

1 **Supplemental 1. Bacterial species-specific primer**

| SPECIES                                  | GENE        | SEQUENCE OF PRIMERS (5' → 3')  |
|--|-------------|--|
| Universal primer (1)                     | 16S rDNA    | F-GTG STG CAY GGY TGT CGT CA<br>R-ACG TCR TCC MCA CCT TCC TC               |
| <i>P. gingivalis</i> (2)                 | <i>rpoB</i> | F-GGA AGA GAA GAC CGT AGC ACA AGG A<br>R-GAG TAG GCG AAA CGT CCA TCA GGT C |
| <i>T. forsythia</i> (3)                  | <i>rpoB</i> | F-GGATTGACCACCGGCGAAGACA<br>R-CGGACACGACGGTTACTCAAATGG                     |
| <i>T. denticola</i> (3)                  | <i>rpoB</i> | F-CGGGCGTGCATCTTGTCTGCTAC<br>R-CTTAACCGGCCGCTCTTTGAA                       |
| <i>P. intermedia</i> (4)                 | <i>rpoB</i> | F-ACC CAT TGG CAG AAG TTA CG<br>R-TCA ATA GGA CAC AAG CGA CCA TAG          |
| <i>P. endodontalis</i> (3) <sup>21</sup> | <i>rpoB</i> | F-AGCAGAGTTCCGTCGTCGTATTCA<br>R-GCGCCCTGCCATCTTGTCTC                       |
| <i>F. alocis</i> (5)                     | 16S rDNA    | F-AACCGGAGCAAACTGAGAA<br>R-CCGTCCGCCACTAACTTCTA                            |
| <i>F. nucleatum</i> (3)                  | <i>rpoB</i> | F-ACCTAAGGGAGAAACAGAACCA<br>R-CCTGCCTTTAATTCATCTCCAT                       |
| <i>P. micra</i> (6)                      | <i>fusA</i> | F-AGAATCAATTTCTCAAGGTGCAG<br>R-CAATGTCCATATTGTCCACTACCA                    |
| <i>R. dentocariosa</i> (3)               | <i>rpoB</i> | F-CTGGGCAAAGCGTCTGGAAAAC<br>R-GAAATCACGAATCGGGGAAATCTC                     |

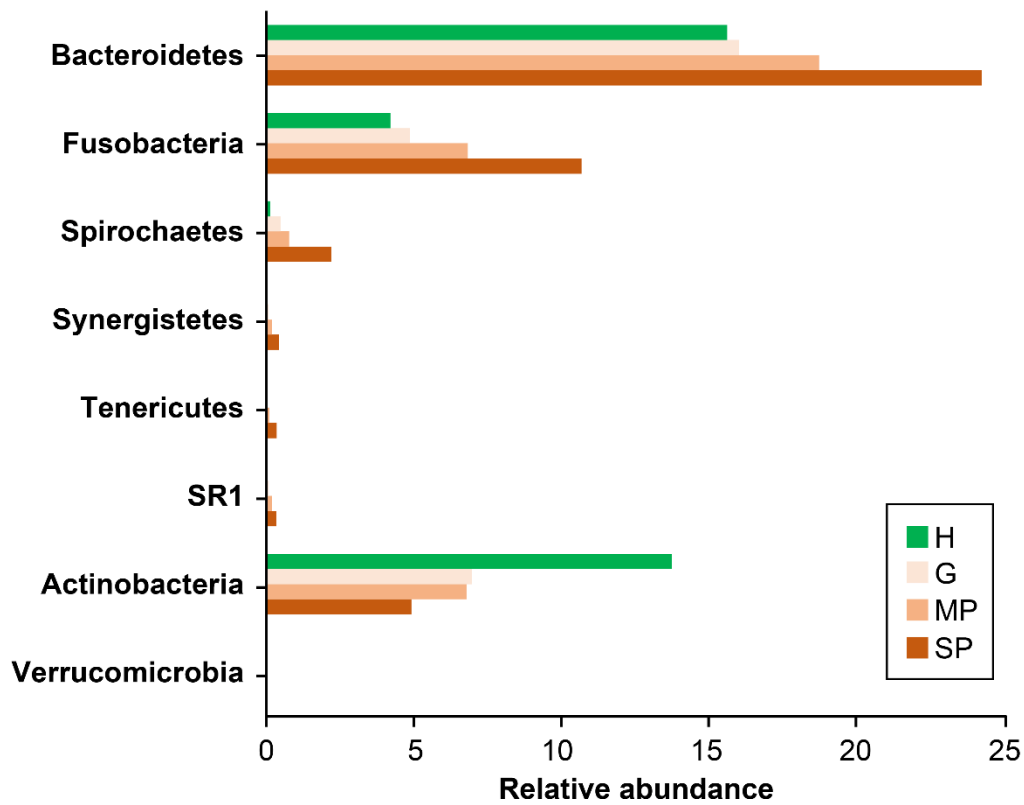
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3 **References**

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1 Supplemental 2.



