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# Meta-transcriptomic analysis of three oral *Veillonella* spp.

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## Introduction

*Veillonella* are Gram-negative, small cocci, isolated from the oral cavity and intestinal tract of humans and animals, that gain energy from the utilization of short-chain organic acids particularly lactate and succinate.

Only 3 of the human oral *Veillonella* have been sequenced (*V. parvula, V. dispar* and *V. atypica*), these were used in this study, to investigate the level of genes expressed by these bacteria in both infected dentine from carious lesions and saliva from caries-free subjects.

We aimed to better understand the basis for the differential intra-oral distribution of these 3 predominant *Veillonella* species, using a meta-transcriptomic approach.

## Materials and Methods

Eleven independent samples (carious dentine and wax stimulated saliva) were obtained for each group and total RNA was extracted using the UltraClean® Microbial RNA isolation kit (MOBIO Laboratories, inc.) (>100ng per sample). RNA was processed using the Illumina® TruSeq™ RNA Sample Preparation Kit and 76bp paired end sequencing was carried out. FASTQ files were imported into CLC Genomic Workbench and the reads mapped to individual genes in 144 annotated bacterial genomes. Reads mapping to *V. parvula* DSM2008, *V. dispar* ATCC 17748 and *V. atypica* ACS-0049-V- Sch6 were extracted, rRNA genes reads were removed, the level of gene expression was calculated as (number of transcript reads count/gene length [in kbases]/total reads count [in millions]) and compared using t-tests and a FDR correction (Benjamini and Hochberg) applied with $p<0.01$ regarded as significant.

## Results and Discussion

In total, 148 genes were found to be significantly up-regulated in *V. parvula* in the carious lesions, while only 46 were up-regulated in *V. dispar*, and 37 in *V. atypica* (data not shown). Some of these genes have been identified as stress-associated (such as chaperonin, heat shock protein and putative peroxide-responsive repressor proteins). In all three species, the genes with the greatest level of expression and up-regulation were those coding for the alpha- and beta-subunits of hydro-lyase, Fe-S type, tartrate/fumarate subfamily protein; these are involved in the production of ATP through catabolism of lactate and succinate (Table 1).

