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Release of periplasmic proteins from *Sphingomonas paucimobilis* strain S ATCC 43388

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Alkaline phosphotase and α-santonin binding proteins were released in the periplasmic shock fluid from *S. paucimobilis* strain S ATCC 43388, on treatment with freeze thaw (FTM), cold osmotic shocking (COSM) and chloroform (CHLM) method. Such cells devoid of periplasmic proteins showed corresponding reduction in the transport of α-santonin. Amongst the methods employed FTM was found to be most effective,

Sphingomonas paucimobilis strain S ATCC 43388 initially identified as *Pseudomonas* sp. is capable of utilizing α-santonin, a hydrophobic and feebly soluble sesquiterpene, as a sole source of carbon[1-5]. Initial interaction of these cells occurs with α-santonin available in soluble form[3]. Periplasmically located substrate binding proteins of gram negative bacteria play an important role in the initial interaction and uptake of various compounds in soluble form[6-9]. Several methods are used for elution of periplasmic proteins to study their role in the transport[7-9]. Organisms are likely to respond differently to individual method used for release of periplasmic proteins, depending on the cell surface composition[10]. The present study has been undertaken to compare different methods employed to elute out periplasmic proteins from *S. paucimobilis*.

Microorganisms and media—*S. paucimobilis* strain S ATCC 43388 was maintained on 0.4% santonin agar slants[1]. The cells grown in 0.4% santonin agar plates at room temperature for 24 hr were suspended to a desired cell density of 4.5 OD at 450nm (12.5 mg wet weight). Wet weight of the cells was determined after decanting the suspending fluid following centrifugation.

Chemicals—α-santonin was obtained from Alchem International Pvt. Ltd., New Delhi. All the chemicals used for media preparations, buffers and reagents were of analytical grade. Tris hydroxy methyl amino methane, potassium dihydrogen orthophosphate and dipotassium hydrogen orthophosphate were obtained from Qualigens.

Extraction of periplasmic proteins—Periplasmic proteins were extracted from the cells by three methods viz cold osmotic shock (COSM)[7], chloroform extraction (CHLM)[8] and multiple cycles of freezing (−20°C) and thawing (FTM)[9].

Analysis—The shock fluid obtained by each of the above methods was passed through 0.45μm Millipore filter and analyzed for protein content[11], alkaline phosphatase activity[12].

α-santonin binding activity of shock fluid was determined by equilibrium dialysis binding[13]. Shock fluid was dialyzed overnight against saturated α-santonin solution in 0.05 *M* phosphate buffer (*p*H 7) at room temperature, with constant stirring. The contents from the bag were extracted with chloroform and analyzed for α-santonin colorimetrically[3,14].

Rate of uptake of α-santonin by treated and untreated cells was measured by estimating the amount of undepleted α-santonin in the reaction mixture, by the method reported earlier[3,14].

Substrate binding proteins situated in periplasmic space play important role in nutrient transport, demonstrated by their specific binding to the substrates and lack of transport of substrate in the cells devoid of such transport proteins. Efficient release of periplasmic proteins from the organisms in entirety/is desirable. Depending on cell surface characteristics, each organism responds differently to the physicochemical treatments and the release of periplasmic proteins thus varies with the method employed[10]. Chloroform is reported to release maximum periplasmic proteins from *Pseudomonas*[10]. However in the present work with *S. paucimobilis strain S* FTM was more efficient and released more periplasmic proteins than COSM and CHLM (Table 1).

Alkaline phosphatase, a periplasmic enzyme is often used to monitor the release of periplasmic proteins[10]. Eluant of FTM showed release of 10.5 μmoles of *p*-nitro phenol, PNP/ml as compared to COSM and CHLM, the trend corraborating the total proteins in the shock fluid, released by each of the three methods (Table 1). Invariant specific activity of

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the enzyme in shock fluids indicated that the methods differed in their efficiency to release periplasmic proteins only quantitatively. High α-santonin binding activity (2.40μmole/mg of protein) in the periplasmic proteins released by FTM reduced the α-santonin transport in the concerned shocked cells by 90%, on comparison to only 70% by COSM (Table 1). This confirms the periplasmic location of α-santonin binding factor and the efficiency of the FTM in eluting them. This method also has selective advantages over other conventional methods, as it does not involve any chemical treatment such as lysozyme, EDTA, chloroform or toluene and releases selectively periplasmic proteins without loss of apparent cell damage.

Effect of physicochemical parameters on release of periplasmic proteins by FTM

Cell density—Amount of protein released by FTM increased significantly with increase in the cell density (Fig. 1A) from 2.6-12.5 mg/ml and plateaued thereafter, possibly due to the decrease in inter cellular solute space and cell to cell surface contact. Cell density with absorbance of 4.5 optimally elutes periplasmic proteins.

Table 1—Analysis* of periplasmic proteins from *S. paucimobilis* strain S ATCC 43388 cells released by three methods

		Method used		
		FTM	COSM	CHCM
A. Analysis of Released Proteins				
1.	Total Protein released (mg/g of wet cell)	8.0	3.8	0.89
2.	Alkaline Phosphotase[a] (μmole/ml supernatant)	10.5	5.0	1.3
3.	Alkaline phosphotase[a] (μmole/mg of protein)	26.2	26.3	29.2
4.	α-santonin binding activity (μmole santonin/mg protein)	2.40	1.61	0.30
B. Transport activity of the shocked cells				
1.	Rate of uptake[b] (μg α-santonin/ml/min)	0.7	1.5	ND
2.	% Reduction in transport	90	70	ND

*mean values of observations in triplicates.
[a]expressed in terms of Paranitrophenol (PNP) released from PNP phosphate.
[b]Rate of uptake of α-santonin by untreated wild type cells is 7.3 μg/min.

