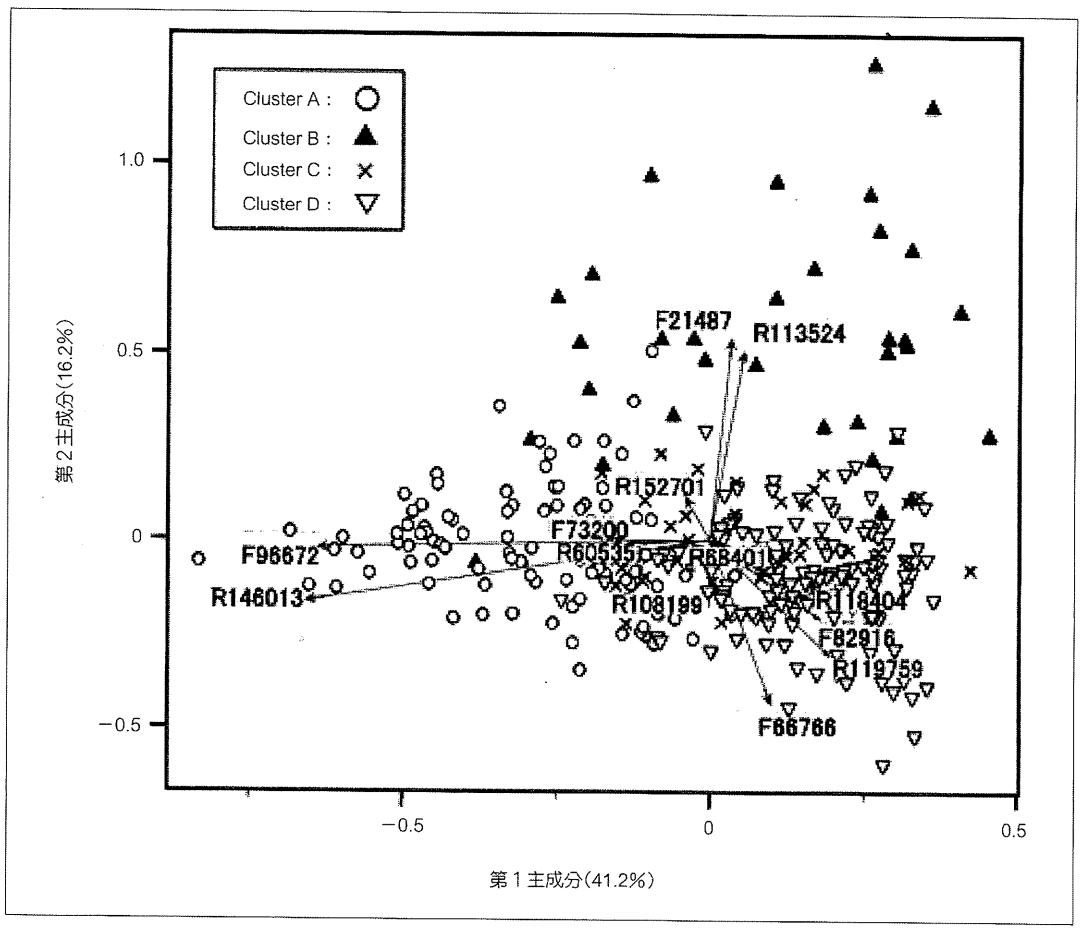

図　T-RFLP パターンの主成分分析による第１，２主成分の散布図

x 軸は第１主成分（寄与率 41.2%），y 軸は第２主成分（寄与率 16.2%）を示す。

T-RFLP：Terminal restriction fragment length polymorphism

（筆者作成）

種の特徴を調べたところ，クラスターＡでは連鎖球菌（*Streptococcus*）と *Rothia* 属が，クラスターC，Dでは *Prevotella* 属，*Veillonella* 属，*Treponema* 属が他のクラスターに比較して優位であることがわかった。さらに，クラスターＣとＤに限って２つのクラスターの相違を見てみると，クラスターＤでは *Neisseria* 属，*Haemophilus* 属，*Aggregatibacter* 属の存在がクラスターＣに比べてより優位であることがわかったが，これら２つのクラスターは高齢者の肺炎関連疾患に対する影響に大きな差が認められないことから，臨床的にこれらのクラスターを分類する意味は今のところ不明である。また，クラスターC，Dのいずれにも肺炎の起因菌として通常あげられている細菌種の存在を検出することはできなかった。

Prevotella 属，*Veillonella* 属および *Treponema* 属が高齢者の肺炎発症や発熱にどのような役割を果たしているのかについては今のところ明らかではないが，*Prevotella* 属，*Veillonella* 属および *Treponema* 属が優位な舌苔では肺炎を発症しやすく，反対に，連鎖球菌と *Rothia* 属が優位な舌苔では肺炎を発症し難いことが本研究の結果から示された。すなわち，高齢者の健康管理を行う上で口腔細菌叢の分析が有益な情報をもたらし，口腔細菌叢を

表2　診査項目と肺炎および発熱日数との関連性

診査項目	肺炎	発熱日数
	ハザード比（95%信頼区間）	オッズ比（95%信頼区間）
クラスター		
A	1	1
B	2.7（0.6～12.6）	10.5（2.0～55.5）**
C	4.0（1.1～15.1）*	4.4（1.1～17.8）*
D	4.9（1.2～21.1）*	11.6（2.3～57.8）**
舌苔		
なし・少量	1	1
中等量・多量	3.2（1.4～7.3）**	3.7（1.4～9.6）**
舌の湿潤度		
5.0 mm以上	1	1
1.0～4.9 mm	2.9（1.1～8.0）*	2.7（1.0～7.3）*
1.0 mm未満	7.9（2.8～22.4）**	10.3（2.9～36.4）**
身体活動レベル		
歩行可	1	1
車椅子使用	2.6（0.7～10.1）	4.6（0.8～25.3）
寝たきり	1.3（0.3～6.4）	6.7（1.1～39.4）*
嚥下障害		
なし	1	1
軽度	1.4（0.5～3.9）	1.5（0.5～4.1）
重度	7.0（1.7～28.9）**	9.5（2.1～42.9）**
抗菌薬の使用		
0	1	1
1～4日	2.7（0.9～8.2）	4.0（1.1～15.1）
5日以上	2.8（1.0～7.5）*	3.6（0.8～15.6）

ハザード比はCox比例ハザード回帰分析を用いて算出した。オッズ比は多重ロジスティック回帰分析を用いて算出した。* $P < 0.05$, ** $P < 0.01$

（筆者作成）

健康な状態で維持する上で有用なガイドラインとなることが期待される。

一方，不思議なことに，クラスターBを特徴づけるTRFsには口腔内に一般に検出されているどの細菌も割り振ることができなかった。確かに，クラスターBの肺炎発症率を単純にクラスターAと比べると著しく高い数値を示している。しかし，多変量解析を行った結果では，クラスターBの患者の細菌叢はクラスターCやDの細菌叢ほど肺炎の臨床診断と密接にかかわっておらず，クラスターBの患者の多くが嚥下障害および摂食困難を示していることを考慮すると，むしろ，嚥下障害が交絡した結果を示していると考えられる。

Ⅳ　おわりに

本稿では高齢者の健康に大きく影響する肺炎あるいはこれに類似の健康問題を取り上げ，口腔細菌との関連性を考察した。従来，肺炎の発症については特定の病原細菌の有無に焦点を当てた議論が盛んに行われ，原因菌の探究が大きな研究の目

標とされてきた。しかし，健康状態が必ずしも良好でない高齢者では，病原性の高い細菌感染がなくても肺炎に類似した健康障害を生じ得ることを考慮すると，病原性の低い細菌が健康障害に与える影響も加味する必要がある。特に，口腔には数百に上る細菌種が共生していることを考えると，細菌叢全体としての病原性の評価という観点から，常在細菌叢の疾病への関与を考え直す必要がありそうである。

近年，細菌叢の解析技術は本稿で紹介したT-RFLP法を始め，さまざまな技術が開発されているが，得られた莫大なデータをどのように分析するのかについては，いまだその方向性は定まっていない。今後，そのような分析方法の統一見解が見出されることで，これまでにない新規の感染症の概念が確立されることが期待される。

文　献

1) El-Solh AA, Pietrantoni C, Bhat A, et al: Microbiology of severe aspiration pneumonia in institutionalized elderly. Am J Respir Crit Care Med **167**: 1650-1654, 2003
2) Didilescu AC, Skaug N, Marica C, et al: Respiratory pathogens in dental plaque of hospitalized patients with chronic lung diseases. Clin Oral Invest **9**: 141-147, 2005
3) Raghavendran K, Mylotte JM, Scannapieco FA: Nursing home-associated pneumonia, hospital-acquired pneumonia and ventilator-associated pneumonia: the contribution of dental biofilms and periodontal inflammation. Periodontology 2000 **44**: 164-177, 2007
4) Yoneyama T, Yoshida M, Ohrui T, et al: Oral care reduces pneumonia in older patients in nursing homes. J Am Geriatr Soc **50**: 430-433, 2002
5) Terpenning MS, Taylor GW, Lopatin DE, et al: Aspiration pneumonia: dental and oral risk factors in an older veteran population. J Am Geriatr Soc **49**: 557-563, 2001
6) Takeshita T, Nakano Y, Yamashita Y: Improved accuracy in terminal restriction fragment length polymorphism phylogenetic analysis using a novel internal size standard definition. Oral Microbiol Immunol **22**: 419-428, 2007
7) Takeshita T, Nakano Y, Kumagai T, et al: The ecological proportion of indigenous bacterial populations in saliva is correlated with oral health status. ISME J **3**: 65-78, 2009
8) Takeshita T, Suzuki N, Nakano Y, et al: Relationship between oral malodor and the global composition of indigenous bacterial populations in saliva. Appl Environ Microbiol **76**: 2806-2814, 2010
9) Takeshita T, Tomioka M, Shimazaki Y, et al: Microfloral characterization of the tongue coating and associated risk for pneumonia-related health problems in institutionalized older adults. J Am Geriatr Soc **58**: 1050-1057, 2010