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3 **Differences in taxa distribution among four groups at the phylum.** Eight phyla significantly  
4 different in saliva among four groups \* $p < 0.05$  by Kruskal–Wallis H test among groups.  $p <$   
5  $0.05$  was considered statistically significant by Kruskal–Wallis H test.

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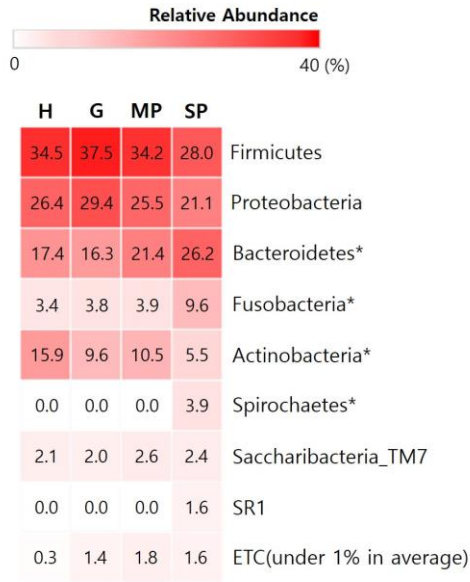
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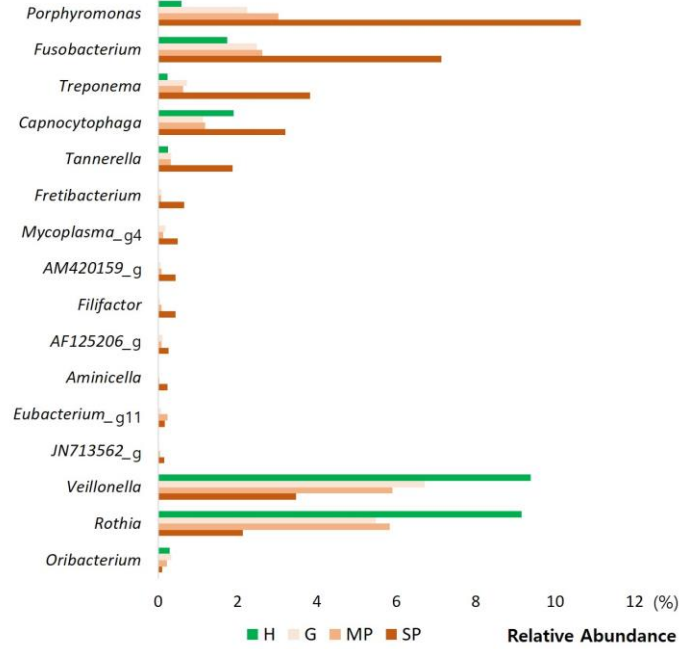
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# 1 Supplemental 3.

