DNA from alkalophilic *Bacillus* can transform *B. subtilis* to alkalophily

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On incubation of *B. subtilis* RM125(*arg15 leuA8* r_M^- m_M^-) with DNA from alkalophilic *Bacillus*, the transformants (Arg^+Leu^- or Leu^-Arg^+) appeared at pH 10. The transformants were able to grow even at pH 7. Alkalophilic *Bacillus* was resistant to bacteriophages ϕ105D1C2·1012 grown on *B. subtilis* 1012($r^-m_M^+$) and ϕ105D1C2·ISMR4 grown on *B. subtilis* ISMR4($r_M^+r_R^+m_M^+m_R^+$), but the recipient *B. subtilis* and the transformant(Arg^+Leu^-) were susceptible to both of the bacteriophages. The results indicate that the transformant is a *B. subtilis* derivative and that alkalophilicity of alkalophilic *Bacillus* was transferred to *B. subtilis*.

Alkalophilic Bacillus *DNA* Bacillus subtilis *Transformation* *Alkalophilic property*

1. INTRODUCTION

The microorganisms which prefer extreme environments for optimum growth are considered to be important tools for elucidating many areas of microbial physiology and evolutionary adaptation. Alkalophilic bacilli which grow optimally at pH 10–11 are one group of these microorganisms, and their physiology and extracellular enzymes have widely been studied [1]. Treatment of *B. alcalophilus* with ethylmethane sulfonate yields a non-alkalophilic mutant [2]. This finding may indicate that a small number of genes are related to the alkalophily of an alkalophile. If so, the characterization of the genes (or the factors expressed by the genes) relating to the alkalophily may afford some interesting insights into the mechanisms of alkalophily and evolutionary adaptation of an alkalophile. We report here that DNA from an alkalophilic *Bacillus* can transform a strain of *B. subtilis* generating transformants which are able to grow at pH 10.

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2. MATERIALS AND METHODS

The bacteria and bacteriophages used were *B. subtilis* RM125(*arg15 leuA8* $r_M^-m_M^-$) [3], obligately alkalophilic *Bacillus* YN-1 [4], facultatively alkalophilic *Bacillus* YN-2 [5] and bacteriophages ϕ105D1C2·1012 grown on *B. subtilis* 1012($r^-m_M^+$) [6] and ϕ105D1C2·ISMR4($r_M^+r_R^+m_M^+m_R^+$) [7].

Alkalophilic *Bacillus* YN-1 and YN-2 were grown in the peptone medium as in [4,5], being kept at pH 10 with 3 M NaOH during the culture. DNA was prepared according to [8].

Preparation of competent *B. subtilis* cells and transformation were carried out according to [9]. The *B. subtilis* was aerobically grown at 37°C in the following, chemically defined medium of pH 7.5; 0.6% KH_2PO_4, 1.4% K_2HPO_4, 0.19% Na-citrate, 0.2% $(NH_4)_2SO_4$, 0.144% $MgSO_4 \cdot 7\,H_2O$, 0.5% glucose, 25 μg/ml of histidine, tryptophan, valine, lysine, threonine, glycine, aspartic acid and methionine, and 50 μg/ml of arginine and leucine. The cells cultured for 1–2 h after the logarithmic phase were used as competent cells. The competent

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cells (~10^9 cells/ml) were treated with DNA (100 μg/ml) at 37°C in the medium (pH 7.5) above for 4 h. The cell culture was then mixed with the peptone medium (1:9) and incubated at pH 9.5 for 1.5 h. The cells thus obtained were spreaded on the minimal medium (1% KH_2PO_4, 0.05% Na-citrate, 0.1% $(NH_4)_2SO_4$, 0.01% $MgSO_4 \cdot 7\,H_2O$, 0.4% glucose) agar plates (pH 7.5 or 10.0) unsupplemented or supplemented by either arginine or leucine (50 μg/ml) and incubated at 37°C for 40 h. The pH of the culture (liquid or agar) in the above alkaline region was adjusted with Na_2CO_3, and only slightly decreased after culture (0.2–0.4).