Ⅱ. 歯

5. 歯周病とメタボリックシンドローム

Association between periodontal diseases and metabolic syndrome

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我々は歯周病とメタボリックシンドロームとの関連性に着目して，両者の関連性を久山町コホート研究の対象者について調べてみた．その結果，メタボリックシンドロームの陽性項目数が増えるに従い歯周病患者の割合が段階的に増加し，メタボリックシンドロームの項目が4つ以上陽性になると，陽性項目が全くない健常者に比べ6.6倍も歯周病患者が増えていた．さらに2010年，歯周病がメタボリックシンドロームを悪化させることを示唆するコホート疫学研究が報告されており，歯周病患者ではメタボリックシンドロームになりやすい，あるいは悪化しやすいと考えられる．

key words

歯周病
メタボリックシンドローム
耐糖能
肥満
生活習慣病

はじめに

歯周病はう蝕(むし歯)と並ぶ歯科の二大疾患の一つであり，歯科医学の中で重要な位置を占める疾患であるが，一般にはその重要性が十分に認識されていないように思われる．たとえば，幼児のう蝕予防を中心として普及してきた1歳6ヵ月児歯科健診や3歳児歯科健診の受診率は，2008年度でそれぞれ92.6%と89.8%と極めて高い値を示すのに対し，成人を対象とした歯周病の検診の受診率はいまだに数%台にとどまっていることをみれば，そのことは火をみるより明らかである．確かに，1961年に開始された3歳児歯科健診や，1977年に開始された1歳6ヵ月児歯科健診に比べ，歯周病の検診の歴史が浅いことがその低受診率の原因となっている事実は否めない．しかし，老人保健法に基づく老人保健事業の一環として2000年に40歳および50歳の者を対象とした歯周病検診が開始されて以来，検診対象年齢層の拡充などの手が打たれて検診者数は増加してはいるものの，10年が過ぎた現在でも受診率はさほど変化していないのが実状である．さらに老人保健法が廃止され，2008年からは歯周病の検診は新しく健康増進事業に組み入れられたが，検診を継続するための財源の担保は十分とはいえず，今後の各市町村における検診の継続性も不透明な状況である．テレビでは歯周病予防を目的とした歯磨き剤や洗口剤のコマーシャルが放映され，歯周病の脅威が日々訴えられているが，前述のように歯周病検診の受診率が一向に向上しない現状では，実際に国民の心にどの程度響いているのかは今ひとつ定かではない．

本稿では，歯周病と近年メタボ検診として注目されているメタボリックシンドロームとの関連性に焦点をあて，読者にこれからの歯科医療において歯周病対策がいかに重要であるかを認識していただく契機としたい．

歯周病と全身疾患

古くから局所の慢性炎症が全身の健康に影響を及ぼすことが少なからず認識されており，1900年には英国のW. Hunterが歯科の慢性炎症が原病巣となり遠隔の組織に炎症性の疾患を惹起

する可能性を“Oral Sepsis（口腔敗血症）”として提唱した[1]．この提唱には賛否両論があったが，米国のF. BillingらはW. Hunterの考えを裏付ける研究を進め，“Focal Infection（病巣感染）”という概念を確立した[2]．歯科医療における病巣感染の考え方は特に歯性病巣感染として取り上げられた．その歴史的変遷については最近，秀逸な総説[3]が報告されているので詳細についてはその総説に譲るが，歯性病巣感染という考え方はある意味で保存的な歯科医療を否定する側面もあり，ややもすると抜歯優先の考え方に偏向しかねない危惧もあったようである．この辺りに，歯性病巣感染の概念が歯科界で十分に成熟してこなかった理由があるのかもしれない．しかし，1980年代になると歯周病が特異的な細菌の感染症であるとする考え方が広まるとともに，歯周病細菌の研究が飛躍的な進歩を遂げ，これらの細菌が全身に及ぼす影響についても科学的な根拠が示されるようになった．

さらに1990年代に入り，数々の疫学研究によって口腔の健康と全身の健康との関連性が明らかにされて，かつての歯性病巣感染を連想させる概念が復活し，その妥当性が見直された．その結果，米国から，歯周病が虚血性心疾患，低体重児出産，呼吸器系疾患，糖尿病などの全身の健康に影響するとする“Periodontal Medicine”の概念が提唱され，その後も口腔保健が全身の健康の維持・増進に大きく寄与することを示す多数の疫学研究が続々と報告されている．このようななか，米国のHealthy People 2000，同2010や，わが国の健康日本21[4]でも口腔保健が単に口腔の健康維持にとどまらず，健康寿命の延伸のために重要であることが謳われている．このPeriodontal Medicineの考え方で興味深い点は，かつての病巣感染から波及する二次疾患には主に炎症に関連した疾患が並んでいたのに対し，この新しい概念では直接炎症に関連しない糖尿病や虚血性心疾患などの生活習慣病に歯周病が関連するとしたことである．

メタボリックシンドローム

先進諸国の健康問題はかつての感染症から，心疾患，脳卒中，悪性新生物などの生活習慣関連病に移行しており，なかでも心疾患や脳卒中の多くは動脈硬化症が原因となっている．メタボリックシンドロームは，生活習慣病に関連する肥満，耐糖能異常，脂質異常症，高血圧などの重複によって動脈硬化症を発症するリスクが増大する事実に基づいて提唱された概念である．わが国では，2005年4月に8つの学会の合意に基づき，内臓脂肪蓄積（内臓脂肪面積100cm^{2}以上）の代替マーカーとして，腹囲が男性で85cm，女性で90cm以上であることを必須項目とし，かつ①血清脂質異常（トリグリセリド値150mg/dL以上，またはHDL-コレステロール値40mg/dL未満），②血圧高値（最高血圧130mmHg以上，または最低血圧85mmHg以上），③高血糖（空腹時血糖値110mg/dL以上）の3項目のうち2項目以上を満たす場合をメタボリックシンドロームとした．同じく2005年に，国際糖尿病学会（IDF）から提唱された基準はわが国の基準と同様に腹囲を必須項目としているが，米NCEP-ATP Ⅲ（National Cholesterol Education Program Adult Treatment Panel Ⅲ）の基準（2001年）では，各項目の比重は皆等しく，腹囲（肥満），中性脂肪，HDL-コレステロール，血圧，空腹時血糖の5つの項目の中からいずれか3つ以上の項目が陽性となった場合にメタボシックシンドロームと判定するなど，その基準については必ずしも世界的に統一されていない．また，腹囲についてもさまざまな議論の余地があり，IDFの基準では各国の事情で異なるとされている．先述したわが国の基準についても，久山町コホート研究の結果からは，男性90cm，女性80cm以上とした南アジアで用いられている値を用いたほうが動脈硬化性疾患の発症をよりよく予測できることが報告されており，近い将来にわが国のメタボリックシンドロームの基準値の改訂が行われる可能性がある．

歯周病とメタボリックシンドローム

メタボリックシンドロームの重要な徴候の一つである，血糖値の上昇が主症状となる糖尿病は古くから歯周病と関連することが報告されている．

糖尿病患者の歯周病が重篤であるとの報告は1899年のO.G. Grunertによるものがその始まりとされているが，その後J.B.Williamが1928年に糖尿病患者の歯周病の特徴を“Diabetic

periodontoclasia”として提唱したことが歯周病を糖尿病の合併症に数える今日の概念に大きく影響した[5]．糖尿病患者は一般に易感染性と考えられており，糖尿病患者に歯周病が多発することや，発症した歯周病が重症化することは想像に難くない．実際，糖尿病群は非糖尿病群に比較して歯周炎がより重症であり，糖尿病患者は非糖尿病者に比べ歯周病の発症率が2.6倍高いことが，ピマ・インディアンを調べた米国の疫学研究において報告されている[6]．その後の横断研究でも，2型糖尿病患者は非糖尿病者よりもアタッチメントロス(attachment loss：エナメルセメント境からポケット底までの距離)が大きく，歯周病が悪化しやすいこと[7]，さらに2年間のコホート研究では非糖尿病者に比較して歯槽骨吸収がより多かったことが報告されている[8]．その他にも2型糖尿病が歯周疾患の発症や重症度に影響することを示す多くの研究結果があるが，同じ2型糖尿病患者でも血糖値のコントロールが不良な群では，良好にコントロールされている群に比較して歯槽骨吸収がより進んでいたと報告されている[9]．一方，血糖値のコントロールが良好な場合には非糖尿病者と比べて歯周病の有病率に有意な差はないとの報告もあり[10]，メタボリックシンドロームの徴候の一つである血糖値が歯周病の発症に強く影響することが示唆されている．しかし，これらのデータのほとんどが日本人のものではなく，さらに日本人が欧米人に比較してインスリン分泌能が低く，糖尿病に対する感受性が高いことを考えると，これらのデータを鵜呑みにしてそのまま日本人に適用することは慎むべきであろう．

そこで，久山町コホート研究のデータから日本人における歯周病と耐糖能の関係をみてみたい．表1に久山町の一斉検診(1998年)の際に歯周病検診を行った被験者1,111名中，歯が10本以上あった961名(男性377名，女性584名)について，正常耐糖能(空腹時血糖値＜110mg/dL かつ糖負荷試験後2時間の血糖値＜140mg/dL)，糖尿病(空腹時血糖値＜126mg/dL かつ糖負荷試験後2時間の血糖値＜200mg/dL)，血糖値がいずれにも該当しない耐糖能異常(糖尿病には至らないが血糖値が異常に高値を示すもの)の3群に分けてその歯周組織の状態を比較した結果を示す[11]．

本診査では歯周組織の状態をNHANES Ⅲ (National Health and Nutrition Examination Sarvey Ⅲ)の方法で評価し，歯周ポケット深さやアタッチメントロスをmm単位で比較することが可能であるため，他の多くの疫学研究で用いられているCPI (Community Periodontal Index) に比較して歯周組織の状態をより詳細に分析できる利点がある．多重比較による統計分析では，平均値および最大値のいずれでみても，現状の歯周病の状態を示す歯周ポケット深さおよび過去か

表1　耐糖能別にみた各群間の歯周組織の状態の比較

歯周組織	正常耐糖能群 (n＝669名)	糖尿病群 (n＝101名)	耐糖能異常群 (n＝191名)
	平均[a] (標準偏差)		
平均[b] 歯周ポケット深さ(mm)	1.6(0.5)	1.8(0.6)***	1.7(0.5)**
最大歯周ポケット深さ(mm)	3.5(1.4)	4.0(1.7)**	3.9(1.5)*
平均[b] アタッチメントロス(mm)	1.9(0.8)	2.3(0.9)***,†	2.0(0.9)
最大アタッチメントロス(mm)	4.1(1.7)	4.9(2.1)**	4.5(2.1)

a：集団内における個人の値の平均，b：個人内における複数の診査箇所の平均．
群間の統計学的有意差の比較はボンフェローニ法による多重比較によって行った．
*：正常耐糖能に対して$p<0.05$，**：正常耐糖能に対して$p<0.01$，
***：正常耐糖能に対して$p<0.001$の統計学的有意差を示す．
†：糖尿病と耐糖能異常間で$p<0.05$の統計学的有意差を示す．

