

# Extracellular Glycoside Hydrolase Activities in the Human Oral Cavity

Taichi Inui,<sup>a</sup> Lauren C. Walker,<sup>b</sup> Michael W.J. Dodds,<sup>a</sup> A. Bryan Hanley<sup>c</sup>

## Supplementary data

Wm. Wrigley Jr. Company, Chicago, Illinois 60642, USA<sup>a</sup>; School of Chemistry, University of Edinburgh, Edinburgh, Scotland, UK<sup>b</sup>, The Knowledge Transfer Network, The Roslin Institute, Midlothian, Scotland, UK<sup>c</sup>

**Running Head:** Glycoside Hydrolases in Oral Cavity

**Keywords:** Oral Ecology, glycosidase, extracellular enzyme, ectoenzyme

**Table S1.** Known glycoside hydrolase families among the tested oral micro-organisms in this study

| Glycoside Hydrolase Family                        | 1 | 2                          | 3                               | 8                             | 13                           | 20                | 23                             | 24       | 25       | 27       | 29                              | 31            | 32            | 33                | 35        | 36                            | 38                              | 57                    | 63                               | 66            | 68         | 70                          | 73            | 77                    | 85           | 87                    | 92                      | 97                    | 102                          | 103              | 108              | 109                | 125              |                                |              |                |  |   |    |    |    |    |    |    |
|---|---|----------------------------|---------------------------------|-------------------------------|------------------------------|-------------------|--------------------------------|----------|----------|----------|---------------------------------|---------------|---------------|-------------------|-----------|-------------------------------|---------------------------------|-----------------------|----------------------------------|---------------|------------|-----------------------------|---------------|-----------------------|--------------|-----------------------|-------------------------|-----------------------|------------------------------|------------------|------------------|--------------------|------------------|--------------------------------|--------------|----------------|--|---|----|----|----|----|----|----|
| Examples of enzymes in the corresponding families |   | $\beta$ -glc/ $\beta$ -gal | $\beta$ -gal/exo- $\beta$ -galN | $\beta$ -glc/ $\beta$ -HexNAc | chitosanase ( $\beta$ -glcN) | $\alpha$ -amylase | $\beta$ -glcN/ $\beta$ -HexNAc | lysozyme | lysozyme | lysozyme | $\alpha$ -gal/ $\alpha$ -galNAc | $\alpha$ -fuc | $\alpha$ -glc | invertase/sucrase | sialidase | $\beta$ -gal/ $\beta$ -glcNAc | $\alpha$ -gal/ $\alpha$ -galNAc | $\alpha$ -mannosidase | $\alpha$ -amylase/ $\alpha$ -gal | $\alpha$ -glc | dextranase | $\beta$ -fructofuranosidase | dextranucrase | endo- $\beta$ -glcNAc | amylomaltase | endo- $\beta$ -glcNAc | $\alpha$ -1,3-glucanase | $\alpha$ -mannosidase | $\alpha$ -glc/ $\alpha$ -gal | transglycosylase | transglycosylase | N-acetylmuramidase | $\alpha$ -galNAc | exo- $\alpha$ -1,6-mannosidase | # of enzymes | # of sequences |  |   |    |    |    |    |    |    |
| <i>A. actinomycetemcomitans</i>                   |   |                            |                                 |                               |                              |                   |                                |          |          |          |                                 |               |               |                   |           |                               |                                 |                       |                                  |               |            |                             |               |                       |              |                       |                         |                       |                              |                  |                  |                    |                  |                                |              |                |  |   |    |    |    |    |    |    |
| ANH9381 Taxonomy ID: 754507                       |   |                            | 1                               |                               | 3                            | 1                 | 4                              |          |          |          |                                 | 1             |               |                   |           |                               |                                 |                       |                                  |               |            |                             |               |                       | 1            |                       |                         |                       |                              |                  |                  |                    |                  |                                |              |                |  | 8 | 13 |    |    |    |    |    |
| D11S-1 Taxonomy ID: 668336                        |   |                            | 1                               |                               | 3                            | 1                 | 4                              |          |          |          |                                 | 1             |               |                   |           |                               |                                 |                       |                                  |               |            |                             |               |                       | 1            |                       |                         |                       |                              |                  |                  |                    |                  |                                |              |                |  |   | 8  | 13 |    |    |    |    |
| D7S-1 Taxonomy ID: 694569                         |   |                            | 1                               |                               | 3                            | 1                 | 3                              |          |          |          |                                 | 1             |               |                   |           |                               |                                 |                       |                                  |               |            |                             |               |                       | 1            |                       |                         |                       |                              |                  |                  |                    |                  |                                |              |                |  |   | 8  | 12 |    |    |    |    |
| <i>F. nucleatum</i>                               |   |                            |                                 |                               |                              |                   |                                |          |          |          |                                 |               |               |                   |           |                               |                                 |                       |                                  |               |            |                             |               |                       |              |                       |                         |                       |                              |                  |                  |                    |                  |                                |              |                |  |   |    |    |    |    |    |    |
| ATCC 25586 Taxonomy ID: 190304                    |   |                            |                                 |                               | 2                            |                   |                                |          |          |          |                                 |               |               |                   |           |                               |                                 |                       |                                  |               |            |                             |               | 1                     | 1            |                       |                         |                       |                              |                  |                  |                    |                  |                                |              |                |  |   |    | 3  | 4  |    |    |    |
