High Frequency of Natural Genetic Transformation of *Pseudomonas stutzeri* in Soil Extract Supplemented with a Carbon/Energy and Phosphorus Source

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Agar medium (SME) prepared from aqueous soil extract was used to examine genetic transformation of *Pseudomonas stutzeri* JM302 (his-I) by homologous his⁺ DNA in a plate transformation assay. Growth studies indicated that SME was strongly limited in carbon and nitrogen sources. Transformation was observed on SME supplemented with pyruvate, phosphate, and ammonium. A 25-fold increase of the transformation frequency was obtained with nitrogen limitation when SME was supplemented with only pyruvate plus phosphate. Similar results were obtained with artificial soil extract medium prepared on the basis of the chemical analysis of the soil extract. On a standard minimal medium, transformation frequencies also increased (10- to 60-fold) when ammonium, phosphate, or pyruvate was growth limiting. Limitation of two or three nutrients did not stimulate transformation. The size of the inoculum (2 × 10⁵ to 2 × 10⁷ cells) was irrelevant to the enhanced transformation under nitrogen limitation on SME or standard minimal medium. We further show that *P. stutzeri* can use a variety of carbon and energy sources for competence development. It is concluded that genetic transformation of *P. stutzeri* is possible in the chemical environment of soil upon supply of nutrients and may be strongly stimulated by a growth-limiting concentration of single nutrients including sources of C, N, or P.

The use of genetically engineered microorganisms in the environment has focused interests on gene transfer processes in natural systems such as soil or sediments. It is important to know whether genetically engineered microorganisms and also their recombinant DNAs can persist in such microbial habitats and thereby contribute not only to the intracellular but also to the extracellular bacterial gene pool. DNA may be released from cells during cell lysis or by excretion from living cells, as has been shown for several bacterial species living in soil (e.g., see references 4, 5, and 7) and also for natural bacterial populations (21, 22). Free chromosomal and plasmid DNA is stabilized against enzymatic degradation by adsorption to particulate material (1, 9, 11, 23a). Such adsorbed DNA can be taken up by naturally transformable recipients (10, 13).

Many bacterial species isolated from soil have been shown to undergo natural transformation (12). Transformation experiments are generally done in complex high-nutrition growth media. Early experiments demonstrating the transformation of *Bacillus subtilis* in soil were performed in the presence of complex media (6). However, soil is considered a nutrient-poor environment (14, 27). Potential organic substrates and inorganic nutrients such as ammonium, nitrate, or phosphate may even be unavailable to microbial cells because of their adsorption to humic acids and particulates, including clay minerals (26). Essentially no information is available as to the effect of growth-limiting concentrations of nutrients in natural habitats on transformation. In this work, we investigated transformation of the naturally transformable soil bacterium *Pseudomonas stutzeri* (3, 13) in soil extract. Specifically, the influence of the addition of defined nutrients on transformation was studied. The findings indicate that transformation of *P. stutzeri* in the chemical environment of soil is possible and may even be stimulated if growth is restricted by single-nutrient limitation.

**MATERIALS AND METHODS**

Bacterial strains and DNA. *P. stutzeri* JM302 (his-I) was used for transformation. A noncompetent culture (2 × 10⁹ transformants viable cell⁻¹ at 1 μg of DNA ml⁻¹) grown in modified LB (3) to 6.3 × 10⁹ cells ml⁻¹ was shock frozen (liquid N₂) in the presence of 10% (vol/vol) glycerol and stored at −80°C until use (13). Transforming DNA was isolated from *P. stutzeri* JM375 (his⁺) according to the method of Marmur (15). Both strains were kindly provided by J. L. Ingraham, University of California.

Soil extract. The marsh soil used was collected during winter from a near-shore agricultural area (pastures) at Jadebusen, northwest Germany. The sample was air dried at 30°C for 12 days. To 1 kg of soil 2 liters of deionized water was added, and the sample was extracted at room temperature for 24 h under occasional stirring. The slurry was centrifuged at 4,800 × g for 15 min. The turbid supernatant was filtered through a paper filter (Schleicher & Schuell; no. 595) to remove large particulate and floating material. Small suspended material was sedimented by a high-speed centrifugation step (15 min, 19,200 × g). The clear, brownish supernatant was passed through a cellulose acetate filter (0.45 μm; Sartorius, Göttingen, Germany) and stored in sterile glass bottles at 4°C. The absence of colony-forming bacteria in the extract was verified by plating on rich and minimal medium.