Freeze-thaw cycles—The proteins were released in the shock fluid partially after the first and to a maximum extent after the second FT cycle and with no further increase on subsequent FT cycles (Fig. 1B). A single overnight freezing and thawing[9] of cells at −20°C released less proteins than the two freeze thaw cycles.

Temperature—Cells freezed more effectively at −20 or −70°C and released 110μg/ml of periplasmic proteins, possibly due to rapid ice-crystal formation resulting in alteration of membrane permeability[15], as against release of only 70μg/ml proteins at −4°C (Fig. 2C).

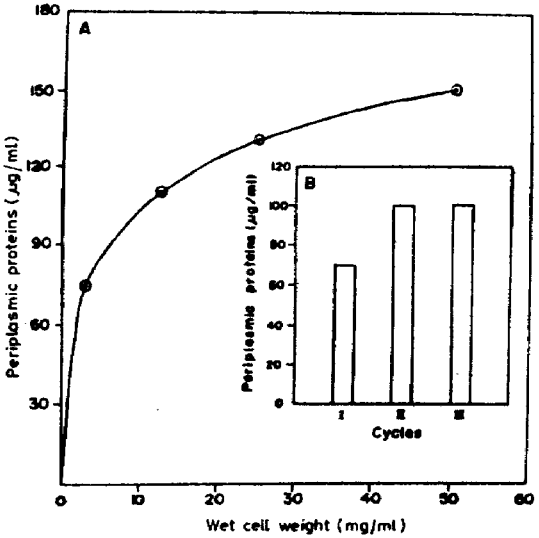

Fig. 1—Standardization of freeze thaw method. (A) Effect of cell concentration on release of periplasmic proteins. (B) Amount of proteins released after each cycle of freeze-thawing from the cells suspended to an absorbance 4.5 at 450nm.

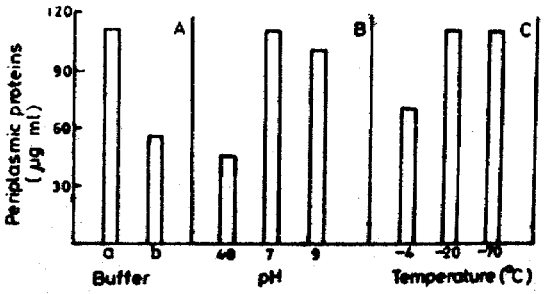

Fig. 2—Effect of A: buffer : (a) phosphate, *p*H 7.0, 0.05*M*, (b) Tris HCl; B: *p*H; and C: temperature, on the amount of periplasmic proteins released by FTM.

Buffer and pH—The amount of proteins released may vary with the solutes in the suspending medium[16]. The cells suspended in Tris HCl *p*H (7.0) eluted only 55μg/ml as compared to 110 μg/ml released by cells suspended in phosphate buffer, *p*H (7.0) (Fig. 2A). At acidic *p*H (4.8), proteins release was reduced significantly to 45μg/ml (Fig. 2B).

The shock fluid eluted from *Sphingomonas paucimobilis* strain S ATCC 43388, suspended in 0.05*M* phosphate buffer by FTM two cycles showed, high periplasmic protein content, alkaline phosphatase activity and α-santonin binding activity. This method is therefore suitable for extraction of periplasmic proteins especially α-santonin binding proteins from *S. paucimobilis* strain S ATCC 43388.

The shock fluid eluted from *Sophingomonas* to MSR.

References

1 Sangodkar U M X & Mavinkurve S, *J Biosci*, 4 (1982) 79.
2 Sangodkar U M X & Mavinkurve S, *Indian J Microbiol*, 24 (1984) 202.
3 Furtado I, Sangodkar U M X & Mavinkurve S, *Biotech Bioeng*, 30 (1987) 991.
4 Colaco D, Furtado I, Naik U P & Mavinkurve S, *Lett Appl Microbiol*, 17 (1993) 212.
5 Furtado I, Sangodkar U M X & Mavinkurve S, *Indian J Microbiol*, 34 (1994) 219.
6 Ames G F L, Mirmura C S & Shyamala V, *FEMS Microbiol Rev*, 75 (1990) 429.
7 Neu H C & Heppel L A, *J Biol Chem*, 240 (1965) 3685.
8 Ames G F L, Prody C & Kostu S, *J Bacteriol*, 160 (1984) 1181.
9 Paoletti L A, Short K A, Blakemore N & Blakemore R P, *Appl Environ Microbiol*, 53 (1987) 2590.
10 Lall S D, Eribio B E & Jay J M, *J Microbiol Methods*, 9 (1989) 195.
11 Lowry O H, Rosebrough N J, Farr A L & Randall R J, *J Biol Chem*, 193 (1951) 265.
12 Belsare D K, Prasad D Y, Belsare S D & Gupta S N, *Proc Nat Acad Sci India*, Section B Part III (1992) 305.
13 Stinson M Z, Cohen M A & Merrick J A, *J Bacteriol*, 131 (1977) 670.
14 Furtado I & Mavinkurve S, *Pafai*, 3 (1981) 16.
15 Calcott P H & Macleod R A, *Can J Microbiol*, 20 (1974) 671.
16 Peter H C & Robert A M, *Can J Microbiol*, 21 (1975) 1924.