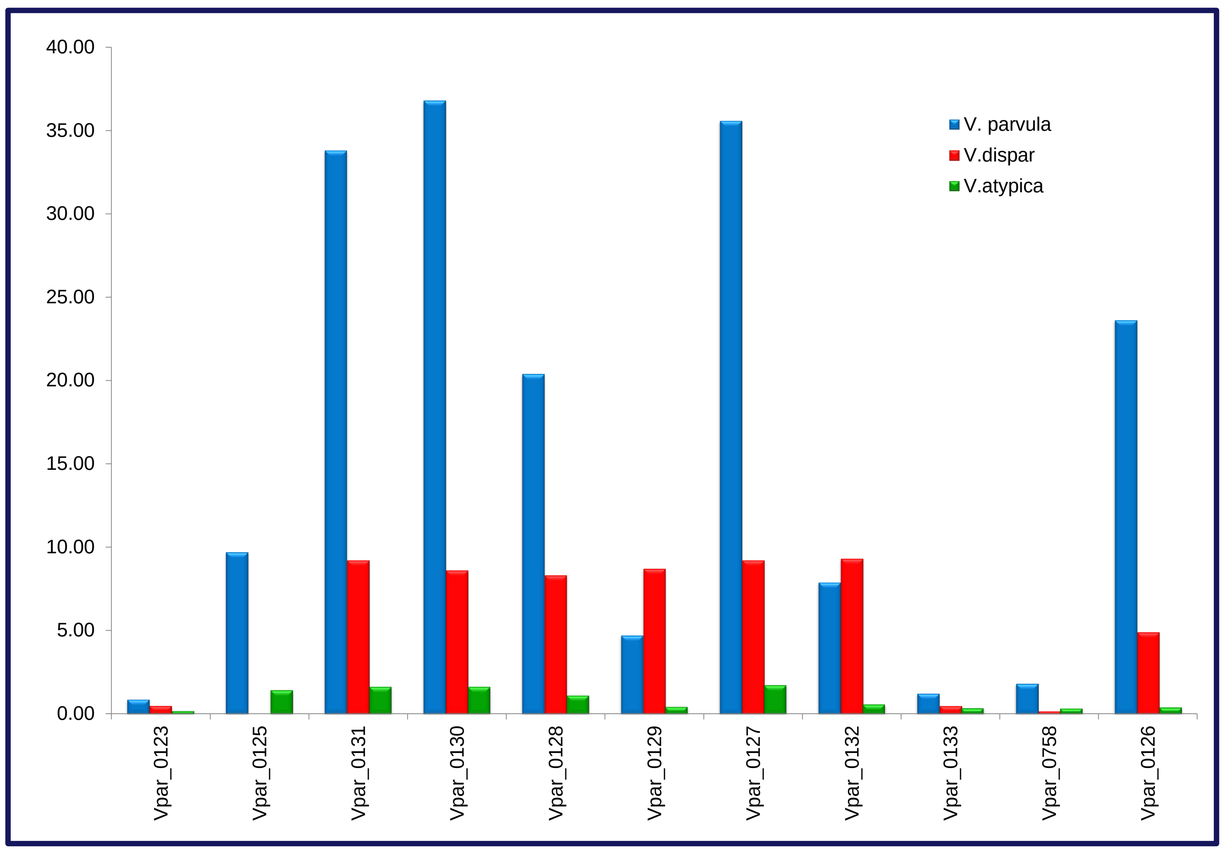


**Fig.1:** Comparison between up-regulated genes in caries, involved in the histidine biosynthesis pathway, in the 3 *Veillonella* species. Y-axis: Ratio between the mean expression value in caries and in health. X-axis: the up-regulated genes listed are as follows, histidinol-phosphatase [Vpar_0123], ATP phosphoribosyltransferase [Vpar_0125], phosphoribosyl-ATP pyrophosphatase [Vpar_0131], Phosphoribosyl-AMP cyclohydrolase [Vpar_0130], phosphoribosylformimino-5-aminoimidazole carboxamide ribotide isomerase [Vpar_0128], imidazole glycerol phosphate synthase cyclase subunit [Vpar_0129], imidazoleglycerol-phosphate dehydratase [Vpar_0127], histidinol-phosphate aminotransferase [Vpar_0132], imidazole glycerol phosphate synthase amidotransferase subunit [Vpar_0133], histidinol-phosphate phosphatase [Vpar_0758], histidinol dehydrogenase [Vpar_0126].

In *V. parvula,* the analysis of genes involved in histidine (His) biosynthesis pathway showed their involvement in the conversion of ribose 5-phosphate to His, especially with the up-regulation of ATP phosphoribosyltransferase, which has a central role in His biosynthesis (Fig. 1).

It can be speculated that the up-regulation of the His operon results in increased intra-cellular levels of His which may contribute to intracellular buffering capacity, hence allowing for intracellular pH control. this may explain the ability of *V. parvula* to be better fitted to growth and proliferation in the acidic environment of carious lesions compared to the other two species.

