Data article

Data on preparation of psychrotolerant bacterium Shewanella olleyana sp. nov. cells for transmission electron microscopy

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A B S T R A C T

This data article contains transmission electron microscopy (TEM) images of psychrotolerant bacterium Shewanella olleyana sp. nov. Cells of S. olleyana were grown following an optimized culture conditions in liquid medium. Procedure for the preparation of cells suitable for TEM is described in detail.

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Specifications Table

| Subject area       | Microbiology |
|--------------------|--------------|
| More specific sub- | Microscopy   |
| ject area          |              |
| Type of data       | Transmission electron microscopy (TEM) images, Tables |
| How data was       | TEM following an optimized cell preparation protocol |
| acquired           |              |
| Data format        | Analyzed     |

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Experimental factors
Bacterial cells were grown at different temperatures in liquid and solid media

Experimental features
Culture conditions and preparation for electron microscopy of the bacterial cells were optimized

Data source location
N/A

Data accessibility
Data are provided in this article

Value of the data

- These data describe the culture conditions and preparation of *Shewanella olleyana* cells suitable for TEM.
- The obtained TEM images of *S. olleyana* can serve as reference data for its morphological features and ultrastructural characteristics.
- To our knowledge, this data article is first to report the ultrastructure of *S. olleyana*.
- The data presented here confirmed the presence of high levels of iron and/or iron sulphide in the broth culture precipitates of *S. olleyana*. Researchers using this psychrotolerant bacterium can take these findings into consideration for its culture propagation and other purposes (e.g. microscopy).

1. Data

The data presented in this article show the morphological and ultrastructural characteristics of psychrotolerant bacterium *Shewanella olleyana* sp. nov. obtained by TEM. Cells were grown and prepared following an optimized procedure. *S. olleyana* cells were grown and propagated in solid medium at temperatures between 4 and 10 °C for 24–48 h. Complete sedimentation of suspended *S. olleyana* cells (grown in solid medium) was achieved following centrifugation at 4000 x g for 10 min at 4 °C. The presence of residual or contaminating reduced form of iron and/or iron sulphide was detected when cells are grown in liquid medium.

2. Experimental design, materials and methods

2.1. Bacterial strain, culture conditions, and cell preparation

*Shewanella olleyana* sp. nov. (LMG 21437) was obtained from BCCM/LMG Bacteria Collection, Laboratory for Microbiology, University Ghent K. L. Ledeganckstraat, Belgium. *S. olleyana* strain was grown routinely in Zobell’s marine agar 2216 [1]. To determine the optimum growth conditions for the culture and propagation of *S. olleyana*, its growth response to varying incubation temperatures (4–10, 25, 30, and 35 °C) was observed for 24–48 h (Tables 1 and 2).

The minimum centrifugation speed and time required to sediment *S. olleyana* cells suspended in MH buffer ((280 mM mannitol/10 mM HEPES (4-(2-hydroxyethyl) piperazine-1-ethanesulfonic acid) pH 7.4) at 1:1 ratio (w/v) was determined. Immediately after harvesting the cells from the agar plates and suspending it in MH buffer, the cell suspension was subjected to varying centrifugation speed (1500, 2000, 4000, and 8000 x g) and sedimentation time (5, 10, 15 and 20 min), as shown in Fig. 1.

For TEM analysis, 24–48 h old *S. olleyana* cells were harvested from agar plates. Cells were flooded with 4 ml of sterile cold physiological saline (0.85% NaCl) and scraped using a glass rod to detach the cells. The cell suspension was centrifuged at 4000 g for 10 min at 4 °C. The resulting pellet was washed with cold physiological saline. Cells were resuspended in MH buffer and stored at −86°C until use. Growth of *S. olleyana* cells in marine broth medium was also observed.

2.2. X-ray fluorescence (XRF) spectroscopy analysis of *S. olleyana* precipitates from broth cultures

To verify the occurrence of iron reduction in the broth medium by *S. olleyana* cells, XRF analysis of the recovered precipitates was done. The PANalytical Epsilon5 EDXRF spectrometer was used for the multi-
elemental analysis of the sample. The instrument is equipped with 600 W-anode x-ray tube and 100 kV generator, up to 15 secondary targets and a high resolution PAN-32 detector. To identify the elemental constituents of the sample, it was analyzed qualitatively using the spectrum generated by the EDXRF. Cells were grown on 10 ml marine broth [1] incubated at 4–10°C for 3–5 days. Formation of black precipitates during incubation was observed (Fig. 2). Precipitates were recovered and dried free of moisture at 110°C for 14 min in an aluminum dish. The precipitate that adhered on the aluminum dish was placed on top of an “XRF insert”, covered with X-ray thin film sample support and inserted in stainless steel cap. The sample assembly was then put inside the EDXRF spectrometer and analyzed using the PANalytical Epsilon Software for elemental analysis. Fig. 3 shows the result of XRF analysis of the black precipitates. The precipitates contain high levels of elemental iron (17%), confirming that this could be a mixture of reduced form of iron (Fe²⁺) and/or iron sulphide precipitates produced by S. olleyana.

2.3. TEM and negative staining of S. olleyana

S. olleyana cells were fixed in buffered 2.5% glutaraldehyde and 4% paraformaldehyde. Cells were washed three times with physiological saline to remove excess fixative and were fixed in unbuffered 1% osmium tetroxide and washed with physiological saline. It was then dehydrated in a graded series of acetone solutions and gradually impregnated in Epon resin with heat polymerization. Semi-thin survey sections were sliced with glass knives, stained with toluidine blue and used to orient sections. Ultra-thin sections were mounted on uncoated copper grids and stained with uranyl acetate and lead citrate. Sections were examined and viewed in a JEOL 1010 TEM. Negative staining of S. olleyana cells was done following a previously published method [2]. Samples were viewed using a JEOL 1200 EX electron microscope (Figs. 4–6).
Fig. 3. XRF spectrum of black precipitates obtained from the broth cultures of *S. olleyana*. Al-Ka1 = background spectra of aluminum dish where the precipitate was adhered for analysis.

Fig. 4. Transmission electron micrograph of intact *S. olleyana* suspended in MH buffer and stored at −86°C. Bar, 0.25 μm.
Fig. 5. Transmission electron micrograph of negatively-stained *S. olleyana* undergoing cell division (Bar, 0.5 μm).

Fig. 6. Transmission electron micrograph of negatively-stained *S. olleyana* showing a single polar flagellum (F). Bar, 0.5 μm.
2.4. Statistical analysis

Each data point represents the mean $\pm$ SD of two trials. GraphPad InStat software was used to determine the differences among the means. Data were compared using one-way analysis of variance (ANOVA) with post-test. Dunnett’s test was used to compare treatment means against the control mean. Statistical significance was determined at $p < 0.05$.

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Transparency document. Supporting information

Transparency data associated with this article can be found in the online version at http://dx.doi.org/10.1016/j.dib.2016.09.049.

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