Soil extract medium. Experiments on soil extract agar medium (SME) were done in petri dishes (diameter, 3.5 cm; Greiner, Nürtlingen, Germany) which were filled with 2.5 ml of SME. The preparation of the agar medium was as follows: to a tube filled with 4 ml of warm (45°C) soil extract, 0.05 ml of histidine (4 μg ml⁻¹), hot (80°C) agar (0.5 ml of 10%...
The tube was sealed with Parafilm (American Can Co., Greenwich, Conn.) and vigorously shaken. The contents were poured into two cell culture petri dishes (2.5 ml in each). Supplementation of the soil extract with nutrients was done by adding 100-fold-concentrated solutions of pyruvate, potassium phosphate (pH 6.8), and/or ammonium chloride, giving final concentrations as listed in the table. Towards agar, histidine, and water were added to give a volume of 5 ml. The medium was poured into petri dishes as mentioned before.

Artificial soil extract was prepared on the basis of the chemical analysis of the natural soil extract. The following inorganic ions were present at the indicated concentrations (in millimoles): PO₄³⁻, 0.28; NO₃⁻, 0.01; NO₂⁻, 0.03; NH₄⁺, 0.23; K⁺, 3.84; Na⁺, 1.0; Ca²⁺, 1.75; Mg²⁺, 0.37; Fe³⁺, 0.01. Total organic carbon was 35.3 mg liter⁻¹; the pH was 8.5. K, Na, and Ca and Mg and Fe were measured by flame and atomic absorption spectrometry, respectively. A Scalar Analyzer (Erkelenz, Germany) was used for NO₃⁻ and NH₄⁺ quantification. P was measured colorimetrically (2), and K was measured by ion chromatography (Dr. onex Equipment, Mülheim, Germany). Inorganic ions and pyruvate as a substitute for the organic carbon present in the soil extract were used at the concentrations indicated above. Nitrate and nitrite were included as sodium salts and phosphate as K₂HPO₄. Ammonium, potassium, sodium, and calcium were present as chlorides in artificial soil extract agar medium (ASME) and magnesium and iron(III) were present as sulfates. The pH was adjusted to 8.5 with 0.01 N NaOH. ASME was prepared in the manner described for SME (see above).

Minimal media. Minimal succinate (MS) medium (3) including 1.5% agar was used for selection of His⁺ transformants. Viable counts were determined on MS agar plus histidine (40 µg ml⁻¹). MS contains 8.1 g of sodium succinate liter⁻¹, 100 mM NaNO₃, 10 mM NH₄Cl, 2.5 mM K₂HPO₄, 2.5 mM KH₂PO₄, 1 ml of trace mineral solution (per liter of 0.1 M HCl, 2.5 g of EDTA, 5 g of FeSO₄·7H₂O, 1.54 g of MnSO₄·H₂O, 0.1 g of CuSO₄·5H₂O, 0.24 g of Co(NO₃)₂·6H₂O, 0.095 g of Na₂B₄O₇, and 1.189 g of Na₃MoO₄·2H₂O), and 1 ml of 20% (wt/vol) of MgSO₄·7H₂O liter⁻¹. The pH was adjusted to 6.8 with 0.1 N KOH.

The influence of different carbonaceous substrates on growth and transformation was studied by replacing succinate in MS by the organic substrates indicated in Table 3. Histidine (40 µg ml⁻¹) was always included in the media. Organic acids (sodium salts), L-valine, and alcohols were included at 0.8% (wt/vol), and saccharides were included at 0.5%. Experiments on the effect of varying concentrations of one nutrient, i.e., carbon, phosphorus, or nitrogen, on growth and transformation (see Fig. 1) were done with standard minimal medium, which is MS containing pyruvate instead of succinate. Nitrate was omitted from the medium when the influence of ammonium on growth and transformation was studied. In some experiments (see Table 2) more than one nutrient was used at concentrations limiting growth with nitrate omitted from the medium. All studies were done in normal petri dishes filled with 25 ml of agar media.

