Data Article

Dataset of single nucleotide polymorphisms and comprehensive proteomic analysis of Streptococcus equi subsp. equi ATCC 39506

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A R T I C L E   I N F O

Article history:
Received 31 August 2021
Revised 13 September 2021
Accepted 16 September 2021
Available online 23 September 2021

Keywords:
Comprehensive proteomics
Extracellular vesicle purification
Immunoprecipitation–MS
Label-free proteomics
Shotgun proteomics
Subcellular fractionation
Vaccine candidate

A B S T R A C T

Streptococcus equi subsp. equi (S. equi) is an opportunistic pathogen and a major causative agent of equine strangles, a contagious respiratory infection in horses and other equines. In this study, we provide the dataset associated with our research publication “Streptococcus equi-derived extracellular vesicles as a vaccine candidate against Streptococcus equi infections” [1]. We describe the genomic differences between S. equi 4047 and S. equi ATCC 39506 and outline the comprehensive proteome information of various fractions, including the whole cell lysate, membrane proteome, secretory proteome, and extracellular vesicle proteome. In addition, we included a dataset of highly immunoreactive proteins identified through immunoprecipitation. The specifications table provides a detailed summary of the gene annotation and quantitative information obtained for each proteome. The proteomics data were analyzed using shotgun proteomics with LTQ Velos and Q Exactive mass spectrometry in the data-

DOI of original article: 10.1016/j.vetmic.2021.109165
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https://doi.org/10.1016/j.dib.2021.107402
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Specifications Table

| Subject                  | Veterinary Science and Medicine |
|--------------------------|---------------------------------|
| Specific subject area    | Genomics and Proteomics         |
| Type of data             | Table                           |
| How data were acquired   | Instrument: UltiMate 3000 RSQC nano HPLC System (Thermo Fisher Scientific, Waltham, MA, USA) coupled with LTQ Velos and Q Exactive mass spectrometer (Thermo Fisher Scientific) Software: kSNP3, MASCOT (version 2.4, Matrix Science, Boston, MA, USA) |
| Data format              | Raw and analyzed                |
| Parameters for data collection | Reference genome was used for comparative genomic analysis. *S. equi* protein samples were obtained from different fractions (including whole cell lysate [WCL], membrane proteome [MEM], secretory proteome [SEC], and extracellular vesicle proteome [EV]) or were immunoprecipitated with mock and convalescent mouse sera. |
| Description of data collection | The genome was annotated using the NCBI Prokaryotic Genome Annotation Pipeline. Single nucleotide polymorphisms were identified using the kSNP3 software. The subcellular fractionated and immunoprecipitated proteomes were profiled using LC–MS/MS. All cellular fractions were analyzed in triplicate. Proteins identified more than twice in those replicates were considered positive. The Percolator algorithm was used to recalculate the ion score to reduce the false positives in the IP samples. |
| Data source location     | Institution: Research Center for Bioconversion Analysis |
|                         | Country: Republic of Korea      |
|                         | City/Town/Region: Korea Basic Science Institute, Ochang 28119 |
| Primary data sources:   | The genomes of *S. equi* 4047 (NCBI reference sequence, NC_012471.1) and *S. equi* ATCC 39506 (GenBank, CP021972.1) |
| Data accessibility      | Name of Repository: PRIDE [2]  |
|                         | Data identification number: PXD025152 (Fractionated proteome) |
|                         | Direct URL to data: https://www.ebi.ac.uk/pride/archive/projects/PXD025152 |
|                         | Data identification number: PXD025527 (Immunoprecipitated proteome) |
|                         | Direct URL to data: https://www.ebi.ac.uk/pride/archive/projects/PXD025527 |
| Related research article | Lee, H., and Kim. S. I. *Streptococcus equi*-derived extracellular vesicles as a vaccine candidate against *Streptococcus equi* infections, Vet. Microbiol. 259 (2021) 109165. https://doi.org/10.1016/j.vetmic.2021.109165 |

Value of the Data

- This proteomics study performed on *S. equi* subsp. *equi* reveals a comprehensive baseline map of the bacterial proteome under different conditions, which is a valuable resource for future studies.
- Protein expression of bacterial components and gene annotation data obtained from various databases can be useful to investigate the bacterial pathogenesis or specific cellular proteome.
- The antigens isolated from mouse serum through immunoprecipitation can be attractive targets and facilitate further discovery of vaccine candidates.
- These datasets would be a valuable baseline for further proteogenomic studies.

dependent acquisition mode. We have deposited the acquired data, including the mass spectrometry raw files and exported MASCOT search results, in the PRIDE public repository under the accession numbers PXD025152 and PXD025527.