3. RESULTS AND DISCUSSION

The results of the transformation experiments are summarized in table 1. Several transformants appeared on the pH 10.0 plate containing arginine or leucine. It was shown that all the Arg^+ transformants remained Leu^- and the Leu^+ transformants Arg^-. No transformant was obtained when selection was made for growth on the pH 10.0 plate without arginine nor leucine. The Arg^+Leu^- or Leu^+Arg^- transformants also appeared at pH 7.5, but much more frequently than at pH 10.0. No colonies appeared at pH 10.0 and 7.5, when *B. subtilis* or DNA from alkalophilic *Bacillus* was omitted from the transformation mixture. All the transformants appeared at pH 10.0 were stable, and grew well at pH 10.0 retaining their amino acid requirement properties, even after 10 successive transfers in the chemically defined medium of pH 10.0 used for selection of the transformants. The neutral Arg^+Leu^- or Leu^+Arg^- transformants were unable to grow on the pH 10.0 plates. The alkalophilic Arg^+Leu^- or Leu^+Arg^- transformants, on the other hand, could also grow at pH 7.5. To further characterize the alkalophilic transformants, one of the Arg^+Leu^- transformants obtained with YN-1 DNA(Arg^+Leu^- YN-1) was selected and used for the following experiments.

B. subtilis was unable to grow at pH > 9, while alkalophilic *Bacillus* YN-1 was unable to grow at pH < 8 (fig.1). The Arg^+Leu^- YN-1 transformant grew well over pH 7–10 (fig.1). This may indicate that the transformant possesses the properties concerning the pH-dependent growth of both *B. subtilis* and alkalophilic *Bacillus* YN-1.

The susceptibility to bacteriophages ϕ105D1C2·1012 [6] and ϕ105D1C2·ISMR4 [7] of the Arg^+Leu^- YN-1 transformant was examined, and compared to those of the recipient *B. subtilis*

Table 1

Transformation for alkalophilic growth (no. transformants/10^9 cells)

Recipient *B. subtilis*	Donor DNA	Exp. no.	Alkaline plates (pH 10.0 growth)		Neutral plates (pH 7.5 growth)	
			+ Arg	+ Leu	+ Arg	+ Leu
+	–	I	0	0	0	0
		II	0	0	0	0
–	YN-1	I	0	0	0	0
		II	0	0	0	0
–	YN-2	I	0	0	0	0
		II	0	0	0	0
+	YN-1	I	0	2	101	108
		II	0	0	36	42
		III	1	1	237	197
+	YN-2	I	1	2	213	141
		II	1	2	48	19
		III	1	5	1600	1400