(文献11)より引用)

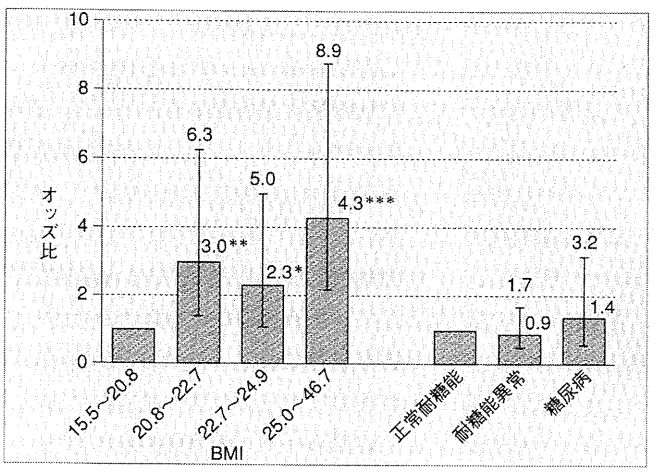

図1　多変量解析によるBMIおよび耐糖能と歯周病との関連性
歯周病を目的変数，年齢，歯垢スコア，性別，運動の頻度，アルコール摂取量，喫煙，職業，BMIおよび耐糖能を説明変数とした多重ロジスティック回帰分析結果のBMI（左側）と耐糖能（右側）のオッズ比を示す．平均歯周ポケット深さが1.9mm以上を歯周病とした．
*：p<0.05，**：p<0.01，***：p<0.001
（文献12)より改変引用）

らの歯周病の進行状態を示すアタッチメントロスの両者が糖尿病群では正常耐糖能群に比較して有意に悪化しており，糖尿病患者に歯周病が多いとする過去の通説を裏付ける結果となった．また，糖尿病には至らないが，血糖値が高値を示す耐糖能異常群では，アタッチメントロスでは正常耐糖能群との間に有意差は認められなかったが，歯周ポケット深さは正常耐糖能群よりも有意に深く，血糖値と歯周組織の二者の関係に限定すれば，日本人においても糖尿病は歯周病に関連していた．

しかし，その一方で歯周病には糖尿病以外の喫煙や肥満などのさまざまな要因が影響を与えることが明らかになっており，これらの交絡因子の影響を加味して糖尿病と歯周病の関連性を調べる必要がある．そこで，同じ対象者について歯周病に関連すると報告のある因子［年齢，性別，BMI（Body Mass Index），運動の頻度，アルコール摂取量，喫煙］を独立変数とした多重ロジスティック回帰分析により，耐糖能と歯周病の関係を調べた．その結果，耐糖能異常や糖尿病が歯周病と有意に関連していることが示唆されたことから，日本人においても糖尿病患者に歯周病の患者が多いと結論付けることはできそうである[11]．

ところが，先述の調査対象者から女性（584名）のみを抽出して，年齢，歯垢スコア，性別，BMI，耐糖能，運動の頻度，アルコール摂取量，喫煙，職業を同時に独立変数とした多重ロジスティック回帰分析を行うと，BMIは歯周病に関連した有意な独立変数であったが（図1左），耐糖能異常は糖尿病も含めて歯周炎と有意な関連性は認められなかった（図1右）[12]．

さらに，BMIを肥満と関連する体脂肪率やウエストヒップ比に置き換えて，同様の多変量解析を行っても歯周病と耐糖能異常とは有意な関連性を示さなかった．また，アタッチメントロスにより歯周病を判定した場合は，耐糖能異常のみならず肥満関連因子さえも歯周病とは有意な関連を認めなかった．これらの結果に基づけば，これまでの多くの報告のように「糖尿病があれば歯周病になりやすい」と単純に結論付けることはできないことになる．ただし，有意差はないがオッズ比には両者に関連のある傾向があることから，調査対象者を女性に絞り込んだことで被験者数が減少したことがこのような結果を生んだ可能性もある．また，女性ホルモンと骨代謝には密接な関係があることから，そのような性差が歯周炎の感受性に影響しているのかもしれない．これらの点に関しては今後の研究課題といえる．

一方で，メタボリックシンドロームは虚血性心疾患のリスクとして捉えられているが，歯周病は虚血性心疾患にも関連するという疫学研究が多数報告されている．そこで，我々は耐糖能のみに固執するのではなく，歯周病とメタボリックシンドロームとの関連性についても先述の対象者について調べてみた．この研究ではメタボリックシンドロームの定義は米国のNCEP ATP

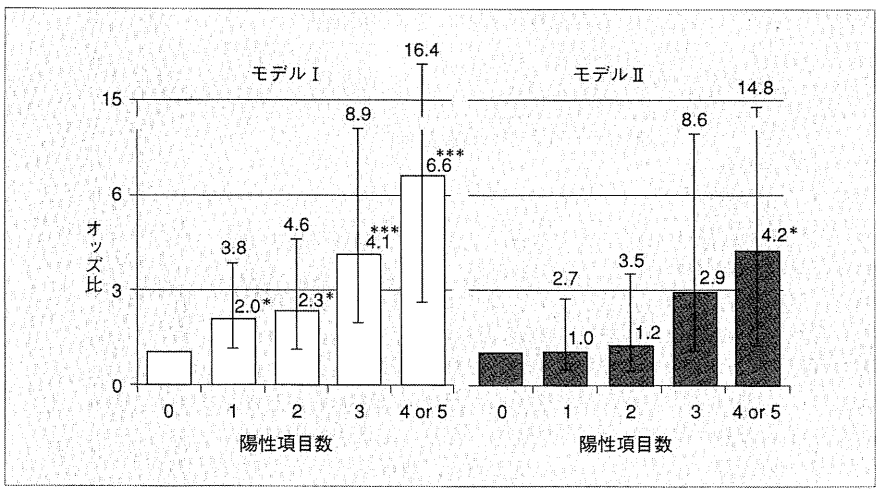

図2　メタボリックシンドロームの陽性項目数と歯周病との関連性

歯周病を目的点数，年齢，喫煙，脂質抑制剤の服用，総コレステロールを説明変数とした多重ロジスティック回帰分析結果を示す．モデルⅠでは平均ポケット深さが2mm以上を歯周病とし，モデルⅡでは平均アタッチメントロスが3mm以上を歯周病とした．
*：$p<0.05$，***：$p<0.001$

（文献13）より改変引用）

Ⅲの基準に従った．その結果，メタボリックシンドロームの陽性項目数が増えるほど歯周病患者の割合が段階的に増加した．歯周病を平均歯周ポケット深さで定義したモデルⅠでは，メタボリックシンドロームの項目が4つ以上陽性になると陽性項目が全くない健常者に比べ，実に6.6倍も歯周ポケットの深い歯周病患者が増える結果となった（図2）[13]．

その後，他の多くの研究においても同様の結果が報告されている（表2）[14)-17)]が，これらのほとんどは時間軸のない横断的な疫学研究結果であり，その因果関係の考察は難しい．

しかし2010年，歯周病がメタボリックシンドロームを悪化させることを示唆するコホート研究が報告され[17]，その因果関係の解明に一歩を進めた．この研究ではベースラインの産業健診の受診者を歯周病の有無の2群に分け，4年後にメタボリックシンドロームの陽性項目数の増加に2群間で差があるか否かを調べている．その結果，歯周病があった群において陽性項目の増加数が有意に高いことが示されており，歯周病があるとメタボリックシンドロームになりやすい，あるいは悪化しやすいことを強く示唆している．しかし，歯周病をアタッチメントロスで定義したモデルⅡではモデルⅠほど歯周病とメタボリックシンドロームとの関連性は強く表れていない．アタッチメントロスは歯周病の過去の病歴も蓄積して評価するが，現時点の歯周ポケットの有無のほうがよりメタボリックシンドロームと関連性が強いという結果は，歯周ポケット内の細菌からの持続的な刺激が大きな意味を持つことを示唆しているのかもしれない．この点についても今後の検討が必要である．

おわりに

超高齢社会を迎えるわが国の健康問題として，生活習慣病は避けて通れない重要な課題である．歯周病と生活習慣病の関連は古くから語り継がれてきたにもかかわらず，その成果が実際の医療現場において十分に生かされてこなかったきらいがある．医科歯科の連携がわが国の健康問題の喫緊の課題であることを，小論を通して幅広い分野の方々に感じていただければこの上のない喜びである．

文　献

1) Hunter W：Oral sepsis as a cause of disease. Br Med J **1**：215-216, 1900
2) Billings F：Focal Infection as the cause of general disease. Bull N Y Acad Med **6**：759-773, 1930
3) 奥田克爾：「歯性病巣感染」温故知新．歯科学報 **110**：288-291, 2010
4) 厚生労働省ホームページ：http://www1.mhlw.go.jp/topics/kenko21_11/s0.html
5) Manouchehr-Pour M, Bissada NF：Periodontal disease in juvenile and adult diabetic patients；a review of the literature. J Am Dent Assoc **107**：766-770, 1983
6) Nelson RG, Shlossman M, Budding LM, et al：Periodontal disease and NIDDM in Pima Indians. Diabetes Care **13**：836-840, 1990
7) Cerda J, Vázquez de la Torre C, Malacara JM, et al：Periodontal