Other growth and transformation tests (see Table 4) were performed on minimal media supplemented with minimal pyruvate (MP) agar medium (2.5 ml in cell culture petri dishes). MP contains pyruvate (4 g liter⁻¹), KPO₄ (0.5 mM, pH 6.8), NH₄Cl (40 µM), agar (1% wt/vol), 1 ml of trace mineral solution liter⁻¹, and 1 ml of 20% MgSO₄·7H₂O liter⁻¹.

Plate transformation procedure. The plate transformation method (8) was modified for quantitative analysis of transformation on agar medium. The frozen noncompetent culture of JM302 was thawed in a water bath at room temperature and centrifuged in a Biofuge A centrifuge (Heraeus, Germany) at 13,000 rpm for 10 min. After washing with saline (0.9% [wt/vol] NaCl) the cell pellet was finally resuspended in saline. Saturating amounts of JM375 DNA (final concentration, 18 µg ml⁻¹) were added, and 50 µl of the suspension (containing 2.3 × 10⁶ viable cells and 0.9 µg of DNA) was spotted on a single agar plate with 25 ml of medium. In experiments with SME, ASME, and MP, 5 µl of the suspension (2.3 × 10⁶ viable cells, 0.09 µg of DNA) were applied to 2.5 ml of agar media. After the spots had dried, cell cultures petri dishes (maximum, 12) were placed into 2-liter beakers containing a wet paper towel and sealed with Parafilm to prevent drying of the plates during incubation. In all experiments inoculated agar plates were kept at 37°C for 3 days. Then the agar with the growth area was cut out with a sterile spatula. The piece of agar with the cells coming from 25–ml plates was transferred to a glass tube with 1 ml of MS plus 10 µg of D.Nase ml⁻¹. Cells on agar cut out from 2.5 ml of medium were transferred to 0.1 ml of MS plus 10 µg of D.Nase 1 ml⁻¹ in Eppendorf tubes. The tubes were vigorously vortexed for 15 s, incubated for 10 min at 37°C, and vortexed again. Samples of the suspension were plated for determination of the number of transformants and viable cells. Transformation frequencies are given as His⁺ transformants per viable cell.

RESULTS

Transformation on soil extract agar. Transformation of P. stutzeri on SME was investigated by a modified protocol of the plate transformation assay previously described (8). Stationary-phase cells (2.3 × 10⁷) together with saturating amounts of transforming DNA were spotted on SME and incubated at 37°C for 3 days. Then viable cells and transformants were quantified. Transformations were performed in parallel on SME and SME supplemented with defined nutrients (pyruvate, phosphate, and ammonium) which were added to SME in various combinations. For comparison, growth and transformation were studied on ASME, which was prepared according to the data of the chemical analysis of the soil extract (see above) and on nutrient-supplemented ASME.

Growth on SME and ASME was poor, giving rise to only one to two generations (Table 1, experiment 1). Supplementation with pyruvate, phosphate, and ammonium (Table 1, experiment 2) increased growth, resulting in 6- to 10-fold-higher viable counts in both media. This shows that SME and ASME were limited for nutrients. On SME (Table 1, experiment 1) no transformants were detected. However, supplementation with all three nutrients (Table 1, experiment 2) resulted in transformation. Some transformants were also found on ASME without supplementation, indicating that conditions on ASME were more favorable for transformation than on SME. However, fully supplemented ASME gave 13-fold-higher transformation frequencies than unsupplemented ASME (Table 1, compare experiments 1 and 2), demonstrating that nutrient supply was suboptimal for transformation in the unsupplemented synthetic medium. Incubation of cells without adding DNA on supplemented media yielded no His⁺ colonies.