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1. Data Description

The data presented in this paper are the results of a comparative genomic analysis of *Streptococcus equi* (S. equi) 4047 and ATCC 39506 and proteomic analysis of four different fractions (WCL, MEM, SEC, and EV) and EV samples treated through immunoprecipitation (IP). For comparative genomics analysis, the reference genomes of *S. equi* 4047 (NCBI reference sequence, NC_012471.1) and *S. equi* ATCC 39506 (GenBank, CP021972.1) were used. We identified 246 single nucleotide polymorphism (SNP) sites. Twenty-one of the identified SNP sites caused synonymous or non-synonymous mutations (Table 1). Other SNP sites were involved in the mutation of either phages or pseudogenes. The fractionated proteome dataset obtained from LC–MS/MS analysis included locus tags, UniProt accessions, description of the identified proteins, official gene symbols, enriched cluster information, predicted localization, the results of reverse vaccinology, and normalized exponentially modified protein abundance index (emPAI) values. Each accession of *S. equi* ATCC 39506 was matched with that of *S. equi* 4047 using BLASTp. Differentially expressed proteins (DEPs) underwent Gene Set Enrichment Analysis for prokaryotes (GSEA-Pro) (http://gseapro.molgenrug.nl/). The raw data were submitted to the ProteomeXChange database under the accession numbers PXD025152 and PXD025527.

| Locus_tag         | Description                                                                 | Coding region change(s) | Amino acid change(s) |
|-------------------|-----------------------------------------------------------------------------|-------------------------|----------------------|
| SE071780_00014    | cell division protein FtsH                                                  | A->G                    | H366R                |
| SE071780_00045    | putative phosphoribosylformylglycinamidine synthase protein                 | A->G                    | -                    |
| SE071780_00193    | putative autolysin regulatory protein                                        | A->C                    | P53H                 |
| SE071780_00367    | type I glyceraldehyde-3-phosphate dehydrogenase                             | T->G                    | C65F                 |
| SE071780_00369    | phosphoglycerate kinase                                                      | G->A                    | K234E                |
| SE071780_00390    | putative exported protein                                                    | C->T                    | -                    |
| SE071780_00420    | endonuclease MutS2                                                          | T->C                    | T453I                |
| SE071780_00530    | transcription termination/antitermination protein NusA                      | A->G                    | -                    |
| SE071780_00836    | ArpU family transcriptional regulator                                        | A->C                    | P53H                 |
| SE071780_00878    | hypothetical protein                                                         | C->T                    | I66L, V81A, S95P     |
| SE071780_00900    | PTS fructose transporter subunit IID                                          | G->A                    | H147V                |
| SE071780_00908    | voltage-gated chloride channel protein                                        | A->C                    | H91N                 |
| SE071780_00925    | putative exported protein                                                    | G->A                    | I31V                 |
| SE071780_00944    | glycogen phosphorylase                                                       | T->C                    | -                    |
| SE071780_00966    | collagen binding putative ancillary pilus subunit Cne                        | G->A                    | T574A                |
| SE071780_00967    | fimbrial protein                                                             | T->C                    | -                    |
| SE071780_01063    | ABC transporter permutase                                                     | A->C                    | -                    |
| SE071780_01237    | triphosphoribosyl-dephospho-CoA synthase CitG                                | C->A                    | N271H                |
| SE071780_01600    | DNA adenine methylase                                                        | G->A                    | -                    |
| SE071780_01920    | collagen-like surface-anchored protein ScII                                  | G->C                    | D332E                |
| SE071780_02280    | cell surface protein                                                         | A->G                    | R217K                |

The genome sequence of *Streptococcus equi* subsp. *equi* 4047 was used as the reference genome. 1 Proteins identified by proteomic analyses are highlighted in bold.
2. Experimental Design, Materials and Methods

2.1. S. equi culture conditions

*S. equi* ATCC 39506 was procured from the Korean Culture Center of Microorganisms (Seoul, Republic of Korea). The bacteria were cultivated overnight in Todd–Hewitt broth supplemented with 1% yeast extract at 37.5 °C in an atmosphere with 5% CO₂. Bacteria were further inoculated in fresh broth for 7–8 h until the mid-logarithmic growth phase (optical density at 600 nm = 0.4–0.6) was achieved for protein sample preparation.