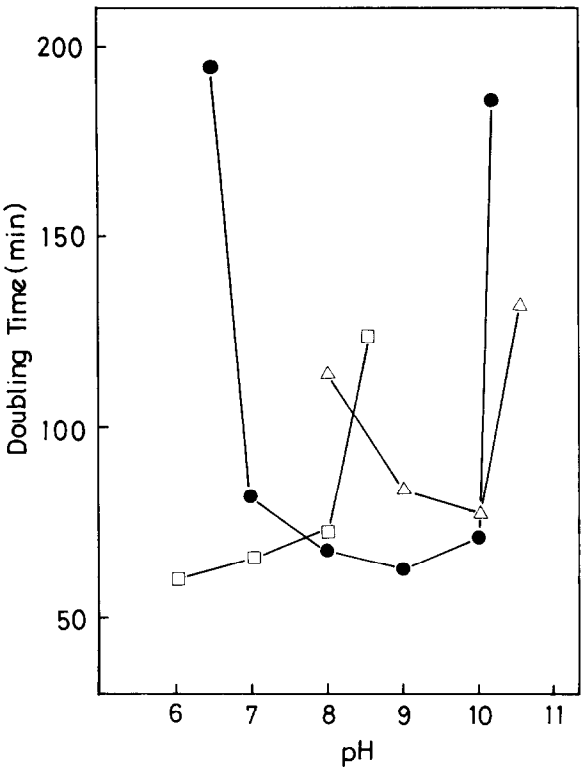

Fig.1. Effect of pH on the growth of *B. subtilis* RM125 (□), Arg^+Leu^- YN-1 transformant (●) and alkalophilic *Bacillus* YN-1 (△). The cells were grown in the peptone medium [4], the pH being kept at the values indicated with NaOH.

and alkalophilic *Bacillus* YN-1 by dilution spot method [6]. *B. subtilis* RM125 was susceptible to both of the bacteriophages at pH 8.5 (fig.2). Alkalophilic *Bacillus* YN-1 (DNA donor) was not susceptible to either of the bacteriophages at pH 9.5 (fig.2) and also at pH 8.5. The Arg^+Leu^- YN-1 transformant was susceptible to both of the bacteriophages at pH 9.5 (fig.2) and to a lesser ex-