Analysis of nutrient requirement for transformation on soil extract agar. In a series of experiments, we examined which
of the nutrients added were required for transformation on SME. Supplementation of SME with C, N, or P did not increase growth, nor did transformants arise (Table 1, experiments 3 to 5). It is concluded that SME was limited in more than a single nutrient. On ASME, there was also no or only a minor response in growth upon supplementation with pyruvate, phosphate, or ammonium (Table 1, experiments 3 to 5). However, transformation occurred on ASME when supplemented with pyruvate or ammonium (Table 1, experiments 3 and 5). With two nutrients added to SME or ASME simultaneously, growth only in the case of supply with pyruvate plus ammonium (Table 1, experiment 7) was comparable to that on media amended with all three nutrients (Table 1, experiment 2). Addition of pyruvate plus phosphate or phosphate plus ammonium did not support growth (Table 1, experiments 6 and 8). These findings suggest that SME as well as ASME was limited in a metabolizable substrate and a nitrogen source. Transformation on SME and ASME was observed upon supplementation with pyruvate in combination with phosphate or ammonium (Table 1, experiments 6 and 7). With extra pyruvate plus phosphate (Table 1, experiment 6), transformation frequencies were substantially higher than on SME or ASME supplemented with all three nutrients, indicating some stimulating effect of these media. The absence of transformants on media amended with phosphate or phosphate plus ammonium (Table 1, experiments 4 and 8) is striking, because unsupplemented ASME gave transformation (Table 1, experiment 1).

Relationship of transformation frequency to nutrient limitation in standard minimal medium. The unexpected finding that SME and ASME supplemented with pyruvate plus phosphate gave very high transformation frequencies was further investigated. Growth and transformation were measured in standard minimal medium which contained decreasing concentrations of pyruvate, phosphate, or ammonium. Figure 1 shows that with decreasing concentrations of each of the nutrients, growth was continuously reduced until a minimum level of one to two generations was reached. With decreasing growth, transformation frequencies increased, showing a maximum at a specific low nutrient supply.

Figure 2 shows the time courses of growth and of the appearance of transformants on both standard and nitrogen-limited minimal medium. On standard minimal medium the cells reached the stationary phase after 12 h at about $3 \times 10^9$ ml$^{-1}$ (approximately four generations). Under nitrogen limitation, the cell titer doubled only once within the first 6 h and then remained constant at about $5 \times 10^8$ ml$^{-1}$. These cells were N starved. Their transformation frequency increased for 48 h and remained at the high level during the next 24 h. On standard minimal medium the transformation frequency reached a lower level during the exponential growth and subsequently declined. In summary, transformation was stimulated by limitation of one nutrient, which can be N, C, or P (Fig. 1 and 2).

On standard minimal medium with limiting concentrations of two or three nutrients (Table 2), transformation was low or not detectable. This corresponds to low or absent transformation in Table 1, experiments 1, 3, 4, and 5. Thus, stimulation of transformation by low nutrient supply appears to be restricted to the lack of a single nutrient.

**Influence of substrate utilization and cell number on transformation.** The results obtained so far indicate that transformation on SME depended on the supply of pyruvate plus phosphate and/or ammonium. We analyzed whether *P. stutzeri* could use substrates other than pyruvate for transformation. Table 3 shows that JM302 was able to develop competence on many substrates. A clear correlation between the extent of growth on different C sources and the transformation frequency was not observed. In one case,

### Table 1. Plate transformation assay on marsh extract (SME) and ASME agar media

<table>
<thead>
<tr>
<th>Expt no.</th>
<th>Medium</th>
<th>Addition of nutrients (final concn)</th>
<th>Transformation frequency ($10^6$)</th>
<th>Viable count (0.1 ml$^{-1}$) ($10^{-7}$)</th>
</tr>
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<tbody>
<tr>
<td>1</td>
<td>SME</td>
<td>- 4 g liter$^{-1}$</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>ASME</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>2</td>
<td>SME</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>ASME</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>3</td>
<td>SME</td>
<td>+</td>
<td>-</td>
<td>-</td>
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<tr>
<td></td>
<td>ASME</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>4</td>
<td>SME</td>
<td>-</td>
<td>+</td>
<td>-</td>
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<tr>
<td></td>
<td>ASME</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>5</td>
<td>SME</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>ASME</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>6</td>
<td>SME</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>ASME</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>7</td>
<td>SME</td>
<td>+</td>
<td>-</td>
<td>+</td>
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<tr>
<td></td>
<td>ASME</td>
<td>+</td>
<td>-</td>
<td>+</td>
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<tr>
<td>8</td>
<td>SME</td>
<td>-</td>
<td>+</td>
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</tr>
<tr>
<td></td>
<td>ASME</td>
<td>-</td>
<td>+</td>
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</tr>
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</table>

* Agar media (2.5 ml) were inoculated with $2.3 \times 10^7$ cells and 0.09 μg of DNA in 5 μl; after 3 days at 37°C, the growth area with agar was cut out and cells were resuspended in 0.1 ml of MS for determination of viable count and transformants (see Materials and Methods).

