

Contribution of GCF to the Saliva Proteome: Quantitative Proteomic Analysis

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Overview

- Saliva is a complex biological fluid containing proteins derived from the salivary glands, gingival crevicular fluid (GCF) and bacteria. It is a rich source of protein biomarkers.

- Here we ask: What proteins in saliva are derived from GCF?

Introduction

- Saliva is a complex biological fluid with a large dynamic range. It is a rich source of proteins and collection is non-invasive. However the proportion of GCF derived proteins is currently poorly defined.
- Tandem mass spectrometry is one of the most powerful tools for analysing complex protein mixtures. High mass accuracy and fast acquisition speed allow hundreds of peptides to be identified in less than an hour.
- We show quantitative online data-dependent liquid chromatography (LC) MS/MS analysis of saliva from healthy dentate and edentulous volunteers performed on a Thermo Fisher Scientific LTQ-Orbitrap Velos mass spectrometer.
- The results show that over 950 proteins were identified with 2 or more peptides. However, none of the peptides were solely observed in the dentate sample.

Method

- Saliva was collected from 10 healthy dentate volunteers and 10 healthy edentulous volunteers.
- The samples were centrifuged for 10 minutes and the supernatant retained. 10 µL of each sample for dentate and edentulous was combined to give two 100 µL samples.
- The proteins in both samples were reduced and alkylated prior to overnight digestion with Lys-C and trypsin.
- The samples were labelled with two iTRAQ (ABSciex) labels from an 8-plex (115 and 118) and combined.
- The peptide mixture was fractionated by strong cation exchange (SCX) chromatography. SCX was performed with a Polysulfoethyl A column. Samples were eluted over a 60 minute salt gradient from 0-50% 500 mM KCl (pH 3). 16 fractions were collected.
- The fractions were loaded onto a 75 µm C18 reversed phase analytical column (LC Packings). Peptides were separated over a 30 minute gradient from 3.2 to 44% acetonitrile (0.1% formic acid).
- Samples were infused by use of an Advion Triversa Nanomate nanospray ionization source into a Thermo Fisher Scientific LTQ-Orbitrap Velos hybrid mass spectrometer.
- The mass spectrometer performed an initial high resolution survey scan in the Orbitrap and the three most intense multiply charged ions were selected for collision induced dissociation (CID) tandem mass spectrometry, detected in the ion trap. The same three ions were then fragmented by higher energy collisional dissociation (HCD), detected in the Orbitrap.
- Analysed peptides were placed on an exclusion list for 60 seconds.
- Data were collected with Xcalibur 2.1 (Thermo Fisher Scientific) and analysed using the SEQUEST search algorithm and the IPI human database (v3.75) supplemented with known oral bacteria, concatenated with the reverse sequences.
- CID spectra were used to identify the peptides and HCD spectra were used for quantification.

Conclusions

- We have identified over 950 proteins from saliva with two or more peptides including 74 bacterial proteins (derived from *P.gingivalis* and *T.denticola*.) at a 1% FDR.
- 97% of the proteins were identified in greater abundance from the healthy dentate saliva.
- After normalisation 35 proteins were identified with greater than 2 fold (log2) increase (dentate:edentulous). 29 proteins were identified with greater abundance in the edentulous.
- None of the proteins identified were only present in the dentate saliva suggesting the GCF proteins observed in edentulous saliva may be contributed from tissue transudate.
- We have previously identified 31 of these proteins in GCF suggesting they are either tissue transudates or saliva contaminants in GCF.

Results

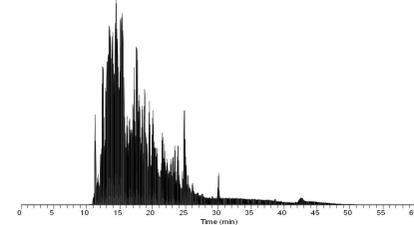


Figure 1: The total ion chromatogram of one of the SCX fractions.

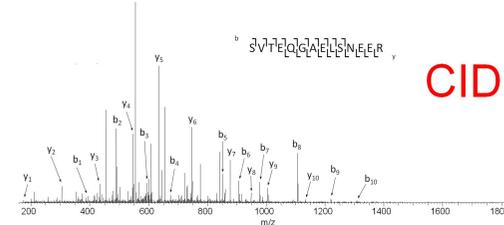


Figure 2: The CID mass spectrum of the triply charged peptide ion [iTRAQ-SVTEQGAELSNEER]³⁺. Complete coverage is observed.

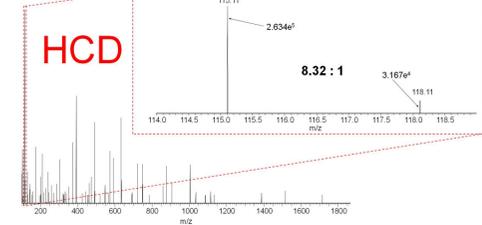


Figure 3: The HCD mass spectrum of the triply charged peptide ion [iTRAQ-SVTEQGAELSNEER]³⁺. Inset: The m/z region containing the iTRAQ labels.

