Transformation of Phage-sensitivity in *Bacillus subtilis*

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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 & Alfoldi, 1954).

Phage-sensitivity was transferable by DNA isolated from *Bacillus subtilis* 168 M trp^+ phs (SPO-I phage-sensitive) to the recipient strain of 168 M trp phr (SPO-I 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-I 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 \times 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 & Alfoldi, 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 $9.6 \times 10^5$ 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. $2 \times 10^6$ 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 $T$, for the control phage-resistant bacterial suspension and $T_1$ for the bacterial suspensions which contained phage-sensitive cells. The difference $T_1 - T$, 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 ($4 \times 10^7$ 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.
**Transformation of phage-sensitivity**

**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).

![Graph](image)

**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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REFERENCES