| <i>P. gingivalis</i>                              |   |                            |                                 |                               |                              |                   |                                |          |          |          |                                 |               |               |                   |           |                               |                                 |                       |                                  |               |            |                             |               |                       |              |                       |                         |                       |                              |                  |                  |                    |                  |                                |              |                |  |   |    |    |    |    |    |    |
| ATCC 33277 Taxonomy ID: 431947                    |   | 3                          | 1                               |                               | 2                            | 1                 | 2                              | 2        |          | 1        | 2                               |               |               |                   | 1         |                               |                                 |                       | 1                                |               |            |                             |               |                       | 1            | 1                     |                         | 4                     |                              |                  |                  |                    |                  |                                |              |                |  |   |    | 15 | 24 |    |    |    |
| TDC60 Taxonomy ID: 1030843                        |   | 3                          | 1                               |                               | 2                            | 1                 | 2                              | 1        |          | 1        | 2                               |               |               |                   | 1         |                               |                                 |                       | 1                                |               |            |                             |               |                       | 1            | 1                     |                         | 4                     |                              |                  |                  |                    |                  |                                |              |                |  |   |    | 15 | 23 |    |    |    |
| W83 Taxonomy ID: 242619                           |   | 3                          | 1                               |                               | 2                            | 1                 | 1                              |          |          | 1        | 2                               |               |               |                   | 1         |                               |                                 |                       | 1                                |               |            |                             |               | 1                     | 1            |                       | 4                       |                       |                              |                  |                  |                    |                  |                                |              |                |  |   |    | 14 | 21 |    |    |    |
| <i>Pr. intermedia</i>                             |   |                            |                                 |                               |                              |                   |                                |          |          |          |                                 |               |               |                   |           |                               |                                 |                       |                                  |               |            |                             |               |                       |              |                       |                         |                       |                              |                  |                  |                    |                  |                                |              |                |  |   |    |    |    |    |    |    |
| 17 Taxonomy ID: 246198                            |   |                            |                                 |                               | 4                            |                   | 1                              | 1        | 2        |          | 1                               |               | 1             |                   |           |                               |                                 |                       | 1                                |               |            |                             |               |                       | 1            | 1                     |                         |                       | 1                            |                  |                  |                    |                  |                                |              |                |  |   |    |    | 10 | 14 |    |    |
| <i>S. gordonii</i>                                |   |                            |                                 |                               |                              |                   |                                |          |          |          |                                 |               |               |                   |           |                               |                                 |                       |                                  |               |            |                             |               |                       |              |                       |                         |                       |                              |                  |                  |                    |                  |                                |              |                |  |   |    |    |    |    |    |    |
| Challis - CH1 Taxonomy ID: 467705                 | 5 | 1                          |                                 |                               | 6                            | 3                 | 1                              |          | 2        |          | 2                               |               | 3             |                   | 1         |                               | 1                               |                       |                                  |               |            |                             | 1             | 1                     | 1            | 1                     |                         | 1                     |                              |                  |                  |                    |                  |                                |              |                |  |   |    |    |    | 16 | 31 |    |
| <i>S. mutans</i>                                  |   |                            |                                 |                               |                              |                   |                                |          |          |          |                                 |               |               |                   |           |                               |                                 |                       |                                  |               |            |                             |               |                       |              |                       |                         |                       |                              |                  |                  |                    |                  |                                |              |                |  |   |    |    |    |    |    |    |