Protein	Accession	115/118	Normalized
Titin	IPI:IP100455173.5	0.22	-4.29
MORN repeat containing 1	IPI:IP100514478.1	0.28	-3.93
Synaptosomal associated protein, 23 kd	IPI:IP100795513.1	0.41	-3.38
ATP-binding cassette sub-family F member 2	IPI:IP100005045.1	0.44	-3.28
Cell cycle checkpoint kinase	IPI:IP100909845.1	0.53	-3.03
Glucocorticoid receptor DNA binding factor 1	IPI:IP100718985.1	0.53	-3.02
Chromosome X open reading frame 3	IPI:IP100642861.1	0.55	-2.97
Interleukin 9 receptor	IPI:IP100328860.5	0.62	-2.80
SLC2A4 regulator	IPI:IP100221366.3	0.64	-2.75
Mucin 16	IPI:IP100745907.3	0.69	-2.64
Zinc finger protein 609	IPI:IP100853436.1	29.33	2.76
Amino acid kinase family protein	Q73KY2	29.55	2.77
Organic anion transporter F	IPI:IP100792631.1	29.77	2.79
Organic anion transporter 2	IPI:IP100642120.3	30.76	2.83
Zinc finger protein 92	IPI:IP100888059.1	33.11	2.94
H2A histone family member V	IPI:IP100927887.1	34.48	3.00
Iroquois homeobox protein 6	IPI:IP100797780.1	40.39	3.23
Homeobox C12	IPI:IP100010758.2	41.30	3.26
Glutaminyl peptide cyclotransferase	IPI:IP100003919.1	45.80	3.41
Family with sequence similarity 83, member H	IPI:IP100784320.3	60.18	3.80

Table 1: The ten proteins with the greatest increase/decrease between dentate and edentulous (115/118).

Number of CID events	97,659
Number of peptides identified	7,996
Number of human proteins identified	893
Number of bacterial proteins identified	74
Human proteins x2 upregulated (dentate/edentulous)	142
Bacterial proteins x2 upregulated (dentate/edentulous)	11
Proteins previously identified from GCF	31

Table 2: The number of CID events, proteins and peptides identified from the LC-MS/MS analysis of dentate and edentulous saliva.

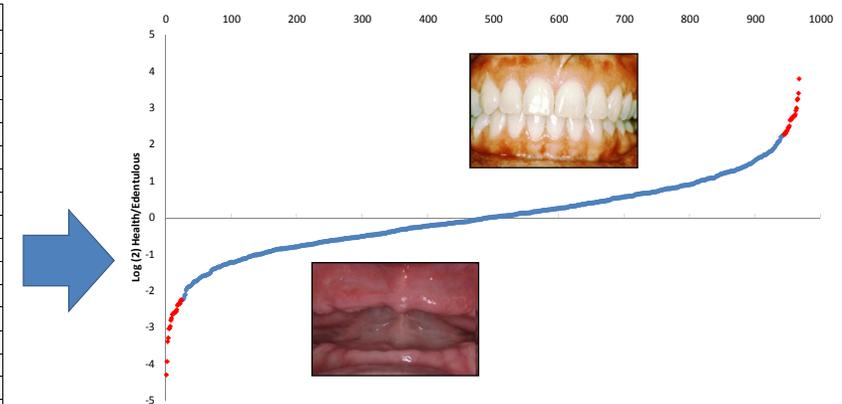


Figure 4: The log (2) curve of the ratios of proteins identified in dentate and edentulous saliva.

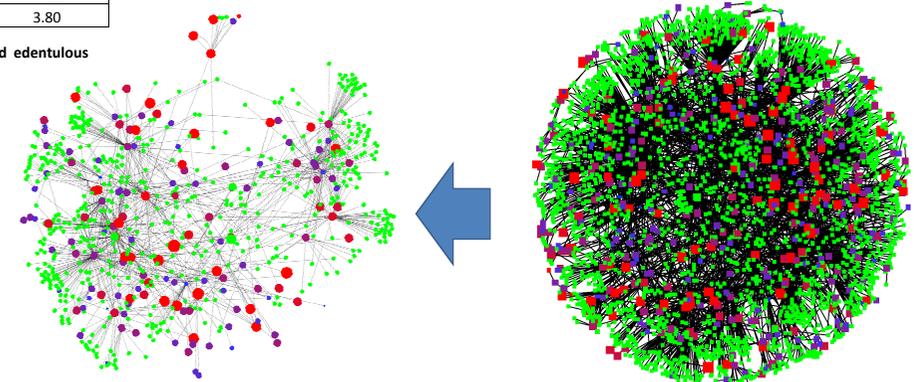


Figure 5: The protein-protein interaction map for the identified proteins.

Figure 6: The extracted protein-protein interaction maps for titin (more abundant in edentulous) a key component of muscle tissue.