表2　歯周病とメタボリックシンドロームの関係を示す主な疫学研究結果

文献番号	調査対象者	年齢	研究手法	主な結果
13	地域住民584名（女性584名，男性0名）	40～79歳 平均年齢：55.7歳	横断研究	図1に結果を要約
14	第3回米国健康栄養調査の受診者の中で歯周病検診を受けた者 13,677名（女性の割合：49.4％）	17歳以上 平均年齢：40.8歳	横断研究	45歳以上で重度の歯周炎の者では，歯周炎のない者に比較して，メタボリックシンドロームである割合が2.31倍（95％信頼区間：1.13～4.73）高い．歯周病の評価は文献13に同じ．
15	産業健診の受診者2,478名（男性2,028名，女性450名）	24～60歳 平均年齢：43.3歳	横断研究	文献13と同様の関係が男女ともに認められる．また，両者の関係は年齢が高いほど強い．歯周病の評価には文献13よりも簡易なCPIを使用．
16	地域住民1,070名（男性281名，女性789名）	40，50，60，70歳	横断研究	文献1と同様の関係が認められる．歯周病の評価には文献13よりも簡易なCPIを使用．
17	産業健診の受診者1,023名（男性727名，女性296名）	20～56歳 開始時の平均年齢：37.3歳	コホート研究	文献15に継続した4年後データによるコホート研究．深さ4 mm以上の歯周ポケットがあると，メタボリックシンドロームの項目が1つ陽性に変化するオッズ比が1.4（95％信頼区間：1.0～2.1），2つあるいはそれ以上の項目が陽性に変化するオッズ比が2.2（95％信頼区間：1.1～4.1）であった．

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disease in non-insulin dependent diabetes mellitus (NIDDM). The effect of age and time since diagnosis. J Periodontol **65** : 991-995, 1994

8) Taylor GW, Burt BA, Becker MP, et al : Non-insulin dependent diabetes mellitus and alveolar bone loss progression over 2 years. J Periodontol **69** : 76-83, 1998

9) Taylor GW, Burt BA, Becker MP, et al : Glycemic control and alveolar bone loss progression in type 2 diabetes. Ann Periodontol **3** : 30-39, 1998

10) Tsai C, Hayes C, Taylor GW : Glycemic control of type 2 diabetes and severe periodontal disease in the US adult population. Community Dent Oral Epidemiol **30** : 182-192, 2002

11) Saito T, Shimazaki Y, Kiyohara Y, et al : The severity of periodontal disease is associated with the development of glucose intolerance in non-diabetics ; The Hisayama study. J Dent Res **83** : 485-490, 2004

12) Saito T, Shimazaki Y, Kiyohara Y, et al : Relationship between obesity, glucose tolerance, and periodontal disease in Japanese women ; the Hisayama study. J Periodont Res **40** : 346-353, 2005

13) Shimazaki Y, Saito Y, Yoneyama K, et al : Relationship of metabolic syndrome to periodontal disease in Japanese women ; the Hisayama Study. J Dent Res **86** : 271-275, 2007

14) D'Aiuto F, Sabbah W, Netuveli G, et al : Association of the metablic syndrome with severe periodontitis in a large U.S.population-based survey. J Clin Endocriol Metab **93** : 3989-3994, 2008

15) Morita T, Ogawa Y, Takada K, et al : Association between periodontal disease and metabolic syndrome. J Pub Health Dent **69** : 248-253, 2009

16) Kushiyama M, Shimazaki Y, Yamashita Y : Relationship between metabolic syndrome and periodontal dsease in Japanese adults. J. Preiodontol **80** : 1610-1615, 2009

17) Morita T, Yamazaki Y, Mita A, et al : A cohort study on the association between periodontal disease and the development of metabolic syndrome. J. Preiodontol **81** : 512-519, 2010

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Relationship between Oral Malodor and the Global Composition of Indigenous Bacterial Populations in Saliva

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Relationship between Oral Malodor and the Global Composition of Indigenous Bacterial Populations in Saliva▽

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Oral malodor develops mostly from the metabolic activities of indigenous bacterial populations within the oral cavity, but whether healthy or oral malodor-related patterns of the global bacterial composition exist remains unclear. In this study, the bacterial compositions in the saliva of 240 subjects complaining of oral malodor were divided into groups based on terminal-restriction fragment length polymorphism (T-RFLP) profiles using hierarchical cluster analysis, and the patterns of the microbial community composition of those exhibiting higher and lower malodor were explored. Four types of bacterial community compositions were detected (clusters I, II, III, and IV). Two parameters for measuring oral malodor intensity (the concentration of volatile sulfur compounds in mouth air and the organoleptic score) were noticeably lower in cluster I than in the other clusters. Using multivariate analysis, the differences in the levels of oral malodor were significant after adjustment for potential confounding factors such as total bacterial count, mean periodontal pocket depth, and tongue coating score ($P < 0.001$). Among the four clusters with different proportions of indigenous members, the T-RFLP profiles of cluster I were implicated as the bacterial populations with higher proportions of *Streptococcus*, *Granulicatella*, *Rothia*, and *Treponema* species than those of the other clusters. These results clearly correlate the global composition of indigenous bacterial populations with the severity of oral malodor.

Oral malodor is one of the major complaints made by patients visiting the dentist, ranking behind only dental caries and periodontal disease (19). It originates mainly from the mouth itself, and the malodorous substrates most commonly are associated with microbial metabolism (32). Major compounds that contribute to oral malodor are volatile sulfur compounds (VSCs) such as hydrogen sulfide (H_2S), methyl mercaptan (CH_3SH), and dimethyl sulfide (CH_3SCH_3) (14, 35). Additionally, short-chain fatty acids, such as propionic acid and butyric acid, cadaverine, indole, and scatole, have been reported to cause oral malodor (8, 16).

The oral surfaces are colonized by large numbers of bacterial species with many members, especially gram-negative anaerobes, which are known to produce malodorous compounds (28). Poor oral hygiene resulting in microbial overgrowth clearly is involved in the development of this condition. Hence, the current major treatment for oral malodor focuses on nonselective antibacterial treatments to reduce the total number of bacteria, with careful attention to anaerobic areas such as the periodontal pockets and tongue dorsum. This approach, however, generally provides short-term benefits, since malodor-causing bacteria quickly recover to their former numbers when treatment is stopped (2).

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An alteration in the bacterial population structure would be necessary to completely cure oral malodor. One general and reasonable approach is to specify the causal agent and directly remove it from the oral cavity. A diversity of bacteria, including *Porphyromonas gingivalis*, *Fusobacterium nucleatum*, *Prevotella intermedia*, and *Treponema denticola*, have been closely associated with oral malodor (19). On the other hand, developing an elimination method that targets various multiple causative agents remains a challenge.

Malodor producers are considered members of the oral microbial ecosystem, which is regulated by numerous interactions among inhabitants. Therefore, adjusting the global composition of indigenous bacterial populations toward a "healthy" pattern may be an alternative approach to effectively prevent oral malodor. Indeed, probiotic treatments have been conducted for the maintenance or manipulation of indigenous bacteria in the gastrointestinal tract (3) and vagina (1), and attempts have been made to apply the same approach to oral malodor (2). However, whether characteristic patterns of healthy and oral malodor-related microbiota or nonspecific bacterial overgrowth result in oral malodor remains unclear. Although the bacterial population structure of tongue microbiota of subjects with and without oral malodor has been revealed comprehensively using a molecular approach (9, 11, 30), a statistical analysis of a larger sample is necessary to correlate the complex microbial community composition with the severity of the condition.

Despite a lack of fully resolved phylogenetic analyses at the species level, terminal-restriction fragment length polymorphism (T-RFLP) analysis is an effective molecular approach

for the rapid assessment and comparison of large numbers of complex bacterial communities (18). The relative differences in microbial community structures are reliably reflected in T-RFLP profiles, although some biases may impair the detection of the actual community structure (10). We previously confirmed that major bacterial genera in the oral cavity can be distinguished roughly by this method using a specific primer set and the restriction enzyme HaeIII. Relative bacterial abundances detected in cloning and sequencing methods were fairly consistent with the peak area proportions of the corresponding fragments in the T-RFLP profile (33).

In the present study, the global composition of bacterial populations found in the saliva of 240 patients complaining of oral malodor, with highly variable levels of severity, was examined using T-RFLP analysis. Although the use of saliva as the most appropriate oral specimen to study the relationship between oral microbiota and health has been questioned, it was considered the sample that would best represent the overall microbial population in the oral cavity. While the bacterial composition in saliva was highly similar to that found in the tongue coating among the different surfaces in the oral cavity (21), bacteria that inhabit other oral surfaces also can be recovered from saliva (4, 15, 20). Microbial community compositions showing a similar pattern were divided into groups using cluster analysis, and the patterns of the microbiota of those exhibiting higher and lower malodor were explored. The significance of malodor severity based on the pattern of microbiota was statistically evaluated using multivariate analysis while controlling for the effect of other oral malodor-associated factors. The aim of this study was to elucidate the relationship between the global composition of indigenous bacterial populations and the severity of oral malodor.

MATERIALS AND METHODS

Subjects and study design. The study population consisted of 240 patients complaining of oral malodor (110 females and 130 males, aged 12 to 79 years with a mean age of 45 ± 14 years) who visited the Oral Malodor Clinic at Fukuoka Dental College Medical and Dental Hospital in Japan. These 240 patients included both subjects with actual oral malodor and those without oral malodor who were unsure if or believed that they had oral malodor. The Ethics Committee of Fukuoka Dental College and Kyushu University Faculty of Dental Science approved the study design. All participants understood the nature of the study and provided informed consent. Previously to the malodor assessment, each subject was asked to refrain from eating, drinking, chewing, smoking, brushing, or rinsing the mouth for at least 5 h.

Malodor assessment. The severity of oral malodor in each individual was determined using an organoleptic test (OLT), a method used for the direct sniffing of expelled mouth air, and gas chromatography (model GC14B; Shimadzu Works, Kyoto, Japan). The OLT scores were estimated on a scale of 0 to 5 (0, absence of oral malodor; 1, questionable odor; 2, slight malodor; 3, moderate malodor; 4, strong malodor; 5, severe malodor) (22). In this study, we divided the subjects into four categories: 0 or 1, 2, 3, and 4 or 5. The gas chromatography was used for measuring the concentrations of VSCs (H_2S, CH_3SH, and CH_3SCH_3) in mouth air. We divided the subjects into four categories based on the sum of the three VSC concentrations: subjects without oral malodor (≤0.20 ppm, lower than the olfactory threshold for VSC concentrations in mouth air [36]), those with low malodor (0.21 to 0.60 ppm; lower one-third of the total number of subjects with oral malodor), those with moderate malodor (0.61 to 1.10 ppm; middle one-third), and those with high malodor (>1.10 ppm; higher one-third).

Clinical examinations. For each patient, the periodontal pocket depth and the amount of tongue coating, two major halitosis-inducing factors, were examined. The periodontal pocket depth was measured at six points around each tooth in all subjects. The total area and thickness of the tongue coating was scored based on conventional criteria (26), with a slight modification (1, no tongue coating or thin, with less than one-third covered; 2, thin, with one-third to two-thirds covered or thick, with less than one-third covered; 3, thin with more than two-thirds covered or thick with more than one-third covered).

