Transformation of Phage-sensitivity in *Bacillus subtilis*

By S. HORVÁTH

Institute of Genetics, Hungarian Academy of Sciences, Budapest, Hungary

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SUMMARY

Two methods were developed to demonstrate a small fraction (10^{-4}) of phage-sensitive cells in a phage-resistant population with a high degree of accuracy, using the plexiglass phage titration method (Horváth & Alföldi, 1954).

Phage-sensitivity was transferable by DNA isolated from *Bacillus subtilis* 168 M *trp*⁺ *phs* (SPO-1 phage-sensitive) to the recipient strain of 168 M *trp* *phr* (SPO-1 phage-resistant) in transformation experiments. The number of 168 M *trp*⁺ *phs* transformants was a function of the concentration of the transforming DNA. The *trp*⁺ and *phs* characters are not linked. The competence curves for the number of 168 M *trp*⁺ *phr* and 168 M *trp*⁺ *phs* transformants were similar. The phenotypic lag was found to be 5 hr 30 min. The maximum frequency of 168 M *trp*⁺ *phs* cells among the transformants was 2 to 5%.

INTRODUCTION

A wide range of bacterial characters are transferable (Ravin, 1961) and Horváth, (1969) succeeded in transforming phage-resistance in *Bacillus subtilis*.

The present investigation demonstrates the transformation of phage-sensitivity.

METHODS

Bacterial and phage strains. For transformation the recipient strain was a spontaneous SPO-1 phage resistant mutant of *Bacillus subtilis* 168 M *trp* *phs* designated 168 M *trp* *phr*. Phage-sensitive spontaneous mutants were found among the 168 M *trp* *phr* bacteria and the rate of mutation was 5.82×10^{-5} mutations per bacterium per generation calculated according to Luria & Delbrück (1943). DNA was prepared from *B. subtilis* 168 M *trp* *phs* and 168 M *trp* *phr* strains after transformation to prototrophy.

Media. Bacterial strains were maintained on potato agar (Spizizen, 1958). The recipient strain was precultivated on minimal glucose yeast agar slope (MGY agar); competent cells were prepared in MGY liquid medium; T medium was used for transformation and MG agar for the selection of 168 M *trp*⁺ *phr* transformants (Horváth, 1967).

Transforming DNA from the donor strains was prepared by the phenol extraction method of Saito & Miura (1963).

The titration of phage was carried out by the plastic tray method (Horváth & Alföldi, 1954).

Transformation procedure. An overnight culture of 168 M *trp* *phr* on MGY agar slope was suspended in 10 ml. liquid medium in a 100 ml. Erlenmeyer flask, fitted with a side arm for densitometry measurement. The optical density (OD) of the

bacterial suspension was 0.025, which contained 9.6×10^5 colony forming u./ml. The culture was incubated in a water bath at 37° on a reciprocal shaker at 100 rev./min. (Horváth, 1967). When the bacterial cells reached the peak of competence (OD 1.5) the cell suspension was diluted in T medium to 0.4 OD. To 0.25 ml. of this suspension 0.75 ml. DNA solution in T medium was added to give a final DNA concentration of 5 µg./ml. and shaken for 30 min. in a water bath at 37°. Finally, 0.25 ml. of this mixture was measured into a 50 ml. Erlenmeyer flask containing 4.75 ml. MG liquid medium with 0.1 % casein hydrolysate and after 14 to 15 hr incubation at 37°, 0.05 ml. of the bacterial suspension was transferred to 2 ml. MG liquid medium containing 0.1 % casein hydrolysate. The suspension was shaken in a water bath at 37°, and after 3 to 4 hr the number of phage-sensitive cells as 168 M *trp*⁺ *phs* transformants was measured according to the following two methods. This transformation procedure yields qualitative rather than quantitative results.

*Measurement of the number of SPO-1 phage-sensitive cells in
phage-resistant populations*

1. *One-step growth curve method.* 168 M *trp phr* and 168 M *trp phs* cell suspensions in early exponential growth were used (OD 0.3). Different numbers of 168 M *trp phs* cells in 0.5 ml. volumes were added to 1 ml. phage resistant 168 M *trp* cells. 2×10^6 SPO-1 phage particles in 0.5 ml. volumes were then added to each tube. These cultures were shaken in a water bath at 37° for 5 min. 0.1 ml. of these suspension were diluted in 10 ml. MGY liquid media and incubation was continued for 1 hr. Samples were taken and one-step growth curves were determined from the plaque forming units (p.f.u.). The higher the number of phage-sensitive cells in the phage-resistant population, the higher the titre of the plateau. The plaque titre at the plateau in log units was designated T_c for the control phage-resistant bacterial suspension and T_s for the bacterial suspensions which contained phage-sensitive cells. The difference $T_c - T_s$, designated D , was used in the transformation experiments to assay the number of phage-sensitive cells present among the 168 M *trp*⁺ *phr* transformants.

