SOP Isolation of surfactant-degrading microorganisms

Protocol first designed by K.A. Cook (1979) and K. Ohwada (1975), modified by Olga Turkovskaya (Turkovskaya and Shub, 1989) and Ekaterina Dubrovskaya
Revised and updated (October 2013) by Ekaterina Dubrovskaya.

1. Methods

1.1. Soil sampling

- Soil (or rhizosphere soil) is sampled from an industrial area contaminated with synthetic surfactants.
- Plants are removed from the contaminated site with a titanium spade; the bulk soil is shaken off the roots, and the plants with rhizosphere soil are placed in plastic bags with labels indicating the plant species, the date, time and place of sampling.

1.2. Isolation of surfactant-degrading microorganisms

- In the laboratory, the roots are separated from the plants, and samples of thin roots with the rhizosphere soil (approximately 1 g) are aseptically collected.
- Root sample is placed into an Erlenmeyer flask with 100 mL of sterile tap water and is shaken for 30 min. The roots are taken off and the suspension is kept to let the soil particles settle out, after which a range of dilutions is prepared for isolation of rhizosphere microorganisms.
- Soil suspension dilutions are plated on nutrient agar and incubated at 30^{o}C for 3-5 days.
- Isolated colonies are transferred to agar medium M9 with a surfactant and nutrient agar by using the replica technique.
- Plates are incubated at 30^{o}C for 5-7 days. Plates with nonionic surfactants are treated with Dragendorff's reagent according to (Cooke, 1978), and plates with anionic surfactants are treated with neutral red reagent according to (Ohwada, 1975) for 10-20 min. Then, the reagents are removed, and plates are washed with running water.
- Transparent areas on orange (for nonionic surfactants) or red (for anionic surfactants) background medium are formed around surfactant-degrading colonies.
- The degradative activity of the isolated surfactant degraders is studied in liquid medium under batch cultivation conditions.

1.3. Identification

- For identification, physiological and biochemical tests, immunochemical analysis, and 16S rRNA gene sequence analysis are used.

1.4. Storage

The cultures are maintained by cryopreservation (at -70ºC) and overlaying with mineral oil.

1.5. Cryopreservation

- A tube with 5 mL of liquid LB medium is inoculated with an 18-48-h culture.
- The tubes are incubated at 30°C till late exponential growth.
- The cell suspension is diluted with fresh medium containing 40% glycerol.
- The cell suspension is distributed into 0.5-1.0 mL sterile Eppendorf tubes. The tubes are labeled with strain name, collection number, and preservation date.
- The tubes are frozen in liquid nitrogen.
- The frozen samples are maintained at -70ºC.

1.6. Overlaying with paraffinic oil

- A tube with 9 mL of semi-liquid LB medium is inoculated with an 18-48-h culture.
- The tubes are incubated at 30°C.
- The agar columns with the inoculants are overlaid with 1-2 mL of paraffinic oil.
- The tubes with the inoculants are stored at 4ºC.

2. Media

Physiological water
Prepare a solution of 0.85% NaCl and sterilize.

M9 medium (Miller, 1972)

Na_2HPO_4	6.0 g
KH_2PO_4	3.0 g
NaCl	0.5 g
NH_4Cl	1.0 g
Distilled water	1.0 L
Agar	2.0 g

Adjust pH to 7.2; Autoclave at 120ºC for 30 minutes.

Dragendorff's reagent (Cook, 1978)

0.17% solution of NO_3 and 4% solution of KI in 0.2N acetic acid; H_3PO_4; C_2H_5OH and 20% solution of $BaCl_2$ are mixed in proportion 10:1:10:5, respectively.

Reagent for isolation of anionic surfactants degraders (Ohwada, 1975)

0.3% solution of neutral red in distilled water and 2.42% Tris solution in distilled water are mixed in proportion 1:1, after that 0.2M HCl is added to adjust pH to 8.6. The mixture is 2-fold diluted with distilled water.

3. References

Turkovskaya O.V., Shub G.M. Microbial degradation of non-ionic surfactants //Appl. Biochem. Microbiol. – 1989. – Vol. 25, N 6. – P. 775-780.

Cooke K.A. Rapid method for the detection of nonionic surfactant – degrading microorganisms // J. Appl. Bacteriol. – 1978. – Vol. 44, N 2. – P. 299-303.

Ohwada K. Agar plate method for detection and enumeration of alkylbensenesulphonate-degrading microorganisms // Appl. Microbiol. – 1975. – Vol. 29, N 1. – P. 40-43.

Miller J.H. Experiments in Molecular Genetics. Gold Spring Harbor Laboratory Press. – 1972. – 466 p.