Saliva sample collection and DNA extraction. The subjects were asked to bite on paraffin wax for 5.5 min, and stimulated saliva samples produced during the last 5 min were collected in sterile plastic tubes. The samples were stored at −80°C until further analysis. Bacterial DNA extraction was performed as described previously (34).

Quantification of total bacteria by real-time PCR. Quantitative real-time PCR was performed using a QuantiFast SYBR green PCR kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. The bacterial universal primers 806F (5′-TTA GAT ACC CYG GTA GTG G-3′) and 926R (5′-CCG TCA ATT YCT TTG AGT TT-3′) were used, and the details of the procedure have been described previously (33). The relative amounts of total bacteria were calculated using the comparative C_T method, and DNA extracted from *Streptococcus mutans* Xc was used as a real-time PCR control.

T-RFLP analysis. From each sample, internal regions of 16S rRNA genes were amplified using the universal forward primer 8F (5′-AGA GTT TGA TYM TGG CTC AG-3′) labeled at the 5′ end with 6-carboxyfluorescein (6-FAM) and the universal reverse primer 806R (5′-GGA CTA CCR GGG TAT CTA A-3′) labeled at the 5′ end with hexachlorofluorescein (HEX). PCR amplification, purification, and digestion by the restriction enzyme HaeIII were performed as previously described (33). The restriction digest products were mixed with 10 μl of deionized formamide and 1 μl of the internal standard, which contained GeneScan-500 ROX standard (Applied Biosystems, Foster City, CA) and six additional DNA fragments (541, 600, 663, 730, 799, and 861 bp) labeled at the 5′ end with ROX. The samples were denatured, electrophoresed, and analyzed with GeneMapper version 4.0 (Applied Biosystems). The unit of fragment size instead of number of bases was estimated based on the molecular weight (MW) of each fragment in the internal standard as previously described (34). The terminal restriction fragments (TRFs) with a peak area of less than 0.5% of the total area were excluded from the analysis.

Cluster classification and characterization. Cluster analysis was performed using two T-RFLP profiles obtained per subject using two different fluorescent dyes (6-FAM and HEX). The estimated MWs of all TRFs from each subject were aligned, and the TRFs with MWs that differed by 80 or less were considered identical. The aligned T-RFLP profiles composed of the size of the TRFs and the percentage of the peak area in each profile were clustered by hierarchical cluster analysis using Euclidean distance and Ward's algorithm with R, version 2.8.1 (http://www.r-project.org). The aligned T-RFLP profiles for the 240 subjects also were analyzed by principal component analysis (PCA) and displayed as a biplot diagram to visualize the bacterial composition in correlation with the classified clusters. The PCA compressed the information from the T-RFLP profiles to a small number of dimensions, and they were plotted as dots in the two-dimensional display, of which the x and y axes represented the first and second principal components, respectively, and the original variables (each TRF) were indicated by arrows. The direction and length of the arrows indicated how each TRF contributed to the first two components in the biplot. PCA was performed by using the R library ade4 as described previously (33). Candidate bacterial species corresponding to the combination of TRFs (6-FAM-labeled or HEX-labeled TRFs) were selected based on their size from our oral bacterial database using TRFMAW (http://www.trflp.info/trfmaw) as described earlier (33). Based on our previous work (34), the matching window was set to an MW of ±660. The diversity of the bacterial community was evaluated by the number of TRFs and the Shannon diversity index of T-RFLP profiles. The bacterial candidates corresponding to the TRFs with significantly more or fewer subjects in one cluster than in the other clusters were selected using BP-TRFMA (24). The correlation threshold in BP-TRFMA was set at 0.13, which corresponds to a significant correlation coefficient in this sample size.

Statistical analysis. Tukey's multiple comparison was conducted with R software to look for differences in peak area proportions of TRFs, age, number of total bacteria, mean pocket depth, total number of TRF, Shannon diversity index, and detection frequency of each TRF among the clusters. Fisher's exact test was performed by R to evaluate the statistical differences in gender and tongue coating score. Bivariate and multivariate ordinal logistic regression with the proportional-odds model were used to examine the associations between the intensity of oral malodor and the bacterial population structure in saliva, including other malodor-associated factors that may affect confounding variables. From the ordinal logistic regression model, proportional odds for cumulative probabilities were generated. The statistical analysis was performed using SPSS (version 15.0; SPSS Japan, Tokyo, Japan). The statistical significance was set at $P < 0.05$ to denote a statistically significant difference.

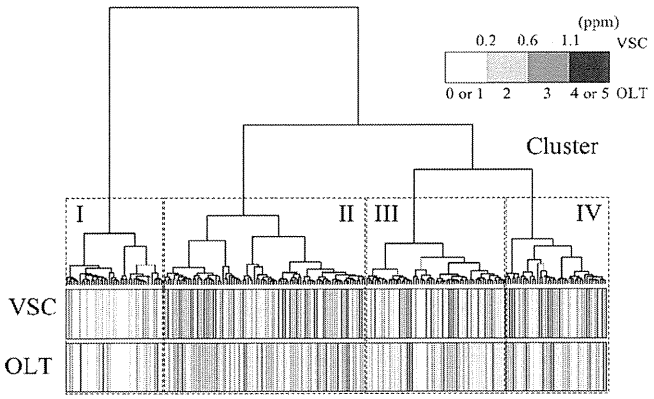

FIG. 1. Cluster analysis of the bacterial population structure in saliva of 240 subjects complaining of oral malodor based on T-RFLP profiles of the 16S rRNA gene. The dendrogram was generated by hierarchical cluster analysis using Euclidean distance and Ward's algorithm. The concentration of total volatile sulfur compounds (VSCs) in mouth air and the organoleptic score (OLT) of each subject were displayed as a gray-scale bar in the lower side of the dendrogram. The bacterial compositions were classified into four clusters: cluster I (n = 43), cluster II (n = 90), cluster III (n = 62), and cluster IV (n = 45).

RESULTS

Differences in malodor productivity associated with T-RFLP profiles. The bacterial composition in the saliva of 240 subjects was assessed using T-RFLP analysis and displayed as peak patterns. The overall profiles contained 164 distinct peaks (TRFs), 99 TRFs in the 6-FAM profiles (F1 to F99) and 65 TRFs in the HEX profiles (R1 to R65). The T-RFLP profiles were compared in a dendrogram generated by hierarchical cluster analysis (Fig. 1). The total concentration of VSCs in mouth air and the organoleptic score of each subject also were displayed. When the bacterial compositions were divided into four groups (clusters I, II, III, and IV), which contained 43, 90, 62, and 45 subjects, respectively, striking differences in malodor productivity were observed among the groups (Fig. 1). Both oral malodor parameters clearly were lower in cluster I, whereas they were higher in clusters III and IV.

Statistical comparison of the severity of oral malodor among clusters. The characteristics of each cluster in terms of age, gender, and other variables possibly associated with oral malodor are shown in Table 1. Significant differences among clusters were observed in mean ages and amount of total bacteria. Tables 2 and 3 show the characteristics of the subjects according to VSC concentrations and organoleptic scores, respectively. To control for the effects of confounding factors, a multivariate analysis was performed that included the above-mentioned variables. After adjusting for potentially confounding variables, subjects belonging to clusters II, III, and IV had significantly higher odds ratios than those for cluster I for both the amount of VSCs in mouth air (Table 2) and organoleptic scores (Table 3). The results suggest that differences in the bacterial colonization pattern were significantly associated with the intensity of oral malodor independently of other variables, including two major halitosis-inducing factors (i.e., an increase in tongue coating and periodontal disease). The adjusted odds ratios for higher levels of VSCs and organoleptic scores were equally high in clusters II (11.0 and 11.4, respectively) and IV (10.8 and 9.8, respectively). Compared to these clusters, the odds ratios were lower for cluster III (4.4 and 3.9, respectively), which exhibited an intermediate pattern in the T-RFLP profile among clusters I, II, and IV.

Mean periodontal pocket depth and tongue coating score also were associated with significantly higher odds ratios for higher levels of VSCs (Table 2) and organoleptic scores (Table 3), as expected. In addition, gender was significantly associated with the organoleptic score (Table 3). Although the total bacterial count in saliva had a significantly increased odds ratio for both a higher VSC level (Table 2) and higher organoleptic score (Table 3) in the univariate analysis, the relationship dissipated after multivariate adjustment.

Bacterial colonization patterns of each predicted cluster based on T-RFLP profiles. To visualize the differences in the T-RFLP profiles among the four clusters, they were analyzed by PCA and represented as four types of dots on a PCA biplot diagram of the first principal component (PC1) and the second principle component (PC2) (Fig. 2). These two components

TABLE 1. Comparisons of general and clinical parameters among four clusters[d]

Variable	Cluster I (n = 43)	Cluster II (n = 90)	Cluster III (n = 62)	Cluster IV (n = 45)	Significant differences between clusters ($P < 0.05$)
Age[a] (yr)	39 ± 15	47 ± 14	47 ± 15	46 ± 14	II > I, III > I
Amt of total bacteria[a] (log deduced CFU[b])	7.6 ± 0.6	8.2 ± 0.7	7.8 ± 0.6	7.8 ± 0.8	II > I, II > III, II > IV
Mean pocket depth[a] (mm)	2.9 ± 0.5	3.0 ± 0.4	3.1 ± 0.6	3.2 ± 1.0	NS
Gender[c]					NS
Female	22 (51.2)	52 (57.8)	34 (54.8)	22 (48.9)	
Male	21 (48.8)	38 (42.2)	28 (45.2)	23 (51.1)	
Tongue coating score[c]					NS
1	23 (53.4)	28 (31.1)	20 (32.2)	17 (37.7)	
2	12 (27.9)	41 (45.5)	32 (51.6)	21 (46.6)	
3	8 (18.6)	21 (23.3)	10 (16.1)	7 (15.5)	

[a] Significant difference between clusters were evaluated by Tukey's multiple comparison.
[b] The copy number of the total bacterial 16S rRNA gene was divided by that of *Streptococcus mutans* UA159, corresponding to 1 CFU per 500 μl.
[c] Significant difference was evaluated by Fisher's exact test.
[d] Data are given either as means ± standard deviations or as the number of data with the percentage in parentheses. NS indicates lack of significance.

