JOURNAL OF BACTERIOLOGY, Mar. 1971, p. 1200-1201

Vol. 105, No. 3
Printed in U.S.A.

NOTES

Selection of Cells Transformed to Prototrophy for Sporulation Markers

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Received for publication 9 December 1970

A method is presented for reproducible quantitative selection of recombinants for spore markers in transformation analysis.

Genetic analysis of *Bacillus subtilis* mutants defective in the process of sporulation was hampered by the lack of suitable techniques for direct selection of sporulating recombinants. This deficiency required the use of selectable auxotrophic markers for linkage analysis in crosses with sporulation defective (*spo*$^-$) mutations. This requirement has not presented much difficulty in PBS-1 transduction crosses since the large size of the transducing fragment increases the probability of linkage of *spo*$^-$ mutations to auxotrophic mutations. Because of this large transducing fragment, the probability of recombination between closely linked markers, when compared to transformation, is greatly decreased and, thus, accordingly, is the usefulness of PBS-1 transduction for fine-structure analysis. Since *spo*$^-$ mutations are infrequently linked to auxotrophic mutations when using transformation analysis, it became imperative to develop a method for the quantitative selection of *spo*$^+$ transformants to utilize the recombination index method (5) of fine-structure analysis. Ionesco (4) described three methods for selection of *spo*$^+$ transformants. We experienced difficulty in obtaining reproducible results with these methods when utilizing mutants blocked early in the sporulation process. Therefore, a modification of Ionesco's method was developed for and used early blocked mutants. In this method, the *spo*$^-$ mutant is transformed with deoxyribonucleic acid and plated on a sporulation agar. The plates are incubated to allow the *spo*$^+$ transformants time to express their phenotype and sporulate. When sporulation is complete, the plates are exposed to chloroform to kill all of the nonsporulated cells. The plates are then incubated for a suitable length of time to allow the spores to germinate, divide, and form discrete colonies.

Recipient cells are brought to competence and transformed in the usual manner (1). Dilutions of the transformation tube are plated on appropriately supplemented minimal media for the selection of auxotrophic requirements. The same dilutions are plated on sporulation plates containing the basal salts of Donellan et al. (2) supplemented with 0.1% glutamate, 0.1% casein hydrolysate, and additions to satisfy the recipient's auxotrophic requirements. After overnight incubation at 37 C, the plates are covered with a thin lawn of growth. The lawn is killed by exposure to 2 ml of chloroform pipetted into the lid of the inverted petri dish. After the chloroform evaporates, the plates are reincubated at 37 C for 24 to 36 hr. At this time the lawn has lysed, and each transformant has given rise to a colony.

The effect of various additions to the sporulation agar on the final yield of *spo*$^+$ transformants is shown in Table 1. In this experiment, JH 109 (*trpC2 spoA9*) was transformed with wild-type deoxyribonucleic acid and plated on the various media. After 19 hr of incubation at 37 C the plates were exposed to chloroform. Both glutamate and acetate were effective in giving high yields of transformants, whereas lactate and citrate decreased the yield. Furthermore, the latter compounds drastically reduced the number of transformants on the lower dilution plates, whereas additivity was maintained with glutamate and acetate. In a similar experiment, the time for expression of sporulation was determined by plating the transformation on basal salts plus 0.1% glutamate and 0.1% casein hydrolysate and selecting with chloroform after various times of incubation. The results (Table 2) show that expression is about 50% complete by 14 hr and complete by 19 hr of incubation.

The addition of *spo*$^+$ cells, at a concentration

TABLE 1. *Effect of carbon sources on the recovery of spo⁺ recombinants in transformation*

Addition to basal salts + 0.1% casein hydrolysate	*spo*⁺ Transformants per ml	*spo*⁺/*trp*⁺ [a]
0.2% Casein hydrolysate	1.0×10^5	0.63
0.1% Glutamate	2.5×10^5	1.56
0.1% Acetate	2.4×10^5	1.50
0.1% Lactate	7.0×10^4	0.44
0.1% Citrate.....	6.5×10^4	0.40

[a] Number of *trp*⁺ transformants was 1.6×10^5/ml.

TABLE 2. *Yield of spo⁺ transformants as a function of expression time*

Hr of expression before selection	*spo*⁺ Transformants per ml[a]
0	<10
8	1.70×10^2
14	1.50×10^6
19	2.74×10^6
26	3.07×10^6
30	2.68×10^6

[a] Number of *trp*⁺ transformants was 1.02×10^6/ml.

equal to the normal level of transformants, to a competent *spo*⁻ culture resulted in a quantitative yield of *spo*⁺ clones by using the above regimen. Thus, no detectable loss of *spo*⁺ cells occurs from the presence of a large excess of *spo*⁻ cells.

We used this method successfully for fine-structure analysis of *spoA* mutants (3) and for another group of early *spo*⁻ mutants (*unpublished data*) which we were unable to link to any auxotrophic marker by PBS-1 transduction.

This investigation was supported by grant GB 15602 from the National Science Foundation and by Public Health Service grant HD 02807 from the National Institute of Child Health and Human Development.

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