2. *The phage, anti-phage serum method.* To 0.5 ml. phage-resistant cell suspension after transformation 0.25 ml. SPO-1 phage (4×10^7 p.f.u./ml.) was added and shaken at 37° for 10 min. Then 0.25 ml. anti-SPO-1 phage serum (K value 25) was added and incubation continued for a further 10 min. at 37°. The number of infective centres was then assayed using 168 M *trp phs* as indicator bacteria.

RESULTS AND DISCUSSION

Transformation of phage-sensitivity

To investigate the transformation of phage-sensitivity, undiluted 168 M *trp phr* competent cells were used in the experiment. The D value was 1.357 when transformation was carried out with DNA isolated from 168 M *trp*⁺ *phs* and 5 % of phage-sensitive cells were found among the *trp*⁺ transformants. When DNA isolated from 168 M *trp*⁺ *phr* was used in transformation the number of phage-sensitive cells was very low ($D = -0.153$) and not higher than with the control strain, 168 M *trp phr*.

When transforming DNA was treated with 50 µg./ml. DNase before transformation, no transformants could be detected.

Effect of DNA concentration

Different quantities of transforming DNA isolated from 168 M *trp*⁺ *phs* cells were added to the undiluted competent 168 M *trp* *phr* cells, and the *D* values were measured. The number of 168 M *trp*⁺ *phs* transformants was a function of the DNA concentration. The dose-effect curve calculated from the number of 168 M *trp*⁺ *phs* transformants was steeper than 45°, which shows that the *trp*⁺ and *phs* markers are not linked. When markers are far apart, double transformants are found only at saturating levels of DNA (Goodgal, 1961; Michel, Sicard & Ephrussi-Taylor, 1964; Kelly, 1967).

The competence curve

The competence curve based upon the number of 168 M *trp*⁺ *phr* transformants was the same as that found earlier. The characteristics of the competence curve for 168 M *trp*⁺ *phs* transformants were also similar to those obtained previously (Horváth, 1967, 1968).

Fig. 1. The number of *trp*⁺ *phr* and *trp*⁺ *phs* cells during transformation. ●—●, 168 M *trp*⁺ *phr* transformants; ○—○, 168 M *trp*⁺ *phs* transformants; 168 M *trp* *phr* bacteria were transformed with DNA from 168 M *trp*⁺ *phs*. The number of *trp*⁺ *phs* transformants was measured using the phage, antiphage serum method. 0 is the time when DNA was added.

Assay of trp+ phr and trp+ phs bacteria during transformation

168 M *trp* *phr* competent cells were used to measure the number of *trp*⁺ *phr* and *trp*⁺ *phs* bacteria during transformation. DNA isolated from 168 M *trp*⁺ *phs* was added to the cell suspension and incubated at 37° for 30 min. 0.5 ml. was then measured into three flasks each containing 9.5 ml. MG liquid medium with 0.1% casein hydrolysate. A control without DNA was included. 0.9 ml. samples were taken during incubation and 0.1 ml. DNase (30 µg.) was then added and incubation continued for 5 min. The *trp*⁺ *phr* transformants were selected on MG agar. The number of *trp*⁺ *phs* transformants was measured by the phage, antiphage serum method (Fig. 1).

After subtraction of the control values, the curve for *trp*⁺ *phs* transformants was obtained. The phenotypic lag was about 5 hr 30 min. A concomitant rise in the number

of *trp⁺ phr* as well as the *trp⁺ phs* transformants was observed during incubation. The frequency of the *trp⁺ phs* among the *trp⁺ phr* transformants was about 3 %, calculated from the corrected values.

Sensitivity of trp⁺ phr and trp⁺ phs transformants to phage SPO-1

Suitable bacterial dilutions were spread on MGY agar to obtain 60 to 80 colonies per plate. 4 to 5 % of these colonies were SPO-1 phage sensitive in tests employing the replica plating technique (Lederberg & Lederberg, 1952). Six phage-sensitive and ten phage-resistant colonies were tested. The phage-sensitive colonies gave high *D* values and phage-resistant colonies gave very low *D* values. Intermediate *D* values were not observed.

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