2.2. Comparative genomic analysis

SNPs in the genomes of *S. equi* subsp. *equi* ATCC 39506 and *S. equi* subsp. *equi* 4047 were identified using kSNP3 with a k-mer length of 31 [3]. SNPs on the coding sequence were selected, and the amino acid variants were identified using BLASTp for comparison with the protein sequences of the two variants.

2.3. Preparation of fractionated protein samples of *S. equi* ATCC 39506

The four subcellular fractions (including WCL, MEM, SEC, and EV) from *S. equi* ATCC 39506 cultures were purified as previously described [4], with slight modifications. Briefly, 6 L of the cultured bacteria were harvested through centrifugation at 8000 × g for 30 min at 4 °C to remove the cell debris. The supernatant and pellet fractions were retained separately for further processing. For the WCL fraction, the pellet was washed thrice with 20 mM Tris–HCl buffer (pH 8.0) and disrupted using a French pressure cell (SLM Aminco, Urbana, IL, USA) at 138 MPa until a transparent solution was obtained. For MEM fraction, the resulting supernatant was collected through centrifugation at 15000 × g for 20 min and precipitated using sodium carbonate precipitation method. The supernatant was mixed in a 10 mM sodium carbonate solution and precipitated through ultracentrifugation at 150000 × g for 1 h at 4 °C. The pellet was resuspended in 20 mM Tris–HCl buffer (pH 8.0). For SEC fraction, the supernatant of the harvested bacteria was filtered using a 0.45-μm bottle-top vacuum filter (Corning, NY, USA). The resulting flow-through was dissolved in 80% ammonium sulfate at 4 °C for 2 h, and then precipitated at 5000 × g for 20 min at 4 °C. Thereafter, the pellet was resuspended in 20 mM Tris–HCl buffer (pH 8.0). Ammonium sulfate was removed through buffer exchange on Vivaspin 20 (Sartorius, Göttingen, Germany) using over 10 volumes of 20 mM Tris–HCl (pH 8.0). For EV fraction, the supernatant of the harvested bacteria was ultrafiltered using a 0.22 μm vacuum filter and concentrated on a 500 kDa hollow fiber membrane mounted on a QuixStand benchtop system (GE Healthcare, Little Chalfont, UK). The collected supernatant was then precipitated through differential centrifugation methods. The sample was centrifuged at 8000 × g for 1 h at 4 °C and the resulting supernatant was further centrifugated at 150000 × g for 3 h at 4 °C. The pellet was resuspended in 20 mM Tris–HCl buffer (pH 8.0). The protein concentration of each sample was determined using a Micro BCA Protein Assay Kit (Thermo Fisher Scientific, Waltham, MA, USA) according to the manufacturer’s instructions. Each subcellular fraction sample was aliquoted and stored at −80 °C until further use.

2.4. Identification of the proteome of each fraction using LC–MS/MS

In-gel digestion was performed using a previously described method [5] with slight modifications. Briefly, an equal amount of protein of each subcellular fraction was separated through sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE). Each lane was cut into
nine slices based on the molecular size. The sliced gels were destained using a destaining solution comprising 50% acetonitrile and 10 mM ammonium bicarbonate and washed thrice with distilled water. The gels were sequentially treated with a reducing solution (10 mM dithiothreitol and 100 mM ammonium bicarbonate) and an alkylating solution (55 mM iodoacetamide). Trypsin digestion was then performed using 50 mM ammonium bicarbonate at 37 °C for 16 h.

The resulting peptides were dissolved in 0.5% trifluoroacetic acid and further fractionated using a 10 cm × 75 μm ID C18 reverse-phase column (PROXENON) at a flow rate of 300 nL/min. Peptides were eluted using a gradient of 0–65% acetonitrile for 100 min. All MS and MS/MS spectral data were acquired in a data-dependent mode in triplicate for each experimental condition using LTQ Velos (Thermo Fisher Scientific). The full MS spectra were acquired in positive mode within a range of 300–2000 m/z. The top six most intense ions in the acquired scan were selected for followed collision-induced dissociation. The maximum ion injection time was 100 ms for the MS/MS scans. The normalized collision energy was 35 and the isolation window employed was 2 m/z. The dynamic exclusion settings utilized were repeat count 1, exclusion duration 30 s, exclusion list size 50, exclusion mass width low and high 1.5, respectively.