| GS-5 Taxonomy ID: 1198676                         | 4 |                            |                                 | 1                             | 6                            |                   | 2                              |          | 2        |          |                                 | 1             | 2             |                   | 1         |                               |                                 |                       |                                  |               | 1          | 1                           | 3             | 1                     | 1            |                       |                         |                       |                              |                  |                  |                    |                  |                                |              |                |  |   |    |    |    | 13 | 26 |    |
| LJ23 Taxonomy ID: 1155071                         | 4 |                            |                                 | 1                             | 6                            |                   | 2                              |          | 2        |          |                                 | 1             | 3             |                   | 1         |                               |                                 |                       |                                  |               | 1          | 1                           | 3             | 1                     | 1            |                       |                         |                       |                              |                  |                  |                    |                  |                                |              |                |  |   |    |    |    | 13 | 27 |    |
| NN2025 Taxonomy ID: 511691                        | 4 |                            |                                 | 1                             | 6                            |                   | 1                              |          | 2        |          |                                 | 1             | 3             |                   | 1         |                               |                                 |                       |                                  |               | 1          | 1                           | 3             | 1                     | 1            |                       |                         |                       |                              |                  |                  |                    |                  |                                |              |                |  |   |    |    |    | 13 | 26 |    |
| UA159 Taxonomy ID: 210007                         | 4 |                            |                                 | 1                             | 6                            |                   | 1                              |          | 2        |          |                                 | 1             | 3             |                   | 1         |                               |                                 |                       |                                  |               | 1          | 1                           | 3             | 1                     | 1            |                       |                         |                       |                              |                  |                  |                    |                  |                                |              |                |  |   |    |    |    | 13 | 26 |    |
| <i>S. salivarius</i>                              |   |                            |                                 |                               |                              |                   |                                |          |          |          |                                 |               |               |                   |           |                               |                                 |                       |                                  |               |            |                             |               |                       |              |                       |                         |                       |                              |                  |                  |                    |                  |                                |              |                |  |   |    |    |    |    |    |    |
| 57.I Taxonomy ID: 1046629                         | 2 | 1                          |                                 | 1                             | 6                            |                   | 2                              |          | 2        |          |                                 |               |               | 3                 |           |                               |                                 |                       |                                  |               | 1          | 1                           | 6             | 4                     | 1            |                       | 1                       |                       |                              |                  |                  |                    |                  |                                |              |                |  |   |    |    |    | 13 | 31 |    |
| CCHSS3 Taxonomy ID: 1048332                       | 3 | 1                          |                                 | 1                             | 6                            |                   | 2                              |          | 2        |          |                                 |               |               | 3                 |           |                               |                                 |                       |                                  |               | 1          | 1                           | 6             | 4                     | 1            |                       | 1                       |                       |                              |                  |                  |                    |                  |                                |              |                |  |   |    |    |    |    | 13 | 32 |
| JIM8777 Taxonomy ID: 347253                       | 2 | 1                          |                                 |                               | 6                            |                   | 3                              |          | 3        |          |                                 |               |               | 3                 |           |                               |                                 |                       |                                  |               | 1          | 1                           | 6             | 4                     | 1            |                       | 1                       |                       |                              |                  |                  |                    |                  |                                |              |                |  |   |    |    |    |    | 12 | 32 |
| <i>Tr. denticola</i>                              |   |                            |                                 |                               |                              |                   |                                |          |          |          |                                 |               |               |                   |           |                               |                                 |                       |                                  |               |            |                             |               |                       |              |                       |                         |                       |                              |                  |                  |                    |                  |                                |              |                |  |   |    |    |    |    |    |    |
| ATCC 35405 Taxonomy ID: 243275                    | 1 | 1                          | 2                               |                               | 3                            | 1                 | 2                              |          |          |          |                                 | 1             |               |                   | 1         |                               | 1                               |                       | 2                                | 1             |            |                             |               |                       |              |                       |                         |                       |                              |                  |                  |                    |                  |                                |              |                |  |   |    |    |    |    | 13 | 18 |