TABLE 2. Effects of each variable on the concentration of VSCs[b]

Variable	Total (n = 240)	Concn of VSCs in mouth air (ppm)				Crude odds ratio (95% CI)	Multivariate odds ratio (95% CI)
		≤0.2 (n = 41)	>0.2 (n = 64)	>0.6 (n = 71)	>1.1 (n = 64)		
Age (yr)	45 ± 14	39 ± 14	45 ± 15	45 ± 12	50 ± 15	1.0 (1.0–1.0)***	1.0 (1.0–1.0)
Amt of total bacteria (log deduced CFU[a])	8.0 ± 0.7	7.8 ± 0.7	7.9 ± 0.7	8.0 ± 0.7	8.1 ± 0.7	1.5 (1.1–2.0)*	1.0 (0.7–1.4)
Mean pocket depth (mm)	3.1 ± 0.7	2.8 ± 0.4	3.1 ± 0.8	3.2 ± 0.6	3.3 ± 0.7	1.9 (1.3–2.8)**	1.8 (1.2–2.8)***
Gender							
Female	110 (45.8)	18 (43.9)	30 (46.9)	27 (38.0)	35 (54.7)	1	1
Male	130 (54.2)	23 (56.1)	34 (53.1)	44 (62.0)	29 (45.3)	0.8 (0.5–1.3)	0.9 (0.5–1.4)
Tongue coating score							
1	88 (36.7)	26 (63.4)	28 (43.8)	15 (21.1)	19 (29.7)	1	1
2	106 (44.1)	12 (29.3)	29 (45.3)	38 (53.5)	27 (42.2)	2.3 (1.4–3.8)**	1.9 (1.1–3.3)*
3	46 (19.2)	3 (7.3)	7 (10.9)	18 (25.4)	18 (28.1)	4.3 (2.2–8.3)***	4.0 (2.0–8.3)***
Cluster of T-RFLP profile							
I	43 (17.9)	21 (51.2)	13 (20.3)	9 (12.7)	0 (0)	1	1
II	90 (37.5)	6 (14.6)	19 (29.7)	33 (46.5)	32 (50.0)	11.1 (5.4–23.2)***	11.0 (5.0–24.2)***
III	62 (25.8)	10 (24.4)	22 (34.4)	14 (19.7)	16 (25.0)	5.0 (2.4–10.5)***	4.4 (2.0–9.7)***
IV	45 (18.8)	4 (9.8)	10 (15.6)	15 (21.1)	16 (25.0)	10.2 (4.5–23.3)***	10.8 (4.5–25.5)***

[a] Copy number of the total bacterial 16S rRNA gene was divided by that of *Streptococcus mutans* UA159, corresponding to 1 CFU per 500 μl.
[b] Data are given either as means ± standard deviations or as the number of data, with the percentage in parentheses. CI, confidence interval. Each odds ratio and P values were calculated by ordinal logistic regression analysis. *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$.

explain 66.5% of the total variance. The four clusters were localized in the right (cluster I), lower-left (cluster II), upper-left (cluster IV), and center (cluster III) areas of the diagram. Of a total of 164 TRFs, 18 were selected that had large loadings (>0.05 in absolute value on the PC1 and PC2) (Table 4) and are represented by arrows (Fig. 2). All of the TRFs were predominately and commonly detected in the T-RFLP profiles of most of the subjects (Table 4).

Five (F42, F57, F68, R10, and R43) of the 18 TRFs had large loadings in the direction in which cluster I was localized (Fig. 2, right area of the diagram). The T-RFLP profiles of cluster I were implicated as bacterial populations, with larger proportions of the bacterial species corresponding to these TRFs, whereas those of clusters II and IV localized in the negative direction were relatively less dominated by these species. The combinations of TRF putatively corresponded to *Streptococcus*, *Granulicatella*, *Rothia*, and *Treponema* (Table 5) based on the size of TRFs predicted from the 16S rRNA sequences in our oral bacteria database (33). Six TRFs (F32, F53, F80, R13, R20, and R29) had large loadings in the direction in which cluster II was localized (Fig. 2, lower left area of the diagram). The TRF combinations corresponded putatively to *Prevotella* and *Veillonella* (Table 5). Seven TRFs (F29, F36, F56, F90, R12, R30, and R39) had large loadings in the direction in

TABLE 3. Effects of each variable on the organoleptic score[b]

Variable	Total (n = 240)	Organoleptic score				Crude odds ratio (95% CI)	Multivariate odds ratio (95% CI)
		0 or 1 (n = 44)	2 (n = 79)	3 (n = 89)	4 or 5 (n = 28)		
Age (yr)	45 ± 14	38 ± 14	44 ± 15	48 ± 14	53 ± 15	1.0 (1.0–1.1)***	1.0 (1.0–1.0)
Amt of total bacteria (log deduced CFU[a])	8.0 ± 0.7	7.7 ± 0.7	7.9 ± 0.7	8.1 ± 0.8	8.2 ± 0.7	1.6 (1.2–2.3)**	0.9 (0.6–1.3)
Mean pocket depth (mm)	3.1 ± 0.7	2.8 ± 0.4	3.0 ± 0.5	3.3 ± 0.7	3.6 ± 0.9	3.0 (2.0–4.5)**	1.8 (1.8–4.5)***
Gender							
Female	110 (45.8)	18 (40.9)	29 (36.7)	42 (47.2)	21 (75.0)	1	1
Male	130 (54.2)	26 (59.1)	50 (63.3)	47 (52.8)	7 (25.0)	0.5 (0.3–0.8)**	0.5 (0.3–0.9)*
Tongue coating score							
1	88 (36.7)	28 (63.6)	34 (43.0)	26 (29.2)	0 (0.0)	1	1
2	106 (44.1)	14 (31.8)	38 (48.1)	41 (46.1)	13 (46.4)	3.0 (1.8–5.2)**	2.9 (1.6–5.3)***
3	46 (19.2)	2 (4.5)	7 (8.8)	22 (24.7)	15 (53.6)	12.0 (5.8–25.2)***	13.1 (5.9–29.0)***
Cluster of T-RFLP profile							
I	43 (17.9)	23 (52.3)	10 (12.7)	7 (7.9)	3 (10.7)	1	1
II	90 (37.5)	5 (11.4)	27 (34.2)	47 (52.8)	11 (39.3)	9.3 (4.5–19.4)***	11.4 (5.0–26.0)***
III	62 (25.8)	11 (25.0)	27 (34.2)	18 (20.2)	6 (21.4)	3.9 (1.8–8.2)***	3.9 (1.7–8.8)**
IV	45 (18.8)	5 (11.4)	15 (19.0)	17 (19.1)	8 (28.6)	8.3 (3.7–19.0)***	9.8 (4.0–24.2)***

[a] Copy number of total bacterial 16S rRNA gene was divided by that of *Streptococcus mutans* UA159 corresponding to 1 CFU per 500 μl.
[b] Data are given either as means ± standard deviations or as the number of data with the percentage in parentheses. Each odds ratio and P values were calculated by ordinal logistic regression analysis. *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$.

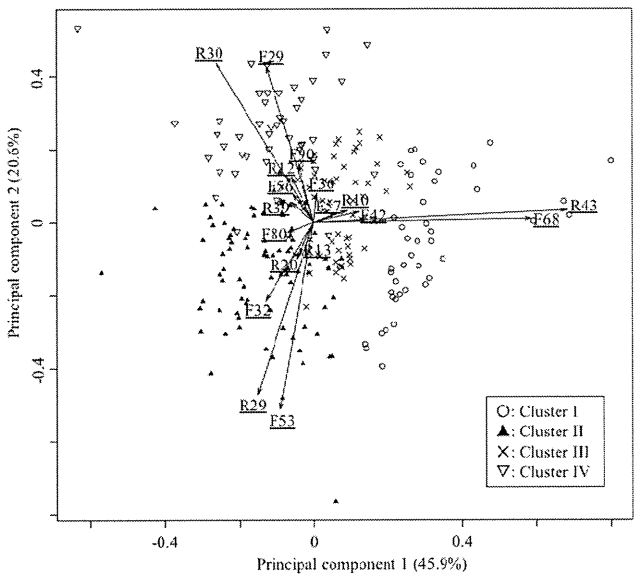

FIG. 2. Principal component analysis (PCA) biplot diagram showing the relationship between variables (each TRF; arrows) and 240 T-RFLP profiles classified into four clusters by hierarchical cluster analysis (four types of dots). Only 18 TRFs with large loadings (>0.05 in absolute value on the first and second principal components) of 164 TRFs were selected and are represented by arrows. These two components explain 66.5% of the point variability.

which cluster IV was localized (Fig. 2, upper-left area of the diagram), and the TRF combinations putatively corresponded to *Neisseria* or *Haemophilus* or *Aggregatibacter*, *Lautropia*, *Fusobacterium*, *Porphyromonas*, and *Parvimonas* (Table 5). The T-RFLP profiles of cluster III localized at the center of the diagram were implicated as the intermediate pattern of the other three clusters.

The peak area proportions of the characteristic TRFs of each cluster, except for F57 and R39, were significantly greater than those of the other clusters (Table 4). The mean peak area proportion of F57 in cluster I and that of R39 in cluster IV also were greater than those of the other three clusters, although statistical significance was not observed in the difference found in one of the three clusters. The distribution of the peak area proportions of these TRFs strongly influenced the cluster classification of this study.