**A**

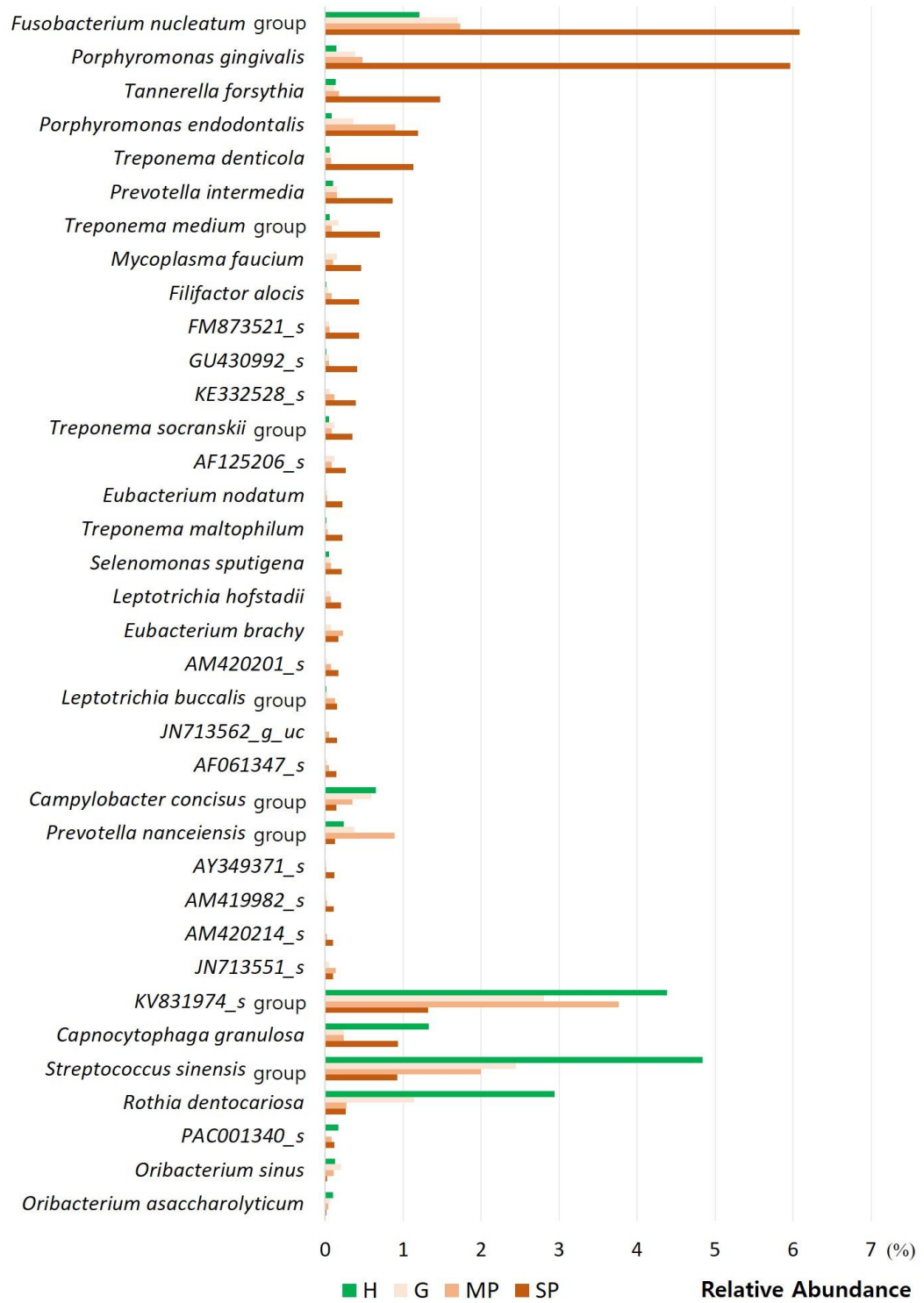


**B**



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1 **Differences in taxa distribution at the phylum, genus, and species levels among four**  
2 **groups of non-smoker females.** The 16S rRNA gene sequencing was analyzed for non-smoker  
3 females consisting of 6 periodontally healthy subjects (H group), 16 patients with gingivitis (G  
4 group), 9 patients with moderate periodontitis (MP group) and 12 patients with severe  
5 periodontitis (SP group). **A.** The distribution pattern of the top 8 phyla except phylum under 1%  
6 in average in each sample group.  $*p < 0.05$  by Kruskal–Wallis H test among four groups **B.**  
7 Genera significantly different among four groups among genera with  $>0.1\%$  relative abundance  
8 in saliva. As the severity of periodontal disease increased, genera more abundant are the top 13  
9 of the graph whereas genera more abundant in the healthy control are the bottom 3. **C.** Species  
10 significantly different between the four groups among genera with  $>0.1\%$  relative abundance  
11 in saliva. As the severity of periodontal disease increased, species more abundant are the top  
12 29 of the graph whereas species more abundant in the healthy control are the bottom 7.  $p <$   
13  $0.05$  was considered statistically significant by Kruskal–Wallis H test. H; healthy group, G;  
14 gingivitis group, MP; moderate periodontitis group, SP; severe periodontitis group.

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1 **Supplemental 4.** Quantitative comparison among periodontal groups of specific bacterial  
 2 species analyzed by qRT-PCR in nonsmokers (bacterial percentage %)

| Variables              | Healthy     | Gingivitis  | Moderate                | Severe                  | <i>p</i> | <i>p</i> Reg |
|------------------------|-------------|-------------|-------------------------|-------------------------|----------|--------------|
|                        | (n=8)       | (n=16)      | Periodontitis<br>(n=17) | Periodontitis<br>(n=18) |          |              |
| <i>P. gingivalis</i>   | 0.125±0.280 | 0.287±0.919 | 0.371±0.574             | 2.297±6.588             | 0.002    | 0.109        |
| <i>T. forsythia</i>    | 0.008±0.013 | 0.014±0.033 | 0.037±0.068             | 0.075±0.092             | 0.009    | 0.004        |
| <i>T. denticola</i>    | 0.012±0.020 | 0.014±0.037 | 0.054±0.084             | 0.033±0.058             | 0.054    | 0.208        |
| <i>P. intermedia</i>   | 0.053±0.106 | 0.026±0.044 | 0.013±0.020             | 0.071±0.092             | 0.048    | 0.316        |
| <i>P. endodontalis</i> | 0.003±0.002 | 0.042±0.062 | 0.040±0.047             | 0.059±0.080             | 0.338    | 0.057        |
| <i>F. alocis</i>       | 0.001±0.002 | 0.016±0.035 | 0.020±0.036             | 0.044±0.076             | 0.003    | 0.033        |
| <i>F. nucleatum</i>    | 0.833±1.168 | 0.375±0.424 | 0.566±0.661             | 0.401±0.601             | 0.748    | 0.347        |
| <i>P. micra</i>        | 0.344±0.335 | 0.497±0.464 | 1.805±3.019             | 0.545±0.712             | 0.182    | 0.546        |
| <i>R. dentocariosa</i> | 0.255±0.464 | 0.113±0.210 | 0.019±0.017             | 0.015±0.021             | 0.076    | 0.005        |