The MS/MS spectra were analyzed through the database search strategy mounted on Mascot version 2.4 (Matrix Science) for the identification of proteins. The genome of *S. equi* ATCC 39506 (GenBank, CP021972.1, 2208 sequences, date of release: 20-Jun-2017) was used as a reference genome for this analysis. The following parameters were used for protein identification: missed cleavages, 2; peptide mass tolerance, ±0.8 Da; peptide fragment tolerance, ±0.8 Da; peptide charge, 2+, 3+, and 4+; static modifications, carbamidomethyl; and dynamic modification, oxidation (Met). A target-decoy search was used to filter out the low-confidence peptides and proteins with a false discovery rate (FDR) of at least 1%. To reduce the number of false positives in the entire result, proteins identified more than twice in the three replicates were considered positive in the fractionation dataset. For comparative analysis, data visualization and statistical analysis were conducted using the Perseus platform [6]. The log2 emPAI value was used as a normalized value. The missing values after filtration were imputed under a normal distribution [7,8]. The DEPs were selected using the significance analysis of the microarray method [9] (FDR < 0.05) through modifying the gene-specific t-statistic calculated by repeated permutation with the background. Heatmap analysis was conducted to visualize the differences in the profiles of each resulting proteome (WCL, SEC, EV, and MEM). Next, the potential vaccine candidates (PVCs) were filtered out using the results of reverse vaccinology and are highlighted in three different colors in the heatmap.

### 2.5. IP and protein identification using MS/MS

IP experiment was conducted to sort the immunogenic antigens present in the EVs. The EV sample (30 μg) was dissolved in IP lysis buffer (Thermo Fisher Scientific) for 1 h at 4 °C and centrifuged at 4000 × g for 3 min at 4 °C, and the supernatant was collected. To reduce false positive reactions, the supernatant was incubated with anti-IgG conjugated with magnetic beads (Dynabeads; Invitrogen, Carlsbad, CA, USA) for 1 h at 4 °C and then separated. Twenty microliter aliquots of pooled mock and convalescent sera (n = 10 each) from the vaccination experiments were incubated with magnetic beads for 30 min at 25 °C as previously described [1]. Each supernatant was incubated with the antibody-bead complex at 4 °C overnight. The beads were vigorously washed with phosphate-buffered saline five times. The bound proteins were eluted with SDS sample buffer and heated at 95 °C for 10 min.

The eluents were cut into five slices based on molecular weight, followed by in-gel digestion, and analyzed through LC–MS/MS using Orbitrap Q Exactive Plus (Thermo Fisher Scientific). The full MS spectra were acquired in positive mode within a range of 150–2000 m/z in 120 min. The top 20 most intense ions in the acquired full mass scan were selected for followed higher-energy collisional dissociation. The maximum ion injection times used were 100 ms for the MS scan and 50 ms for the MS/MS scans. The automatic gain control target settings were 1.0 × 10⁶ for the MS scan mode and 5.0 × 10⁴ for the MS/MS scan mode. The normalized collision energy was
27 and the isolation window employed was 2 m/z. The dynamic exclusion duration was set by 20s. The following parameters were used for protein identification: missed cleavages, 2; peptide mass tolerance, ±10 ppm; peptide fragment tolerance, ±0.8 Da; The other parameters used were as described above. The Peptide score was recalculated using Percolator [10]. Peptide ions with a score above 39, indicating identical or extensive homology matches, were selected. Proteins with more than one independent significant peptide match were selected using the IP dataset.

2.6. Deposition of the MS dataset

The MS proteomics data obtained in this study were deposited in the ProteomeXchange Consortium [11] via the PRIDE partner repository with the identifiers PXD025152 and PXD025527.

Ethics Statements

All animal experiments were reviewed and approved by the Animal Ethics Committee of the Korea Basic Science Institute (approval number KBSI-20-18).

CRediT Author Statement

Hayoung Lee: Conceptualization, data curation, Writing – original draft, Writing – review & editing; Sung Ho Yun: Investigation, and validation; Ju-yong Hyon: Conceptualization, and validation; Sang-Yeop Lee: Conceptualization, Data curation; Yoon-Sun Yi: Investigation; Chi-Won Choi: Investigation; Sangmi Jun: Validation; Edmond Changkyyun Park: Resources, Writing – review & editing; Seung Il Kim: Supervision, Writing – review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

This work was supported by the grants received from the Korea Basic Science Institute research program (grant number K39402) and a National Council of Science & Technology (NST) grant from the Korean government (MSIP) (grant number CRC-16-01-KRICT). The funding sources were not involved in any which way in the research project or in the preparation and submission of this article for publication.

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