Other characteristics of the bacterial population structure of each cluster. Although cluster analysis in this study was influenced mainly by the relative abundance of the dominant TRFs mentioned above, other characteristics of bacterial population structure also were explored from the obtained T-RFLP data. The diversity of the bacterial community, which was determined by the total number of TRFs and the Shannon diversity index of the T-RFLP profile, were significantly lower

TABLE 4. Comparisons of peak area proportions of 18 TRFs with large loadings on the first two components in PCA in each cluster

TRF	Loading of PC		No. (%) of subjects detected in the TRF	Peak area proportions (%, means ± SD) for cluster[b]:				Significant differences between clusters[a]
	PC1	PC2		I	II	III	IV	
6-FAM profile								
F29	−0.13	0.43	237 (98.8)	4.7 ± 3.1	6.8 ± 3.4	9.3 ± 5.7	**15.3 ± 5.7**	IV > III, IV > II, IV > I, III > II, III > I, II > I
F32	−0.13	−0.22	240 (100)	6.6 ± 4.0	**10.9 ± 6.2**	6.5 ± 3.3	5.1 ± 3.3	II > I, II > III, II > IV
F36	0.01	0.08	151 (62.9)	1.4 ± 3.1	0.9 ± 1.4	1.0 ± 6.3	**3.8 ± 6.3**	IV > I, IV > II, IV > III
F42	0.12	0.03	240 (100)	**9.8 ± 4.7**	5.1 ± 2.2	8.0 ± 3.1	6.3 ± 3.1	I > II, I > III, I > IV, III > II, III > IV
F53	−0.09	0.51	236 (98.3)	9.1 ± 5.4	**13.2 ± 5.5**	8.4 ± 3.7	5.4 ± 3.7	II > I, II > III, II > IV, I > IV, III > IV
F56	−0.06	0.08	217 (90.4)	1.0 ± 1.1	2.5 ± 1.6	2.3 ± 2.1	**3.7 ± 2.1**	IV > I, IV > II, IV > III, II > I, III > I,
F57	0.06	0.02	239 (99.6)	4.8 ± 1.9	2.8 ± 1.1	4.2 ± 1.3	3.2 ± 1.3	I > II, I > IV, III > II, III > IV
F68	0.59	0.01	240 (100)	**43.7 ± 6.6**	25.8 ± 5.1	33.4 ± 7.0	27.3 ± 7.0	I > II, I > III, I > IV, III > II, III > IV
F80	−0.07	−0.03	231 (96.3)	2.1 ± 1.5	**5.0 ± 2.3**	3.6 ± 2.5	4.0 ± 2.5	II > I, II > III, II > IV, III > I, IV > I
F90	−0.04	0.16	199 (82.9)	1.8 ± 2.2	3.0 ± 2.7	3.4 ± 3.8	**5.3 ± 3.8**	IV > I, IV > II, IV > III, III > I
HEX profile								
R10	0.09	0.03	234 (97.5)	**5.8 ± 3.5**	2.3 ± 1.3	4.4 ± 2.1	3.4 ± 2.1	I > II, I > III, I > IV, III > II, IV > II
R12	−0.06	0.12	212 (88.3)	1.3 ± 1.5	3.1 ± 2.3	3.1 ± 3.2	**4.7 ± 3.2**	IV > I, IV > II, IV > III, II > I, III > I,
R13	−0.04	−0.09	238 (99.2)	2.2 ± 1.8	**4.1 ± 2.2**	3.1 ± 1.7	2.6 ± 1.7	II > I, II > III, II > IV
R20	−0.05	−0.10	212 (88.3)	2.3 ± 2.2	**3.5 ± 2.9**	1.9 ± 1.5	1.3 ± 1.5	II > I, II > III, II > IV
R29	−0.15	−0.47	238 (99.2)	9.2 ± 5.2	**15.3 ± 4.7**	9.8 ± 3.7	7.5 ± 3.7	II > I, II > III, II > IV
R30	−0.26	0.44	240 (100)	10.4 ± 4.1	16.6 ± 5.0	15.9 ± 5.0	**25.3 ± 5.0**	IV > I, IV > II, IV > III, II > I, III > I
R39	−0.06	0.06	233 (97.1)	1.9 ± 1.4	3.8 ± 1.8	3.4 ± 2.2	4.4 ± 2.2	IV > I, IV > III, II > I, III > I
R43	0.68	0.03	240 (100)	**53.7 ± 8.1**	33.3 ± 6.0	42.7 ± 6.5	34.4 ± 6.5	I > II, I > III, I > IV, III > II, III > IV

[a] Significant difference between clusters were evaluated by Tukey's multiple comparison ($P < 0.05$).
[b] Peak area proportions that are significantly higher than those of the other three clusters are shown in boldface.

TABLE 5. Bacterial candidates corresponding to the TRF combinations

TRF combination	Genus	Species
Cluster I		
F42-R10	*Rothia*	*R. mucilaginosa* and 1 phylotype
F42-R43	*Treponema*	4 phylotypes
F57-R43	*Streptococcus*	*S. anginosus, S. downei, S. pyogenes*, and 1 phylotype
	Granulicatella	*G. adiacens*
F68-R10	*Streptococcus*	*S. mitis* and 1 phylotype
F68-R43	*Streptococcus*	*S. australis, S. cristatus, S. mitis, S. oligofermentans, S. oralis, S. parasanguinis, S. peroris, S. pneumoniae, S. salivarius, S. sanguinis, S. suis*, and 31 phylotypes
Cluster II		
F32-R20	*Veillonella*	*V. dispar, V. parvula*, and 3 phylotypes
F53-R13	*Prevotella*	*P. dentalis, P. denticola, P. loeschei, P. oulorum*, and 16 phylotypes
F53-R29	*Prevotella*	*P. melaninogenica, P. tannerae*, and 8 phylotypes
F80-R13	*Prevotella*	*P. veroralis*
F80-R29	*Prevotella*	1 phylotype
Cluster IV		
F29-R30	*Neisseria*	*N. cinerea, N. elongata, N. subflava, N. weaveri*, and 6 phylotypes
	Haemophilus	*H. influenza, H. parainfluenza, H. pittmaniae, H. quentini*, and 4 phylotypes
	Aggregatibacter	*A. actinomycetemcomitans* and *A. segnis*
F36-R30	*Lautropia*	*L. mirabilis*
F56-R12	*Parvimonas*	*P. micra* and 5 phylotypes
F56-R39	*Fusobacterium*	*F. nucleatum, F. periodonticum*, and 6 phylotypes
F90-R12	*Porphyromonas*	6 phylotypes

in cluster I of both the 6-FAM and HEX profiles (Table 6). Cluster II exhibited the highest values among the four clusters on both parameters, although statistical significance was not observed between clusters II and IV with the total number of TRFs in the HEX profiles (Table 6).

The detection frequencies of each TRF with significantly more or fewer subjects are listed in Table 7. Thirteen TRFs were detected less frequently in cluster I, whereas five and two TRFs in clusters II and IV were detected from more subjects, respectively. The well-known malodor producers, such as *Porphyromonas gingivalis* (F97 and R65), *Porphyromonas endodontalis* (F97, F98, and R65), *Parvimonas micra* (F56 and R12), *Prevotella intermedia* (F19), and *Fusobacterium nucleatum* (F56), were included as candidates corresponding to the TRFs less frequently detected in cluster I.

DISCUSSION

In the present study, the global composition pattern of bacterial populations in saliva was clearly correlated with the severity of oral malodor. The cluster grouping in this study, which was highly influenced by the peak area proportions of the dominant TRFs frequently detected in the T-RFLP profiles of most subjects (Table 4, Fig. 2), classified the bacterial population structure into four patterns with different proportions of indigenous members. The microbial community compositions belonging to cluster I demonstrated significantly lower levels of malodor productivity than the other patterns of microbiota. The T-RFLP profiles of this cluster were associated with bacterial populations with higher proportions of *Streptococcus*, *Granulicatella*, *Rothia*, and *Treponema* species than those of the other clusters.

By using the molecular approach of 16S rRNA sequence analysis, differences in bacterial compositions between subjects with high and no or low odor have been comprehensively evaluated in recent studies (9, 11, 30). Although Kazor et al. also mentioned the predominance of *Streptococcus salivarius* in relation to low malodor, those studies mainly sought to identify bacterial species that were specifically associated with subjects with strong oral malodor. Since most of the bacterial species identified were detected in subjects in both the high and no/low odor groups, Riggio et al. concluded that differences in populations would be quantitative rather than qualitative. Also in

TABLE 6. Diversity of the T-RFLP profiles in each cluster

Profile and category	No. with T-RFLP profile (means ± SD) in cluster:				Significant differences between clusters[a]
	I (n = 43)	II (n = 90)	III (n = 62)	IV (n = 45)	
Total no. of TRFs					
6-FAM	17.9 ± 3.8	23.7 ± 4.0	21.6 ± 3.7	21.9 ± 3.2	II > I, III > I, IV > I, II > III, II > IV
HEX	12.1 ± 2.6	15.9 ± 2.5	14.3 ± 1.8	15.1 ± 1.9	II > I, III > I, IV > I, II > III
Shannon diversity index					
6-FAM	1.9 ± 0.2	2.4 ± 0.2	2.2 ± 0.1	2.3 ± 0.2	II > I, III > I, IV > I, II > III, II > IV
HEX	1.5 ± 0.2	2.0 ± 0.1	1.8 ± 0.1	1.9 ± 0.1	II > I, III > I, IV > I, II > III, II > IV

[a] Significant difference between clusters were evaluated by Tukey's multiple comparison ($P < 0.05$).

TABLE 7. Detection frequencies of the TRFs with significantly more or fewer subjects in one cluster than in the other clusters

TRF	Detection frequency in cluster[a] (%):				Bacterial candidates corresponding to each TRF
	I	II	III	IV	
F19	**41.9**	90.0	82.3	75.6	*Prevotella intermedia, P. nigrescens*, and *P. pallens*
F34	23.3	**55.6**	25.8	6.7	*Dialister pneumosintes*, 3 phylotypes of *Megasphaera*, and 1 phylotype of *Prevotella*
F38	**25.6**	50.0	51.6	**73.3**	*Neisseria polysaccharea* and 2 phylotypes, *Bergeriella denitrificans, Simonsiella muelleri, Vitreoscilla stercoraria*, and 3 phylotypes of *Treponema*
F44	**30.2**	71.1	54.8	48.9	*Actinomyces naeslundii* and 4 phylotypes, *Eubacterium sulci, E. infirmum, E. saphenum* and 6 phylotypes *Mogibacterium timidum* and *M. pumilum, Guggenheimella bovis*, and 1 phylotype of *Peptococcus*
F48	**9.3**	45.6	40.3	44.4	*Leptotrichia hofstadii* and 8 phylotypes, *Kingella denitrificans, Cardiobacterium hominis, Acinetobacter baumannii*, and *Mycoplasma pneumoniae*
F52	46.5	55.6	66.1	**86.7**	Five phylotypes of *Prevotella*, 2 phylotypes of *Treponema*, 1 phylotype of *Bacteroidales*, and 1 phylotype of *Ruminococcaceae*
F56	**69.8**	94.4	93.5	97.8	*Parvimonas micra* and 5 phylotypes, *Fusobacterium nucleatum, F. periodonticum* and 6 phylotypes, and 2 phylotypes of *Shuttleworthia*
F59	81.4	88.9	82.3	**57.8**	*Lactobacillus salivarius*
F71	2.3	**53.3**	24.2	11.1	2 phylotypes of *Lachnospiraceae*
F80	**86.0**	97.8	100.0	97.8	*Prevotella veroralis* and 1 phylotype
F90	**65.1**	84.4	87.1	91.1	Six phylotypes of *Porphyromonas*
F97	**20.9**	58.9	51.6	64.4	*Porphyromonas gingivalis, P. endodontalis* and 5 phylotypes
F98	14.0	**45.6**	21.0	17.8	*Porphyromonas endodontalis* and 5 phylotypes
R12	**67.4**	92.2	93.5	93.3	*Parvimonas micra* and 5 phylotypes, six phylotypes of *Porphyromonas, Leptotrichia hofstadii* and 4 phylotypes, and 2 phylotypes of *Pedobacter*
R13	**83.7**	100.0	98.4	97.8	*Prevotella dentalis, P. denticola, P. loescheii, P. oulorum, P. oris, P. veroralis* and 24 phylotypes
R27	**41.9**	81.1	79.0	86.7	4 phylotypes of *Prevotella*
R41	**11.6**	50.0	45.2	68.9	*Mogibacterium timidum* and *M. pumilum, Flexibacter litoralis*, 2 phylotypes of *Anaerovorax, Guggenheimella bovis*, 3 phylotypes of *Parvimonas*, and 1 phylotype of *Bacteroidales*
R46	69.8	**96.7**	82.3	80.0	*Dialister pneumosintes* and *Selenomonas ruminantium*
R51	20.9	**51.1**	21.0	15.6	*Prevotella tannerae* and 1 phylotype, and 2 phylotypes of *Bacteroidales*
R65	**25.6**	74.4	77.4	77.8	*Porphyromonas gingivalis, P. endodontalis* and 5 phylotypes