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1 **Supplemental 5.** Quantitative comparison between non-smoker and smoker of specific  
 2 bacterial species analyzed by qRT-PCR in SP group (bacterial percentage %)

| <i>Variables</i>       | Non-smoker<br>(n=18) | Smoker<br>(n=11) | <i>p</i> |
|------------------------|----------------------|------------------|----------|
| <i>P. gingivalis</i>   | 2.297±6.588          | 1.530±2.317      | 0.857    |
| <i>T. forsythia</i>    | 0.075±0.092          | 0.059±0.053      | 0.946    |
| <i>T. denticola</i>    | 0.033±0.058          | 0.056±0.081      | 0.982    |
| <i>P. intermedia</i>   | 0.071±0.092          | 0.082±0.128      | 0.500    |
| <i>P. endodontalis</i> | 0.059±0.080          | 0.089±0.146      | 0.753    |
| <i>F. alocis</i>       | 0.044±0.076          | 0.082±0.122      | 0.787    |
| <i>F. nucleatum</i>    | 0.401±0.601          | 0.597±0.788      | 0.893    |
| <i>P. micra</i>        | 0.545±0.712          | 1.338±1.593      | 0.393    |
| <i>R. dentocariosa</i> | 0.015±0.021          | 0.073±0.139      | 0.345    |

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1 **Supplemental 6.** Quantitative comparison between male and female of specific bacterial  
 2 species analyzed by qRT-PCR in MP group (bacterial percentage %)

| <i>Variables</i>       | Male<br>(n=9) | Female<br>(n=10) | <i>p</i> |
|------------------------|---------------|------------------|----------|
| <i>P. gingivalis</i>   | 0.420±0.726   | 0.264±0.352      | 0.327    |
| <i>T. forsythia</i>    | 0.061±0.088   | 0.010±0.008      | 0.683    |
| <i>T. denticola</i>    | 0.066±0.098   | 0.033±0.063      | 0.236    |
| <i>P. intermedia</i>   | 0.018±0.025   | 0.008±0.010      | 0.683    |
| <i>P. endodontalis</i> | 0.042±0.058   | 0.034±0.033      | 0.935    |
| <i>F. alocis</i>       | 0.027±0.050   | 0.012±0.007      | 0.288    |
| <i>F. nucleatum</i>    | 0.667±0.730   | 0.406±0.543      | 0.369    |
| <i>P. micra</i>        | 1.751±1.997   | 1.532±3.625      | 0.369    |
| <i>R. dentocariosa</i> | 0.016±0.023   | 0.021±0.009      | 0.041    |

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- 1 **Supplemental 7.** Quantitative comparison between male and female of specific bacterial  
 2 species analyzed by qRT-PCR in SP group (bacterial percentage %)

| <i>Variables</i>       | Male<br>(n=17) | Female<br>(n=12) | <i>p</i> |
|------------------------|----------------|------------------|----------|
| <i>P. gingivalis</i>   | 1.268±2.031    | 3.051±8.018      | 0.565    |
| <i>T. forsythia</i>    | 0.049±0.045    | 0.097±0.106      | 0.388    |
| <i>T. denticola</i>    | 0.050±0.075    | 0.030±0.057      | 0.825    |
| <i>P. intermedia</i>   | 0.062±0.108    | 0.094±0.102      | 0.376    |
| <i>P. endodontalis</i> | 0.071±0.121    | 0.071±0.092      | 0.626    |
| <i>F. alocis</i>       | 0.069±0.101    | 0.042±0.089      | 0.565    |
| <i>F. nucleatum</i>    | 0.476±0.653    | 0.474±0.726      | 0.825    |
| <i>P. micra</i>        | 1.049±1.342    | 0.559±0.842      | 0.288    |
| <i>R. dentocariosa</i> | 0.056±0.114    | 0.011±0.014      | 0.215    |

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