* Values are means from two experiments.
FIG. 1. Growth (●) and transformation (■) on standard minimal agar medium with decreasing concentrations of pyruvate (A), phosphate (B), or ammonium (C). The abscissa presents concentrations obtained in 1:2 dilution steps of concentrated nutrient solutions.

FIG. 2. Kinetics of growth (open symbols) and transformation (closed symbols) on standard minimal medium containing 10 mM (● and ○) or 40 μM (■ and □) NH₄Cl. At the times indicated, the growth areas were removed from the agar plates and processed as described in Materials and Methods.

transformation occurred even in the absence of growth (valine).

Under optimal conditions for transformation on SME, i.e., limitation of only a nitrogen source (Table 1, experiment 6), similarly high transformation frequencies were found at inoculum cell numbers between 2 × 10⁴ and 2 × 10⁵ (Table 4). Comparable results were also obtained in transformations on ammonium-limited standard minimal medium (Table 4). This shows that the initial number of cells is not a critical factor and that the number of generations is not correlated with the level of transformation (Fig. 1 and 2). Although this finding was not further investigated, one can imagine that at a low inoculum titer, cells are subject to nutrient limitation only after several generations and competence is developed at similar cell concentrations, as in cases in which a high initial titer leads to only one to two generations.

DISCUSSION

Natural genetic transformation is well known from laboratory studies (24). It includes a series of events beginning

<table>
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<tr>
<th>TABLE 2. Plate transformation assay on standard minimal agar medium with combinations of growth-limiting concentrations of nutrients</th>
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<tr>
<td>Limiting nutrients</td>
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<tr>
<td>---------------------</td>
</tr>
<tr>
<td>C and P</td>
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<tr>
<td>C and N</td>
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<tr>
<td>N and P</td>
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<tr>
<td>C, P, and N</td>
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<tr>
<td>Control</td>
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</table>

^a The medium was modified by replacing pyruvate (C), phosphate (P), and ammonium (N) by limiting concentrations of nutrients as specified: pyruvate, 0.5 μl liter⁻¹; KPO₄ (pH 6.8), 7.8 μM; NH₄Cl, 78 μM. The medium (25 ml per plate) was inoculated with 2.3 × 10⁶ cells and 0.9 μg of DNA in 50 μl; after 3 days at 37°C, the growth area with agar was cut out and cells were resuspended in 1 ml of MS for determination of viable count and transformations (see Materials and Methods). The control was standard minimal medium supplied with all nutrients at nonlimiting concentrations.

^b Values are means from two experiments.
with the regulated development of a physiological state called competence, which eventually leads to inheritance of genetic markers derived from free DNA taken up by the cell (24). We are interested in whether gene transfer by transformation occurs in natural bacterial habitats. In this context, a detail important to know is whether the chemical environment of soil is appropriate for transformation. Therefore, we consider the use of soil extract to be a consequential analytical step on the way to transformations in the more complex soil system. Soil extract represents a model environment for the evaluation of transformation in soil, because natural chemical factors influencing transformation can be identified before the effects of physical factors, such as natural solid-liquid interfaces, are studied. Chemical factors may affect transformation at different levels: (i) the concentration of nutrients may determine whether competence is developed; (ii) organic and inorganic substances of soil may interfere with cell-DNA interactions like DNA binding and uptake; and (iii) recombination and expression of the acquired trait may be influenced. For our studies we have chosen *P. stutzeri* JM302. This strain has been isolated from soil and was identified as a naturally transformable pseudomonad (3). Also, strain JM302 has been characterized with respect to its interactions with dissolved and sand-adsorbed DNA (13).