[a] The values significantly higher or lower than those of the other clusters by Tukey's multiple comparison ($P < 0.05$) are shown in boldface.

the present study, a TRF specifically found in subjects with strong oral malodor was not detected (data not shown). On the other hand, we correlated the relative proportions of the major indigenous bacteria with oral malodor rather than the presence or absence of specific bacterial species. The characteristic bacteria in each cluster were genera commonly found in previous studies regardless of malodor intensity (5, 11, 30).

The bacterial population structures suggested by our results are reasonable and consistent with the preceding suggestion from a metabolic perspective (13). The TRFs with characteristically higher peak area proportions in cluster I correspond to gram-positive saccharolytic species, except for four phylotypes of *Treponema*. Although *Treponema* species also were included among the candidates of the corresponding TRFs, it is unlikely that these species were found predominantly in the saliva of many subjects, considering that only a few clones have been obtained from bacterial populations in saliva (12, 25). In both T-RFLP profiles that were strongly associated with oral malodor (clusters II and IV), the TRFs corresponding to gram-positive saccharolytic species also were dominant, but in lower proportions than those of cluster I (Table 4), whereas all of the predicted characteristic bacteria were asaccharolytic or proteolytic gram-negative bacteria. In particular, the *Fusobacterium*, *Porphyromonas*, and *Parvimonas* bacterial species that characterized cluster IV have been found to be active producers of VSCs in vitro (28). The *Prevotella* and *Veillonella* species characteristic of cluster II have been indicated to be the most frequently detected H_2S-producing bacteria in tongue microbiota and were observed in greater amounts among subjects with oral malodor than in healthy patients (37). However, whether these species are directly involved in the development of oral malodor remains uncertain. Considering that the diversity of the bacterial populations was significantly higher in clusters II and IV than in cluster I (Table 6), the patterns of the bacterial communities may simply reflect the suitability of the environment for the growth of minor but more important species for malodor production. Rather, it is remarkable that cluster I, which was implicated as having higher proportions of gram-positive saccharolytic species, was less diverse than the other clusters (Table 6). In addition, the well-known malodor producers, such as *P. gingivalis*, were included as candidates corresponding to the TRFs less frequently detected in cluster I (Table 7). Facilitating the growth of these gram-positive saccharolytic species may prevent the growth of minor species and thus may help to reduce the production of oral malodor.

Most noteworthy in this study were the significant differences in malodor productivity based on the bacterial population structure that were observed following adjustment for potential confounding factors, such as the mean periodontal pocket depth and tongue coating score (Tables 2 and 3). Previous studies indicated that an increase in tongue coating and periodontal disease were two major halitosis-inducing factors (23, 39) that create areas allowing the overgrowth of anaerobic bacteria, many of which have an ability to produce malodorous components. In the present study, differences in bacterial colonization patterns were significantly associated with the intensity of oral malodor independently of these two factors, suggesting that they reflect the oral condition involved in malodor production, which is difficult to evaluate solely by the visual inspection of oral hygiene and periodontal conditions. Considering that the predominant bacterial composition in saliva is relatively stable over time (29, 31), the composition may be implicated in individuals' potential ability to produce oral malodor. On the other hand, the significant relationship between the total bacteria in saliva and oral malodor dissipated after adjustment in the multivariate analysis (Tables 2 and 3). Although it may be controversial to conclude that the total number of bacteria in saliva reflects the amount of bacteria residing in the entire oral cavity, these results indicate that the composition of bacterial populations, rather than the total quantity, is relevant to the development of oral malodor. Current physical or chemical treatments for oral malodor focus on the reduction of the total bacterial count. Although further studies based on the alteration of oral malodor intensity are required, our results suggest the necessity of supplemental treatments to completely cure oral malodor by improving the quality of the indigenous bacterial populations.

In this study, two patterns of bacterial communities (clusters II and IV) were equally implicated as malodor-associated microbiota. Conversely, the total bacterial count was significantly greater in cluster II than in cluster IV (Table 1). The findings suggest that the bacterial populations of clusters II and IV are and are not accompanied by bacterial overgrowth, respectively. The conventional, steadfast mouth-cleaning routine for treating oral malodor may be less effective for subjects possessing bacteria from cluster IV than for those with bacteria from cluster II.

The bacterial prediction made by the present T-RFLP analysis was unable to fully characterize the bacterial community at the species level. Although additional restriction enzyme digestions may reduce the bacterial candidates to those corresponding only to the TRFs, it may interfere with the proportional distribution of bacteria due to the lack of a statistical method capable of dealing with multiple digestion patterns. Cloning and sequencing analyses are more efficient for characterizing bacterial communities at the species level. However, an analysis of 240 subjects remains too laborious and expensive to perform at present, although further insight might be gained by such effort. On the other hand, our main concern in this study was to correlate the patterns of the bacterial community composition with the severity of malodor rather than to identify the causative agents. We consider that the present T-RFLP analysis was sufficient for the purpose intended. Recently, novel high-throughput approaches, such as microbe identification microarrays (27) and bar-coded pyrosequencing (12, 25), have been implemented for the bacterial identification of oral microbial communities. The combinational use of these approaches and innovative cultivation methods may be helpful in identifying the actual malodor producers.

In other organs colonized by commensal microbiota, many studies have reported on the relationship between the global composition of indigenous bacterial populations and human health. For example, the relative proportions of two major bacterial phyla, *Firmicutes* and *Bacteroidetes*, in intestinal microbiota are associated with obesity (17), inflammatory bowel disease (6), and experimental type I diabetes (38). Vaginal microbiota shift from the usual lactobacillus-dominated microbiota to diverse communities with anaerobic and facultative bacteria in bacterial vaginosis (7). A variety of bacteria densely colonize the oral cavity as commensal organisms, whereas the relative proportions of indigenous bacteria have not received much attention in relation to oral health. The results of this investigation clearly demonstrate that oral malodor is a symptom based on the characteristic occupation of indigenous oral bacterial populations, rather than solely on bacterial overgrowth due to poor oral hygiene. The observation of oral bacterial populations from a broad ecological view may provide novel insights into human health and other disorders within the oral cavity.

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REFERENCES

1. **Barrons, R., and D. Tassone.** 2008. Use of *Lactobacillus* probiotics for bacterial genitourinary infections in women: a review. Clin. Ther. **30:**453–468.
2. **Burton, J. P., C. N. Chilcott, C. J. Moore, G. Speiser, and J. R. Tagg.** 2006. A preliminary study of the effect of probiotic *Streptococcus salivarius* K12 on oral malodour parameters. J. Appl. Microbiol. **100:**754–764.
3. **Culligan, E. P., C. Hill, and R. D. Sleator.** 2009. Probiotics and gastrointestinal disease: successes, problems and future prospects. Gut Pathog. **1:**19.
4. **Denepitiya, L., and I. Kleinberg.** 1982. A comparison of the microbial compositions of pooled human dental plaque and salivary sediment. Arch. Oral Biol. **27:**739–745.
5. **Donaldson, A. C., D. McKenzie, M. P. Riggio, P. J. Hodge, H. Rolph, A. Flanagan, and J. Bagg.** 2005. Microbiological culture analysis of the tongue anaerobic microflora in subjects with and without halitosis. Oral Dis. **11**(Suppl. 1):61–63.
6. **Frank, D. N., A. L. St. Amand, R. A. Feldman, E. C. Boedeker, N. Harpaz, and N. R. Pace.** 2007. Molecular-phylogenetic characterization of microbial community imbalances in human inflammatory bowel diseases. Proc. Natl. Acad. Sci. U. S. A. **104:**13780–13785.
7. **Fredricks, D. N., T. L. Fiedler, and J. M. Marrazzo.** 2005. Molecular identification of bacteria associated with bacterial vaginosis. N. Engl. J. Med. **353:**1899–1911.
8. **Goldberg, S., A. Kozlovsky, D. Gordon, I. Gelernter, A. Sintov, and M. Rosenberg.** 1994. Cadaverine as a putative component of oral malodor. J. Dent. Res. **73:**1168–1172.
9. **Haraszthy, V. I., J. J. Zambon, P. K. Sreenivasan, M. M. Zambon, D. Gerber, R. Rego, and C. Parker.** 2007. Identification of oral bacterial species associated with halitosis. J. Am. Dent. Assoc. **138:**1113–1120.
10. **Hartmann, M., and F. Widmer.** 2008. Reliability for detecting composition and changes of microbial communities by T-RFLP genetic profiling. FEMS Microbiol. Ecol. **63:**249–260.
11. **Kazor, C. E., P. M. Mitchell, A. M. Lee, L. N. Stokes, W. J. Loesche, F. E. Dewhirst, and B. J. Paster.** 2003. Diversity of bacterial populations on the tongue dorsa of patients with halitosis and healthy patients. J. Clin. Microbiol. **41:**558–563.