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Study on Transformation of Marine Origin Bacterium Alteromonas 1055—1 Strain

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Abstract

It was found in this study that transformation occurred in a typical marine bacterium *Alteromonas* 1055–1 in a competence medium. The transformation was observed when the culture was kept in the competence medium made with Herbst's artificial sea water containing 0.005% yeast extract, 0.025% polypepton, and Tris buffer pH 7.5 equevalent to 0.05 Mol concentration and at 25 C. Typical minerals usually used for the growth of marine bacteria, namely NaCl, Mg^{++}, and Ca^{++} contributed to the process of transformation.

One of the authors[1] published the results of the study on the variations of the bacteria living in the marine environment in 1968. In the paper, he discussed that 1055–1 strain which was identified as *Pseudomonas*, but later recognized as *Alteromonas haloplanktis*[2], showed the possibility of variant production by the mixed culture with other halophilic bacteria. In this papaer, the authors confirmed a possibility of genetic transformation seemed to be proved by using 1055–1 strain. Since the first observation of transformation was done in *Pneumococcus*[3], a lot of papers[4),5),6),7)] about the same kind of study using various bacteria living in terrestrial environment were published. But, there are no reports till these days as regards to whether transformation is possible in marine bacteria to carry out.

The purposes of this study are to make the possibility of transformation clear by using marine bacteria and to establish the essential factors which presumably introduce the reaction. The transformation was ascertained in this study by means of application of the marker resistant to the antibiotic Leucomycin (LM).

Materials and Methods

Organisms: The derived strain from marine Alteromonas 1055–1 was used in this experiment. This strain was confirmed as marine type organism by Hidaka[8].

Isolation of antibiotic resistant strain (Donor strain): In order to isolate a strain resistant to many antibiotics, 1055–1 strain was placed on the ZoBell 2216E agar plates with various antibiotic disks of Leucomycin, Streptomycin, Tetracycline, etc by the method of Adam's double layer. Among various antibiotics tested LM produced a clear inhibitive zone and a lot of large colonies of resistant strain as compared with their antibiotics used. In this method, the authors were able to obtain a resistant strain

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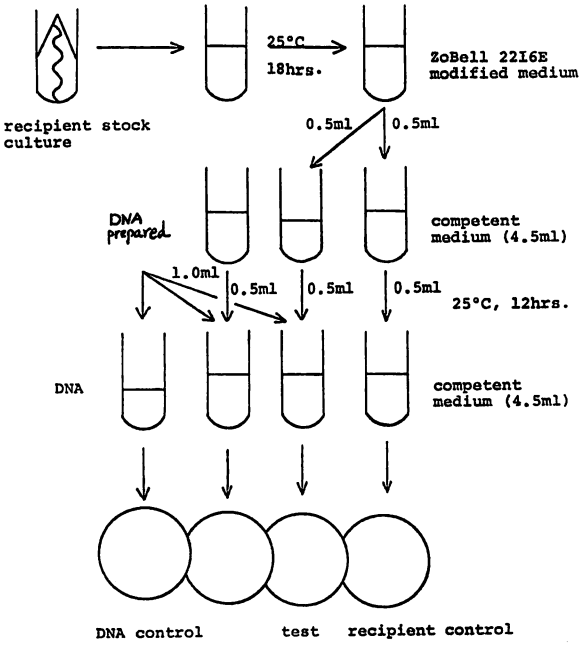
which showed resistance to 230 mg/L LM. To get more stable and stronger strain, this resistant strain was irradiated with UV for 60 sec from the distance of 60 cm. Eventually, some of the organisms treated this way showed a constant resistance to 350 mg/L LM. The organism was used as Donor strain in transformation experiment.

Isolation of LM sensitive strain (Recipient strain): The sensitive strain was isolated from the original strain by replica method which was carried out by replicating the colonies on the ZoBell 2216E agar plate not containing LM. to another plate containing 10 mg/L LM. After incubation., the strain confirmed as sensitive to 10 mg/L LM was picked out. The sensitive strain selected was used as recipient strain.

Culture condition: For isolation and stock of Donor and Recipient strains ZoBell 2216E modified medium yeast extract 1.0 g, Polypepton 5.0 g, Herbst's SAW 1000 ml, and Agar 15 g, pH 7.5 was used.

Competence medium was composed of Herbst's ASW, 1000 ml, Polypepton, 0.25 g, Yeast extract, 0.05 g and 0.05 M Tris buffer (pH 7.5). Incubation was carried out at 25°C for 24 hr except for special purposes.

Chemicals: Leucomycin (Tokyo Jozo Co. LTD), Sodium Laurylsulfate (Nakarai Chemicals LTD), EDTA (Kanto Chemical Co. Inc.), and Chroloform, Isoamylalcohol and so on were all either chemically pure or highest grade.



Transformation procedure

Fig. 1. *Transformation procedure.*

Preparation of DNA: Marmur's method was applied for extracting DNA from the Donor cells.