| | ***V. parvula*** | | | | ***V. dispar*** | | | | ***V. atypica*** | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Gene: RAST annotation** | Feature ID | C_mean | H_mean | C/H | Feature ID | C_mean | H_mean | C/H | Feature ID | C_mean | H_mean | C/H |
| L-lactate permease | Vpar_1596 | 600 | 667 | 0.90 | **VEIDISOL_00332** | 4164 | 620 | 6.50 | **HMPREF9321_1885** | 33 | 133 | 0.25 |
| L-lactate dehydrogenase (EC 1.1.1.27) | Vpar_0498 | 303 | 123 | 2.46 | **VEIDISOL_01515** | 15 | 71 | 0.22 | **HMPREF9321_0650** | 4581 | 885 | 5.18 |
| Predicted L-lactate dehydrogenase, hypothetical protein subunit YkgG | **Vpar_1650** | 1389 | 535 | 2.60 | VEIDISOL_00251 | 506 | 880 | 0.61 | HMPREF9321_1533 | 1112 | 1641 | 0.68 |
| Predicted L-lactate dehydrogenase, Iron-sulfur cluster-binding subunit YkgF | Vpar_1651 | 1800 | 1204 | 1.49 | VEIDISOL_00250 | 582 | 1875 | 0.33 | HMPREF9321_1532 | 1363 | 1721 | 0.79 |
| Predicted L-lactate dehydrogenase, Fe-S oxidoreductase subunit YkgE | Vpar_1652 | 1023 | 767 | 1.33 | **VEIDISOL_00249** | 365 | 1185 | 0.33 | HMPREF9321_1531 | 399 | 1162 | 0.34 |
| Predicted D-lactate dehydrogenase, Fe-S protein, FAD/FMN-containing | **Vpar_1768** | 418 | 51 | 8.23 | **VEIDISOL_00136** | 565 | 77 | 7.44 | **HMPREF9321_1748** | 1037 | 102 | 10.15 |
| Pyruvate carboxyl transferase (EC 6.4.1.1) | **Vpar_0752** | 753 | 382 | 1.97 | VEIDISOL_01339 | 232 | 272 | 0.90 | pyc | 225 | 292 | 0.77 |
| Malate dehydrogenase (EC 1.1.1.37) | **Vpar_1595** | 4526 | 1859 | 2.43 | **VEIDISOL_00334** | 10806 | 1782 | 5.58 | **HMPREF9321_0649** | 9963 | 2472 | 4.03 |
| L(+)-tartrate dehydratase beta subunit (EC 4.2.1.32) | **Vpar_1291** | 6912 | 17 | 417.76 | **VEIDISOL_00680** | 11039 | 17 | 689.18 | **HMPREF9321_1448** | 22784 | 62 | 370.44 |
| L(+)-tartrate dehydratase alpha subunit (EC 4.2.1.32) | **Vpar_1292** | 8034 | 68 | 117.87 | **VEIDISOL_00679** | 5568 | 27 | 213.95 | **HMPREF9321_1447** | 23279 | 56 | 413.72 |
| Fumarate hydratase class I, aerobic (EC 4.2.1.2); L(+)-tartrate dehydratase alpha subunit (EC 4.2.1.32) | **Vpar_1528** | 2072 | 109 | 19.08 | VEIDISOL_00416 | 391 | 446 | 0.91 | **HMPREF9321_1100** | 19053 | 780 | 24.43 |
| Fumarate hydratase class I, aerobic (EC 4.2.1.2); L(+)-tartrate dehydratase beta subunit (EC 4.2.1.32) | **Vpar_1529** | 1429 | 219 | 6.53 | VEIDISOL_00417 | 699 | 337 | 2.20 | **HMPREF9321_1099** | 1568 | 367 | 4.27 |
| Succinate dehydrogenase iron-sulfur protein (EC 1.3.99.1) | **Vpar_1704** | 3073 | 358 | 8.59 | **VEIDISOL_00198** | 8369 | 871 | 9.71 | **HMPREF9321_1671** | 3060 | 614 | 4.98 |
| Succinate dehydrogenase flavoprotein subunit (EC 1.3.99.1) | **Vpar_1705** | 1791 | 170 | 10.53 | **VEIDISOL_00197** | 1859 | 561 | 3.37 | **HMPREF9321_1672** | 10689 | 788 | 13.56 |
| Acetyl-CoA hydrolase( EC:3.1.2.1 ) | **Vpar_1249** | 2377 | 844 | 2.82 | VEIDISOL_00776 | 2324 | 2452 | 0.93 | HMPREF9321_1481 | 1766 | 2604 | 0.68 |
| B12 binding domain of Methylmalonyl-CoA mutase (EC 5.4.99.2) | **Vpar_1247** | 3315 | 608 | 5.46 | VEIDISOL_00778 | 1173 | 3564 | 0.35 | HMPREF9321_1483 | 2209 | 5552 | 0.40 |
| Methylmalonyl-CoA mutase (EC 5.4.99.2) | Vpar_1248 | 4230 | 3963 | 1.07 | VEIDISOL_00777 | 3158 | 3775 | 0.88 | HMPREF9321_1482 | 8624 | 6089 | 1.42 |
| Methylmalonyl-CoA epimerase (EC 5.1.99.1) | Vpar_1245 | 2562 | 1236 | 2.07 | VEIDISOL_00780 | 2893 | 2090 | 1.41 | mce | 2285 | 2932 | 0.78 |
| methylmalonyl-CoA decarboxylase delta-subunit | **Vpar_1244** | 4688 | 2773 | 1.69 | VEIDISOL_00782 | 2418 | 1070 | 2.26 | HMPREF9321_1487 | 2017 | 2398 | 0.84 |
| Methylmalonyl-CoA mutase (EC 5.4.99.2) | Vpar_1762 | 1940 | 1507 | 1.29 | VEIDISOL_00143 | 3190 | 1680 | 1.95 | HMPREF9321_1740 | 9651 | 4962 | 1.94 |
| methylmalonyl-CoA mutase, large subunit (EC:5.4.99.2) | Vpar_1763 | 2307 | 2768 | 0.83 | VEIDISOL_00144 | 748 | 1283 | 0.62 | HMPREF9321_1741 | 64 | 200 | 0.32 |

**Table 1:** List of significantly up-regulated genes in *V. parvula, V. dispar* and *V. atypica,* in caries, that are involved in the catabolism of lactate and succinate. The table includes the genes' feature ID, the calculated mean expression value in caries lesions (C_mean), the calculated mean expression value in stimulated saliva samples from healthy volunteers (H_mean), the ratio between C_mean and H_mean (C/H), and the p values resulted from the corrected t-test p values (Sig.).

## Conclusion

*Veillonella* spp. seem to respond similarly within carious lesions to high lactate levels and low pH.

*V. parvula* exhibited a distinct method of intra-cellular pH control not evident in the other two species investigated which might explain its preponderance in carious lesions and the reduced ability of *V. atypica* and *V. dispar* to proliferate in this acid environment.

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