PROBING THE AI-2 REGULATORY NETWORK IN PORPHYROMONAS GINGIVALIS BY A PROTEOMICS APPROACH

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OVERVIEW

- A Waters Protein Expression System was used to perform protein expression profiling of two strains of the oral pathogen *Porphyromonas gingivalis* (strain ATCC 33277 and strain ΔluxS).
- The initial results from the analyses of tryptic digests of whole-cell lysates revealed that there were 21 up-regulated and 14 down-regulated proteins in strain ΔluxS.

INTRODUCTION

Many bacteria produce extracellular molecules that function in cell-to-cell communication. One of these molecules, autoinducer 2 (AI-2), is shown to be involved in a variety of functions including biofilm formation. AI-2 activity has been discovered in spent culture supernatants of periodontal pathogen *Porphyromonas gingivalis*. However, little is known about the regulation of AI-2 production or turnover.

In this poster, we have presented a novel LC-MS approach to investigate AI-2 dependent regulatory pathways in *P. gingivalis*. For this purpose, a mutant (strain ΔluxS) containing an insertional inactivation of the *luxS* gene was constructed so the mutant is unable to make autoinducer AI-2. Proteins collected from the mutant and a wild type (strain ATCC33277) were digested and analyzed using a novel LC-MS^E methodology.

This LC-MS^E acquisition method records the masses and chromatographic retention times for all detectable, eluting peptides and their corresponding fragment ions. The information was extracted in the subsequent data analysis step to provide time-resolved, accurate mass measurements, which are then used for identification and quantitation of constituent proteins within the complexes of un-fractionated cell lysate of *p. gingivalis*.

The comparison of protein profiles obtained from the two stains showed that there are 21 up-regulated and 14 down-regulated proteins in strain ΔluxS. The effectiveness of the approach is demonstrated by the consistency with the results obtained from previous gene profiling.

METHODS

Protein Extract Preparation & Protein Digest Preparation

Pelletes of *P. gingivalis* cells were collected and re-suspended in a lysis buffer (50 mM Tris buffer, 1.0% RapiGest™). An equal volume of 0.1 mm zirconia/silica beads was added to the bacteria suspension, and the cells were disrupted with a mini bead-beater. Lysates were subsequently reduced with 15 mM DTT for 30 minutes and alkylated with 50 mM Iodoacetamide for 45 minutes before overnight trypsin digestions. The digest mixture was centrifuged, and the supernatant was used for LC-MS analysis.

METHODS

LC/MS^E Configuration

LC/MS^E data sets were acquired using a Waters Q-Tof Premier™ coupled to a Waters nanoACQUITY UPLC™ system. During an LC/MS^E experiment the Q-Tof collision cell is alternated between low (MS) and elevated (MS^E) energy to generate product ions from multiple precursors, in parallel[1]. Thus, precursor ions from all peptides eluting from the UPLC column as well as the corresponding fragmented ions were recorded simultaneously. The related experimental conditions are listed below:

LC Conditions:

Column: Waters 1.7 µm BEH 75 µmX100 mm nanoACQUITY column

Mobile Phases: A: 0.1% Formic Acid
B: 0.1% Formic Acid in Acetonitrile

Gradient: 3—40% B over 90 min

Flow Rate: 300 nL/min

MS Conditions:

Mass Range: 50-1990 m/z

Scan Time: 1.0 sec

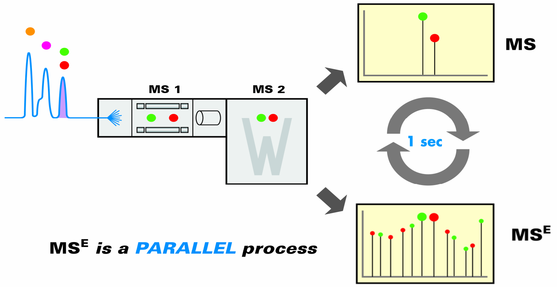
Inter scan delay: 0.1 sec

MS^E Collision Energy: ramp from 15 V to 40 V

Reference Sprayer: 200 fmol/µL [Glu^1]-Fibrinopeptide in 25% ACN/0.1% Formic acid