Confirmation of transformant: The confirmation of transformant was carried out as shown in Fig. 1. The recipient cells were incubated in 8 ml of ZoBell 2216E modified medium and incubated at 25°C for 24 hr. 0.5 ml of this culture suspension was transferred into 4.5 ml of competence medium. After incubation at 25°C for 14 hr ($6.0–9.0 \times 10^8$ cells/ml), 0.5 ml cell suspension was transferred again into 3.5 ml of fresh competence medium ($6.0–9.0 \times 10^7$ cells/ml) with 1.0 ml DNA solution. After this treatment, the cell suspension was inoculated on the ZoBell 2216E agar plate containing 350 mg/L LM, and incubated for 24 hr and the colony appeared was observed. In order to confirm transformation, the recipient control and DNA control were prepared.

Results and Discussion

Factors affecting transformation, especially competence: At the beginning of this study, the authors used the Koser's citrate medium for competence induction, as it is well known that the medium is successiful for transformation of *H. influenza*. But no transformations were observable in such nutrient poor medium. The authors found new one in this experiment which is prepared with Herbst's ASW, yeast extract 0.005%, poplypepton 0.025% and Tris buffer (pH 7.5) of 0.05 Mol. The result is shown in Fig. 2.

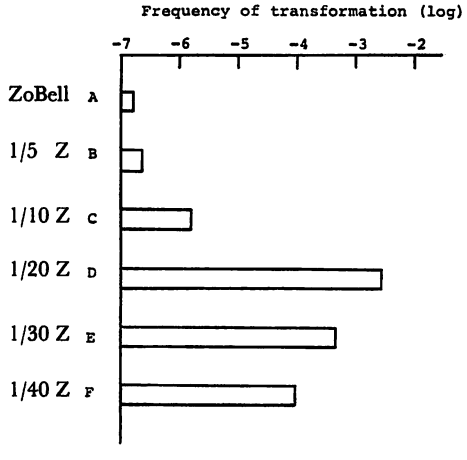


Fig. 2. Effect of concentration of nutrients on competence induction.

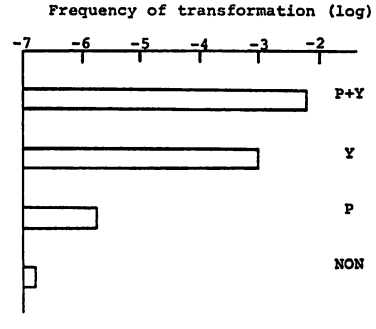


Fig. 3. Effect of organic compounds on competence induction.

Effect of organic compounds on competence induction: The effect on competence induction appeared higher in yeast extract than in polypepton as shown in Fig. 3.

Time course of transformation: After recipient cells were mixed with donor's DNA

in the competence medium, each sample was incubated at 25°C with definite time intervals. As shown in Fig. 4, transformations did not appear at 0 and, 15 mins., but a small number of transformants was observed after 45 mins. That is, the transformants increased rapidly after 45 mins. incubation and reached maximum after 60–70 mins.

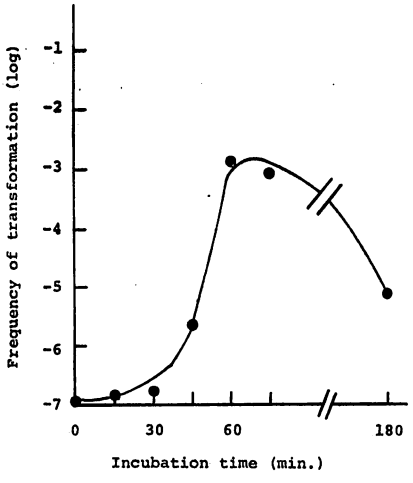


Fig. 4. Time course on competence induction.

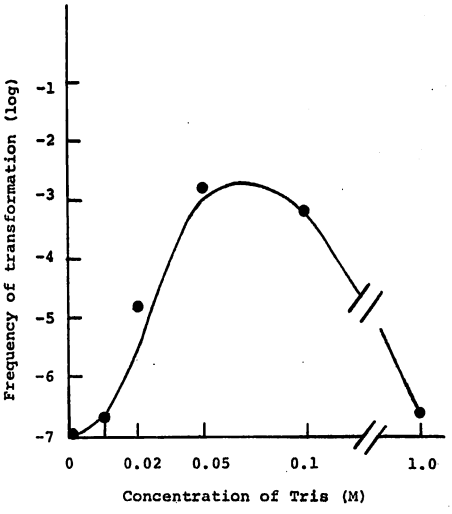


Fig. 5. Effect of Tris conc. on competence induction.

