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Optimisation of DNA extraction from rumen bacteria

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One major condition for estimating rumen bacteria growth rate by ^{32}P incorporation into DNA is the use of a DNA extraction procedure which produces a non selective lysis of cells and gives a high DNA recovery rate. The aim of this study was to adapt a freeze-thaw procedure used in soil biology (Tsai and Olson, 1991, Appl Environ Microbiol, 57, 1070-1074) to the treatment of mixed rumen bacteria.

Six qualitative factors have been considered : the interest of an enzymatic pre-treatment - by proteinase K (concentration of 0.5 g/l) or lysozyme (concentration of 5 g/l) - and the use of five detergents - CHAPS, sodium deoxycholate, SDS, Triton X-100 and sodium lauroyl sarcosine (SLaS). In order to screen efficiently these factors, an asymmetric design of 9 runs was generated among the 96 available combinations of factors and it was applied in duplicate. The characteristics of this design are satisfactory (number of experiments = 18 ; Log determinant (M) = -4.60 ; maximal variance function = 0.47 ; G efficiency = 94 %). The worksheet is available from the authors. The bacterial pellets obtained by centrifugation (27,000 g, 30 min.) were incubated in 300 µl of enzymatic lysis buffer at 37°C for 1 h. Then 400 µl of detergent solution (TE, pH 8.5) was added and a cycle of freezing at -20°C for 30 min and thawing at 37°C for 30 min was conducted. All the detergents but SlaS were screened at their critical micellization concentration (CHAPS : 5mM, sodium deoxycholate : 4 mM, SDS : 2 mM, Triton X-100 : 0.3 mM, SLaS : 17 mM). DNA was extracted using phenol \ isoamyl alcohol \ chloroform and precipitated by addition of spermine. After removal of spermine, the DNA pellet was dissolved in 1 ml of TE buffer (pH 8.0). Two responses have been measured. The proportion of lysed cells was estimated by acridine orange counts. The DNA recovery rate was measured by a fluorometric method using ethidium homodimer (Mordy and Carlson, 1991, Mar Ecol Prog Ser, 73, 283-293).

The main effects of the 6 factors were estimated by fitting a Free Wilson model. The parameter estimates of the simplified model and the Student's probabilities for testing the null hypothesis (P>|T|) are given in the table. The results of the regression analysis were satisfactory (estimated experimental error of 1.45 and 0.92, R-squared of 0.84 and 0.98 respectively for the proportion of lysed cells and the amount of recovered DNA). The use of lysozyme increased by 6 % the proportion of lysed cells and by 45 % the DNA recovery rate. As shown in the table, the two best detergents - SDS and SLaS - had smaller favorable effects. In conclusion, the enzymatic pre-treatment with lysozyme and the use of both SDS and SLaS in the extraction buffer at the final concentration of 15 mM were chosen.

Factor	Lysed cells (%)	P>|T|	DNA (mg/l)	P>|T|
Constant	91.75		26.66	
Proteinase K	2.50	>0.01	0.98	>0.05
Lysozyme	5.65	>0.001	12.08	>0.001
CHAPS	-0.29	0.34	-0.79	0.05
Deoxycholate	0.53	0.24	-0.77	0.06
SDS	0.53	0.24	1.36	>0.01
Triton X-100	-0.06	0.47	0.64	0.09
SLaS	2.36	>0.01	1.14	>0.05