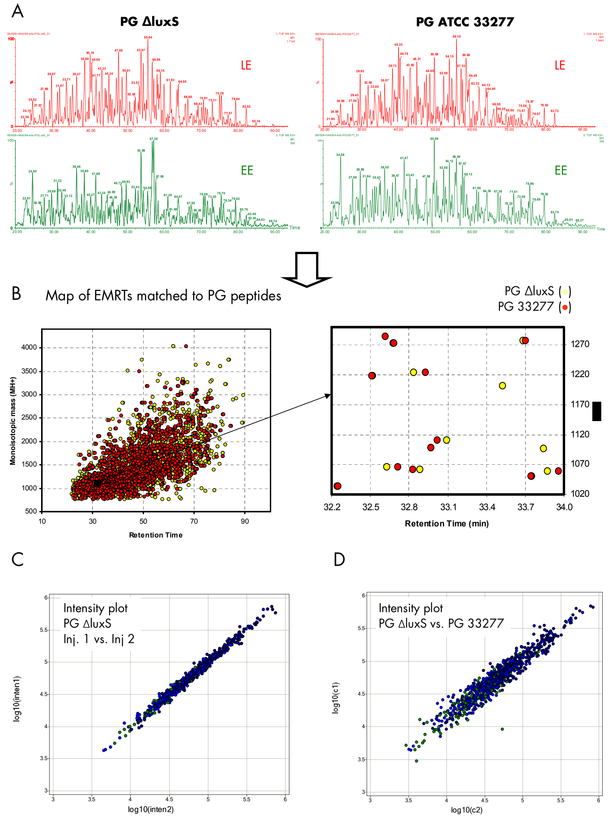
Data Processing and Protein Identification

The continuum LC/MS^E data were processed and searched using ProteinLynx Global Server (prototype, for details, please see poster TP645). Protein identifications were assigned by searching a *P. gingivalis* protein database using the precursor and fragmentation data. The relative quantitation was performed using the precursor intensity measurements. The intensities of all the peptides identified for each protein were used to determine an average relative fold-change of the protein.



RESULTS

1. LC/MS^E analyses of a mutant (PG ΔluxS) and a wild type (PG ATCC 33277) of *Porphyromonas gingivalis*. **(A)** Base peak intensity (BPI) chromatograms of LC/MS^E analyses of tryptic digests of PG proteins from whole lysates from each strain. Each LC/MS^E experiment contains a low-energy (LE) function for the intact precursor ions and an elevated energy (EE) function for the corresponding fragment ions. **(B)** An overlay of the de-isotoped and charge-state-reduced monoisotopic mass (700 – 4000 Da) and apex retention time (25-90 min) of the extracted peptides obtained from PG ΔluxS and PG ATCC 33277. **(C)** An example of the comparison of log intensity measurements from each matched EMRTs from two of replicate injections for PG 33277.**(D)**. Comparison of log intensity measurements obtained from all matched EMRTs for PGΔluxS vs. PG33277.



RESULTS

2. **Selected proteins from 180 identified and quantified proteins in this study are listed. Relative protein quantitation was obtained as a single average measurement from multiple peptides found from each protein. A 95% confidence interval was applied for these proteins that contain more than one peptide.**

Protein Name	Serial Number in TIGR W83 PG Database	Ratios (PGΔluxS:PG33277)
3-Oxoacyl-(acyl-carrier-protein) reductase (fabG)	PG1239	2.13
Lipoprotein	PG0906	1.84
Translation elongation factor Tu (tuf)	PG0387	1.79
Ribosomal protein L10 (rplJ)	PG0392	1.77
Trigger factor	PG0762	1.70
Ribosomal protein L14 (rplN)	PG1928	1.70
Ribosomal protein L17 (rplQ)	PG1910	1.69
Peptidylarginine deiminase	PG1424	1.65
Ribosomal protein L5 (rplE)	PG1926	1.60
Hemagglutinin protein HagC	PG1975	PG ΔluxS
Immunoreactive 61 kDa antigen PG91	PG2102	PG ΔluxS
Pyruvate synthase	PG0429	0.88
Hypothetical protein	PG0179	0.82
TPR domain protein	PG1028	0.82
Cell surface protein	PG0178	0.78
HmuY protein (hmuY)	PG1551	0.76
Oxaloacetate decarboxylase	PG0249	PG ACTT 33277
KH-HDIG domain protein	PG0401	PG ACTT 33277
Chaperonin	PG0521	PG ACTT 33277
Ribosomal protein S9	PG0376	PG ACTT 33277
RNA-binding protein	PG0627	PG ACTT 33277
RecA protein	PG0881	PG ACTT 33277

CONCLUSIONS

- The new LC/MS^E approach has been successfully applied to the qualitative and quantitative proteomics study of periodontal pathogen *P. gingivalis*.
- The study has identified and quantified 180 proteins that are commonly expressed in the strains (strain ΔluxS and ATCC 33277) using the un-fractionated tryptic digests of whole cell lysates.
- The quantitative comparison of protein expression profiles showed that there are 21 up-regulated and 14 down-regulated proteins in strain ΔluxS compared to the wild type pg 33277.
- The effectiveness of the approach is demonstrated by the consistency of the results obtained from previous gene profiling.

References

1. Bateman RH, Carruthers, R.; Hoyes, JB; Jones, C.; Langridge, JI; Milliar, A.; Vissers, J.P.C. J Am Soc Mass Spectrom. 2002;13(7):792-803

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