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Data Article

The proteome of the outer membrane vesicles of an Antarctic bacterium *Pseudomonas syringae* Lz4W



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ABSTRACT

Outer membrane vesicles (OMVs) of gram-negative bacteria are released during all growth phases and play an important role in bacterial physiology. They consist of lipids, proteins, lipopolysaccharides and other molecules. The OMVs of the Antarctic bacterium *Pseudomonas syringae* Lz 4W were isolated and identified their proteins. The mass spectral data set deposited with PRIDE, accession number PXD 000221 is presented in this report. The proteins identified from the OMVs of *P. syringae* Lz4W, data of this study were published in the Journal of proteome research [1].

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Specifications Table

Subject area	Biology, Chemistry
More specific subject area	Microbial Proteomics, Bacterial physiology
Type of data	LC-ESI-MS/MS data
How data was acquired	Mass spectrometry
Data format	MS/MS data obtained from Orbitrap velos (Thermo scientific) was used for the identification of proteins.
Experimental factors	

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	<i>The protein of OMVs isolated from an Antarctic bacterium was identified using SEQUEST, MASCOT and PEAKS programs. The proteins commonly identified in all the three programs were accepted for further functional analysis.</i>
Experimental features	<i>In gel digested proteins with Trypsin were extracted with 5% TFA in 50% acetonitrile. Dried and desalted the peptides with Zip-tip C18 columns and loaded on Nano LC-ESI MS/MS.</i>
Data source location	<i>CSIR-Centre for Cellular and Molecular Biology, Hyderabad, India.</i>
Data accessibility	<i>Achieves of PRIDE, PXD 000221. The identified proteins were published in Journal of Proteome research [1].</i>

Value of the data

- The proteins associated with OMVs of the Antarctic bacterium *P. syringae* Lz4W were identified to understand their structure and functions.
- Outer membrane, periplasm proteins were present as major components of OMVs.
- More than 500 proteins were identified using nano LC-ESI-MS/MS.

1. Data, experimental design, materials and methods

1.1. Bacterial strain growth conditions

The bacterium *P. syringae* Lz4W was isolated from soil samples collected in and around Lake Zub, Schirmacher Oasis, Antarctica. The geographical coordinates of the sampling area are 70°45'12"S and 11°46'E. This bacterial strain can grow between 0 °C and 30 °C with an optimum growth temperature of 22 °C [2]. It is a well studied bacterium in our laboratory whose genome sequence was recently published [3]. The bacterium *P. syringae* Lz4W was grown at 22 °C in Antarctic bacterial Medium (ABM) that contains 0.5% peptone and 0.2% yeast extract with aeration (by continuous shaking up to late stationary phase (OD 600 nm=1.1–1.2) for the preparation of OMVs.

1.2. Preparation of OMVs

The OMVs were prepared from bacteria cultures by using the method described in literature [4,5], with minor modifications. In brief, the cultures of all bacterial strains were grown up to stationary phase in respective growth conditions. The cells were separated from the culture by centrifugation at 10,000 g for 10 min. at 4 °C. The further steps of purifications involved filtration of the supernatant, ultracentrifugation and density gradient ultracentrifugation. The supernatant was filtered by using 0.45 µm membrane (Millipore). The OMVs in the filtrate were pelleted by ultracentrifugation at RCFmax of 150,264 g (36,000 rpm for 'type 45 Ti' rotor, Beckman) for 3 h at 4 °C in Beckman ultracentrifuge in polycarbonate tubes. The pellet of OMVs was obtained which was subsequently re-suspended in 10 mM phosphate buffer saline (pH 7.4). This preparation of OMVs was further purified by sucrose density gradient centrifugation. In polyallomer tubes, equal layers of sucrose solutions (prepared in 10 mM phosphate buffer saline pH 7.4), 70%, 60%, and 20% were added from the bottom to top. The suspension of OMVs was layered on the top of it. The tubes were ultracentrifuged at RCFmax of 164,609 g (35,000 rpm for 'SW 60 Ti' rotor, Beckman) at 4 °C for 6 h. All the fractions were collected from the gradient. Aliquot from each of them was diluted 25 times. They all were tested for the presence of OMVs by using dynamic light scattering (DLS). The fractions that were detected to contain OMVs were pooled together, diluted to 4 mL and subjected to ultracentrifuge at RCFmax of 163,202 g (55,000 rpm for 'TLA-100.3' rotor, Beckman) at 4 °C for 2 h. The pellet of purified OMVs was resuspended in 10 mM phosphate buffer (pH 7.4) for OMVs of *P. syringae* Lz4W and in 10 mM phosphate buffer saline (pH 7.4), and was stored at –20 °C until used for experiments.

1.3. Dynamic light scattering

The size distribution of the OMVs was monitored by using DLS. The preparations of OMVs were diluted with phosphate buffer (10 mM, pH 7.4) to a protein concentration of 0.04–0.06 µg/mL and were subjected to measure the size distribution. The scatter of the diluted OMVs from *P. syringae*

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