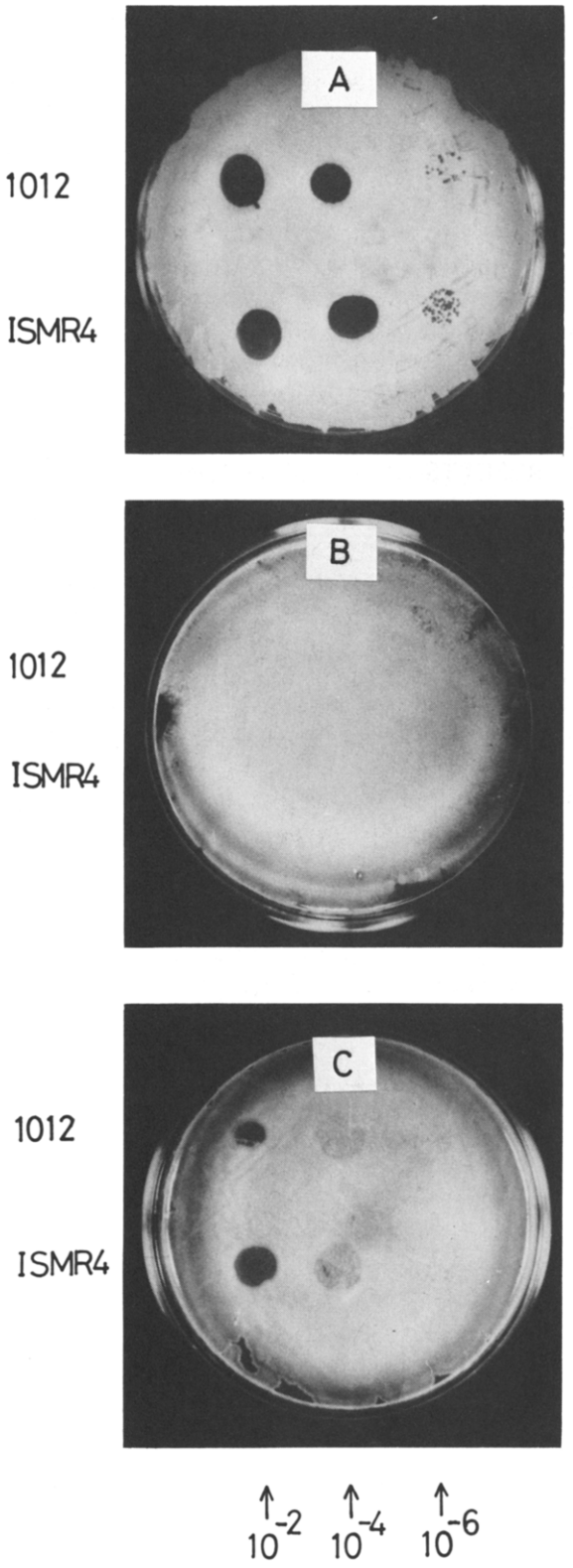

Fig.2. Susceptibility of *B. subtilis* RM125, alkalophilic *Bacillus* YN-1 and Arg^+Leu^- YN-1 transformant to bacteriophages. Suspensions (10 μl each) of bacteriophages φ105D1C2·1012 and φ105D1C2·ISMR4 at 10^{-2}, 10^{-4} and 10^{-6} dilution (original titers, 1 and 3 × 10^9/ml, respectively) were spotted on LB plates seeded with *B. subtilis* RM125, pH 8.5 (A), alkalophilic *Bacillus* YN-1, pH 9.5 (B) and Arg^+Leu^- YN-1 transformant, pH 9.5 (C). After incubation for 12 h the plates were examined for a clear zone of lysis.

tent at pH 8.5. The results indicate that the property of lacking the restriction–modification system in the recipient *B. subtilis* is maintained in the transformant. It is conclusive that the Arg^+Leu^- YN-1 transformant is indeed *B. subtilis* derivative.

The mechanism of alkalophily (or alkalostability) of alkalophilic *Bacilli* has been considered to function on membranes of the bacteria, because the intracellular pH of the bacteria is lower than the environmental pH [10], alkalophily and alkalostability of the bacteria are possibly related to some membrane proteins [5,11] and the Na^+/H^+ antiporter which may be located on membranes is supposed to be related to alkalostability

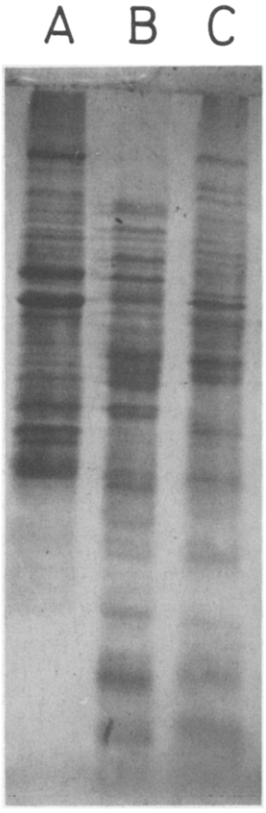

Fig.3. SDS–polyacrylamide gel electrophoresis of the membrane proteins from *B. subtilis* RM125 (A), Arg^+Leu^- YN-1 transformant (B) and alkalophilic *Bacillus* YN-1 (C). *B. subtilis* was cultured at pH 7.5 and alkalophilic *Bacillus* YN-1 and Arg^+Leu^- YN-1 transformant at pH 10.0, the pH values being kept with NaOH. The cultures attained late-logarithmic phase, were rapidly cooled with ice and centrifuged. Membranes were prepared from the cells as in [4], and membrane proteins were solubilized by SDS [5]. Electrophoresis was done on a 12–21% slab gel.

[2,12]. Some membrane proteins in alkalophilic *Bacillus* YN-1 may be transferred to the Arg^+Leu^- YN-1 transformant. The membrane proteins of the Arg^+Leu^- YN-1 transformant were then compared to those of alkalophilic *Bacillus* YN-1 and also to those of *B. subtilis*. Almost all of the membrane proteins of smaller M_r in alkalophilic *Bacillus*, which do not exist in *B. subtilis*, were observed in the Arg^+Leu^- YN-1 transformant (fig.3). Some membrane proteins other than those of smaller M_r as shown above in the transformant, may originate from alkalophilic *Bacillus*. Therefore, some membrane proteins in the transformant from alkalophilic *Bacillus* may be related to alkalophily.

To identify the proteins or genes responsible to the alkalophily, transformation of *B. subtilis* to alkalophily is now being attempted by DNA recombination.

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