Effect of Tris buffer solution on competence induction: Fig. 5 shows the effect of Tris buffer solution. The composition of competence medium was the same as previously described except the concentration of Tris buffer solution. The concentrations were from 0 to 1.0 Mol. No transformants were observed without Tris buffer, and a few transformants appeared in 1.0 Mol and 0.01 Mol in Tris buffer. The optimum concentration was ranged from 0.05 Mol to 0.2 Mol for transformation and also Tris buffer was essential factor on the transformation. To make it clear whether this result depends on only the ability to stabilize the pH, 0.05 Mol veronal buffer solution was used instead of Tris buffer. But very few transformants were observed in this case. This evidence showed that Tris buffer solution played an unknown role besides buffering effect in bringing about transformation.

Effect of temperature on competence induction: The temperatures used in the experiment were from 16°C to 39°C at definite intervals. The competence medium was the same as previously used. Fig. 6 indicates that optimum temperature of competence induction was 25°C and that no transformants were observed at 16°C, 34°C, 39°C. The temperature required for competence induction coincided with optimum temperature of this organism.

Effect on NaCl on competence induction: Competence medium in this experiment was the same as previously used except for NaCl concentrations. Fig. 7 shows the effect

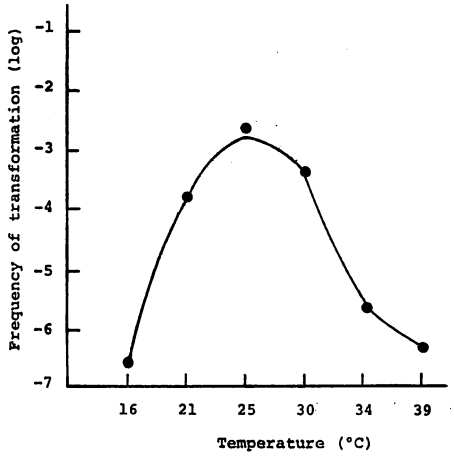


Fig. 6. Effect of temperature on competence induction.

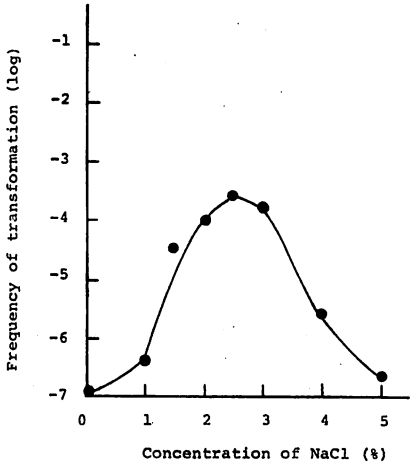


Fig. 7. Effect of NaCl conc. on competence induction.

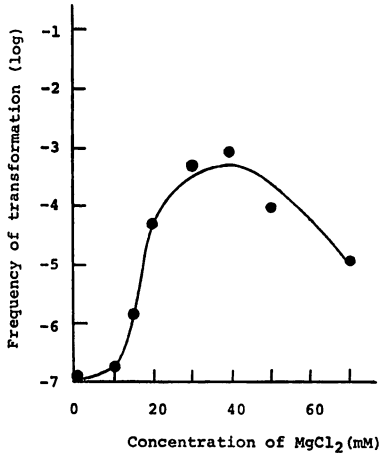


Fig. 8. Effect of $MgCl_2$ conc. on competence induction.

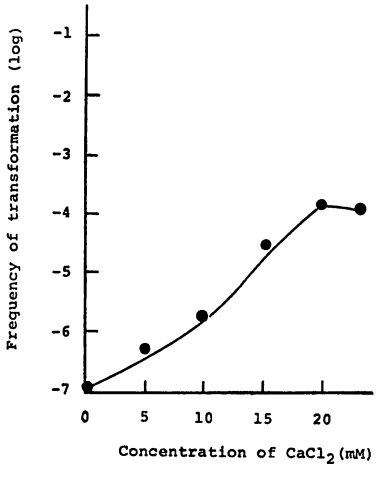


Fig. 9. Effect of $CaCl_2$ conc. on competence induction.

of NaCl concentration on competence induction. Transformation was inhibited at 1% or less and 4% or more concentration of NaCl. The optimum concentration was found between 1.5% and 3%.

Effect of $MgCl_2$ on competence induction: Fig. 8 shows the effect of $MgCl_2$ concentrations on competence induction. Competence medium was the same as previously used except that various concentrations of $MgCl_2$ were added and $CaCl_2$ was omitted from Herbst's ASW. As shown in the figure, there was increase in competence induction with the increase of $MgCl_2$ concentration until the concentration reached

40 mM. after which it decreased. Optimum concentration seemed to be in the ranges from 20 mM to 40 mM.

Effect of $CaCl_2$ on competence induction: Fig. 9 shows the effect of $CaCl_2$ on transformation. The experimental method was the same as previously described in $MgCl_2$ experiment. The frequency of transformant production increased linearly with the increase of $CaCl_2$ concentration, but decreased at 20 mM concentration.

Reference

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