SHORT COMMUNICATION

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A SIMPLE METHOD FOR OBTAINING MULTIPLE MARKED STRAINS OF *BACILLUS SUBTILIS*

HIDEO TAKAHASHI AND YONOSUKE IKEDA

*Institute of Applied Microbiology, University of Tokyo,
Tokyo, Japan*

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The microbiologists studying genetics of *Bacillus subtilis* frequently feel the need of strains with multiple genetic markers, but preparation of definitely marked strains is generally difficult.

The method described below is based on two principles. The first is the use of a phenomenon of the double event transformation for introducing a new marker to a recipient, and the second is the alternative use of streptomycin-resistant marker (*Str-r*) and erythromycin-resistant marker (*Ery-r*) for the selection of transformants and multiplication of genetic markers. The *Str* and *Ery* genes are located close to each other on the chromosome.

Fig. 1 shows results of a transformation experiment performed between *B. subtilis* W23 (*Str-r*) and *B. subtilis* YS-11 (*Ade₆Leu₈Arg₁₅Str-s*). Among the three types of double transformants, *Str-r Ade⁺* type transformant was scored. As this graph indicates, the curve representing the frequency of double-event transformation approaches, at higher DNA concentrations, one-tenth of that of single-event transformation. This result suggests that an *X-Str-r* type double marked strain can be selected easily among the *Str-r* transformants if an *X-Str-r* donor is used. The transformation was performed according to the method of H. TANOOKA and Y. SAKAKIBARA (*Biochim. Biophys. Acta*, **155**, 130 (1968)).

The *X-Str-r* strain will be exposed, in the second step, to a high concentration of *Y-Ery-r* type DNA. As the *Str* and *Ery* loci are linked on the chromosome at a co-transfer index of about 0.45 (I. TAKAHASHI, *J. Bacteriol.*, **89**, 1065), the isolation of *X-Y-Str-s Ery-r* strain is easy. In the third step, an experiment will be done between a *Z-Str-r* (DNA donor) and an *X-Y-Str-s Ery-r* (recipient) to obtain *X-Y-Z* strains. In this fashion, markers may be multiplied.

We have obtained a strain with 9 markers (*Ade₆Leu₈Arg₁₅Met₅Ind₁₆₈His₁Cys₁₄Lys₂₁Thr₅*) starting from a strain with 3 markers (*Ade₆Leu₈Arg₁₅*). The crosses were performed between two isogenic strains. In the above stated

Fig. 1. Single event transformation and double event transformation (DNA donor=W23, recipient=*Ade₆Leu₈Arg₁₅Str-s*).

The DNA was prepared by the method of H. SAITO and K. MIURA (*Biochim. Biophys. Acta*, **72**, 619 (1963)).

Table 1. Two examples of experiments.

Expt.	Donor strain used	Recipient strain used	Marker used for selection	Strain newly produced
I	<i>Ade₆ Leu₈ Met₅</i> <i>Str-s Ery-s</i>	<i>Ade₆ Leu₈ Arg₁₅</i> <i>Str-s Ery-r</i>	<i>Ery-r</i>	<i>Ade₆ Leu₈ Arg₁₅</i> <i>Met₅ Str-s Ery-r</i> (6/60)
II	<i>Ind₁₅₈ Met₅</i> <i>Str-r Ery-s</i>	<i>Ade₆ Leu₈ Arg₁₅</i> <i>Str-s Ery-r</i>	<i>Str-r</i>	<i>Ade₆ Leu₈ Arg₁₅</i> <i>Met₅ Ind₁₅₈</i> <i>Str-r Ery-s</i> (8/227)

About 2 μ g/ml of DNA was used for the double-event transformation. The donor and recipient markers are isogenic. The map position of these markers are described in the papers of D. DUBNAU *et al.* and A. O'SULLIVAN and N. SUEOKA.

strain, the 9 markers are scattered over the whole area of chromosome (D. DUBNAU *et al.*, *J. Mol. Biol.*, **27**, 163 (1967); O'SULLIVAN and N. SUEOKA, *J. Mol. Biol.*, **27**, 349 (1967)). Table 1 shows two examples of the intermediate operation. The figures in the parentheses indicate the ratio of the number of new strains to number of colonies tested.