This table summarizes the previously reported GH families in the tested oral microorganisms. The number in each cell indicates the number of sequences reported for the corresponding enzymes. Data was extracted from [www.cazy.org](http://www.cazy.org). Examples of enzymes in each GH family are also shown, although not a complete list.

**Table S2.** Primers used for qPCR experiment

| Bacteria                                     | Sequence (5->3)              | Direction | Name  | Ref |
|--|------------------------------|-----------|-------|-----|
| <i>Aggregatibacter actinomycetemcomitans</i> | ATTGGGGTTTAGCCCTGGT          | forward   | Aact1 | [1] |
|  | GGCACAAACCCATCTCTGA          | reverse   |       |     |
| <i>Fusobacterium nucleatum</i>               | CGCAGAAGGTGAAAGTCCTGTAT      | forward   | Fnuc1 | [2] |
|  | TGGTCCTCACTGATTCACACAGA      | reverse   |       |     |
| <i>Porphyromonas gingivalis</i>              | AGGCAGCTTGCCATACTGCG         | forward   | Pgin1 | [3] |
|  | ACTGTTAGCAACTACCGATGT        | reverse   |       |     |
| <i>Prevotella intermedia</i>                 | GTGGCGCGTATTTTATGTATGTG      | forward   | Pint1 | [4] |
|  | ATCCGCCATACGCCCTTAG          | reverse   |       |     |
| <i>Streptococcus gordonii</i>                | AGACACGGCCCAGACTCCTAC        | forward   | Sgor1 | [5] |
|  | CTCACACCCGTTCTTCTTTACAA      | reverse   |       |     |
| <i>Streptococcus mutans</i>                  | GGTCAGGAAAGTCTGGAGTAAAAGGCTA | forward   | Smut3 | [6] |
|  | GCGGTAGCTCCGGCACTAAGCC       | reverse   |       |     |
| <i>Streptococcus salivarius</i>              | CGGTCAAGATAACGTTGACCT        | forward   | Ssal2 | [7] |
|  | CTGCTACGATACCGTAACGTG        | reverse   |       |     |
| <i>Treponema denticola</i>                   | AGAGCAAGCTCTCCCTTACCGT       | forward   | Tden1 | [2] |
|  | TAAGGGCGGCTTGAATAATGA        | reverse   |       |     |

1. Psoter, W., Ge, Y., Russell, S., Chen, Z., Katz, R., Jean-Charles, G., Li, Y.: PCR detection of *Streptococcus mutans* and *Aggregatibacter actinomycetemcomitans* in dental plaque samples from Haitian adolescents. *Clin. Oral Invest.* **15**, 461-469 (2011)
2. Kato, H., Yoshida, A., Awano, S., Ansai, T., Takehara, T.: Quantitative detection of volatile sulfur compound-producing microorganisms in oral specimens using real-time PCR. *Oral Diseases* **11**, 67-71 (2005)
3. Kirakodu, S.S., Govindaswami, M., Novak, M.J., Ebersole, J.L., Novak, K.F.: Optimizing qPCR for the Quantification of Periodontal Pathogens in a Complex Plaque Biofilm. *Open Dent. J.* **2**, 49-55 (2008)
4. Price, R.R., Viscount, H.B., Stanley, M.C., Leung, K.P.: Targeted profiling of oral bacteria in human saliva and in vitro biofilms with quantitative real-time PCR. *Biofouling* **23**, 203-213 (2007)
5. Jakubovics, N.S., Kerrigan, S.W., Nobbs, A.H., Strömberg, N., van Dolleweerd, C.J., Cox, D.M., Kelly, C.G., Jenkinson, H.F.: Functions of Cell Surface-Anchored Antigen I/II Family and Hsa Polypeptides in Interactions of *Streptococcus gordonii* with Host Receptors. *Infect. Immun.* **73**, 6629-6638 (2005)
6. Rupf, S., Merte, K., Eschrich, K.: Quantification of Bacteria in Oral Samples by Competitive Polymerase Chain Reaction. *J. Dent. Res.* **78**, 850-856 (1999)
7. Morikawa, M., Chiba, T., Tomii, N., Sato, S., Takahashi, Y., Konishi, K., Numabe, Y., Iwata, K., Imai, K.: Comparative analysis of putative periodontopathic bacteria by multiplex polymerase chain reaction. *J. Periodontal Res.* **43**, 268-274 (2008)