A main result of this work is the observation that transformation was possible in soil extract supplemented with nutrients (Table 1). This means that the absence of transformants on unsupplemented SME was due to an insufficient supply of nutrients for the cells, which most probably prevented competence development. Indeed, the soil extract was a nutrient-poor environment. The extensive growth only on SME supplemented with pyruvate plus ammonium (Table 1, experiments 6 to 8) indicated a strong limitation of metabolizable carbon/energy and nitrogen sources. Such conditions of double limitation are unfavorable for transformation: on standard minimal medium, limitation of pyruvate together with ammonium gave nearly undetectable levels of transformants (Table 2). Other double and also triple limitations virtually abolished transformation (Table 2). In contrast, limitation of a single nutrient highly stimulated transformation (Fig. 1 and 2). Stimulation of transformation by nitrogen limitation was also observed on SME and ASME (pyruvate plus phosphate added; Table 1, experiment 6). The reason for the specifically high transformation efficiency under defined single-nutrient limitation is not understood. Yet we assume that the development of competence is enhanced. Perhaps *P. stutzeri* possesses a sensor for fluctuations of nutrient supply which may direct or modulate competence development.

ASME was applied to test transformation under defined conditions comparable to those used with natural soil extract. The applicability of ASME is demonstrated by the similar transformation frequencies observed on SME and ASME supplemented with pyruvate plus phosphate and pyruvate plus ammonium (Table 1, experiments 6 and 7). The sevenfold-lower transformation efficiency on SME compared with that on ASME under conditions of full supplementation (Table 1, experiment 2) may be the result of the action of some transformation inhibitor endogenous to soil. Adverse effects on transformation by nutrient addition were also detected with ASME. For instance, the low transformation obtained on ASME (Table 1, experiment 1) was abolished by addition of phosphate or phosphate plus ammonium (Table 1, experiments 4 and 8). This finding is not understood but demonstrates the complexity of transformation-related physiological reactions to changes in environmental conditions, including the supply of nutrients.

Supplementation of SME with single nutrients did not allow transformation, presumably because nutritional conditions of SME were not improved, i.e., the other two nutrients were still limited, which prevented growth and transformation. For instance, the absence of transformants on solely pyruvate-amended SME indicates an insufficient supply of phosphate (in addition to strong nitrogen limitation), although the P amount determined by chemical analysis (see "Soil extract medium" above) should be optimal for growth (Fig. 1B). Probably most of the phosphate is unavailable to the cells because of complexation with humic or other sorptive substances in SME. Hence, this medium was limited in two nutrients, which in turn prevented transformation (Table 2). Perhaps there was only weak limitation of phosphate which hindered transformation in combination with strong nitrogen limitation but enabled cells to develop competence when ammonium was supplied additionally (Table 1, experiment 7).

Nutritional factors influence the competence of another
soil bacterium. Azotobacter vinelandii developed competence on a variety of saccharides (17), with a 200-fold difference in levels of competence on glucose or mannitol. As shown here, transformation of P. stutzeri was 4 to 20 times more effective when the bacterium was grown on pyruvate than when it was grown on other substrates (Table 3). A. vinelandii became equally competent with several organic and inorganic ammonium salts and dinitrogen (16). Limiting concentrations of phosphate and magnesium reduced the level of competence (19), whereas under iron limitation (18, 20) or iron limitation plus molybdenum starvation the cells became highly competent (17). Therefore, it was suggested that A. vinelandii may develop competence in soil (18).

With P. stutzeri we demonstrated that the quality and quantity of the nutritional composition of the environment determine transformability. In artificial and natural soil extracts, we observed impressive stimulation by single-nutrient limitation. Inhibitory effects of an extra N plus P supply were also detectable. Many organic substances can be used by P. stutzeri as substrates for the development of competence (Table 3). These data, together with the finding that the cell number was not critical for transformation (Table 4), are consistent with the assumption that transformation can occur in natural habitats such as soil and sediments (23) where nutrients are frequently present at suboptimal concentrations for growth and where the cell number of a transformable bacterial species may be generally low. Whether competence is developed, as well as the eventually achieved extent of competence (Fig. 1 and 2), depends on the actual supply of organic and inorganic nutrients. Recent observations in model microcosms show that P. stutzeri and also B. subtilis can take up mineral-adsorbed DNA (10, 13). This fact may be ecologically important in terms of gene exchange in soil and sediments, because there extracellular DNA is thought to be mostly associated with mineral surfaces which protect DNA against enzymatic degradation (1, 9, 11, 12, 23a). The results obtained so far, including transformation in marine sediments (25), support the idea that transformation may be a relevant gene transfer mechanism of soil bacteria in their natural environments.

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REFERENCES