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ORIGINAL PAPER

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Physiological regulation of competence induction for natural transformation in *Acinetobacter calcoaceticus*

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Abstract *Acinetobacter calcoaceticus* induced competence for natural transformation maximally after dilution of a stationary culture into fresh medium. Competence was gradually lost during prolonged exponential growth and after entrance into the stationary state. Growth cessation and nutrient upshift were involved in the induction of competence. The level of competence of a chemostat culture of *A. calcoaceticus* was dependent on the nature of the growth limitation. Under potassium limitation a transformation frequency of $\pm 1 \times 10^{-4}$ was obtained. This frequency was independent of the specific growth rate. In phosphate-, nitrogen-, and carbon-limited chemostat cultures, in contrast, the transformation frequency depended on the specific growth rate; the transformation frequency equalled $\pm 10^{-4}$ at dilution rates close to $\mu_{\max}$ of 0.6 h^{-1} and decreased to $\pm 10^{-7}$ at a dilution rate of 0.1 h^{-1} . We conclude that (1) DNA uptake for natural transformation in *A. calcoaceticus* does not serve a nutrient function and (2) competence induction is regulated via a promoter(s) that resembles the *fis* promoter from *Escherichia coli*.

Key words DNA uptake · Growth phase · Dilution rate
fis Promoter · Stringent promoter · Nutrient upshift
Growth cessation

Introduction

Natural transformation has been observed in a wide range of organisms. Among these are representatives of gram-

positive bacteria [e.g., *Bacillus subtilis* (for a review see Dubnau 1991); *Streptococcus pneumoniae* (Avery et al. 1944)], gram-negative bacteria [e.g., *Haemophilus influenzae*, *Neisseria gonorrhoeae*, *Azotobacter vinelandii*, and *Pseudomonas stutzeri* (for a review see Stewart and Carlson 1986)], and even archaeobacteria [e.g., *Methanobacterium thermoautotrophicum* strain Marburg (Worrell et al. 1988); *Methanococcus voltae* PS (Bertani and Baresi 1987)]. Competence for natural transformation is inducible in a number of naturally transformable organisms, since nearly every transformable organism has its own specific set of conditions that induces competence. For instance, *B. subtilis* induces competence at the end of the exponential growth phase when grown on a mineral medium with glucose as a carbon source (Dubnau 1991). *A. vinelandii* induces competence also at the end of the exponential growth phase, but only if growth cessation is the result of an iron limitation (Page and von Tigerstrom 1979). *S. pneumoniae* induces competence in response to growth in the presence of 1 mM calcium (Trombe et al. 1992). These are examples in which competence is induced in a certain phase of growth and in which the cells remain competent for only a relatively short period (e.g., *S. pneumoniae* remains competent for about 8 min). In contrast, *N. gonorrhoeae*, *Deinococcus radiodurans*, *Synechococcus* and *Chlorobium* are competent throughout the exponential growth phase (Biswas et al. 1977, 1989; Tigrari and Moseley 1980; Ormerod 1988; Essich et al. 1990; and Wahlund and Madigan 1991). *Acinetobacter calcoaceticus* is a gram-negative and metabolically versatile organism (Juni 1978). Some *A. calcoaceticus* strains are naturally transformable (Juni and Janik 1969; Ahlquist et al. 1980). Of these, strain BD413 is suitable for use as a model system to study natural transformation in gram-negative bacteria because high transformation frequencies can be obtained and the organism is easily grown into the competent state (Juni and Janik 1969; Juni 1972; Sawula and Crawford 1972; Palmen et al. 1993). Nevertheless, with respect to competence development, conflicting observations have been reported (compare Juni 1978 and Cruze et al. 1980).

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The biological function of natural transformation has not yet been elucidated. It has been suggested that natural transformation has a function in (1) horizontal gene transfer (Redfield 1988; Elgar and Crozier 1988), (2) repair of DNA damage (Bernstein et al. 1981; Michod et al. 1988), and (3) nutrient supply (Stewart and Carlson 1986). A better understanding of the physiological regulation of competence induction may help to reveal its biological function. The differences in conditions necessary for induction of competence between different organisms suggests that the function of natural transformation varies among transformable bacteria. In this study we characterized the physiological regulation of competence development in *A. calcoaceticus*.

Materials and methods

Bacteria, media and chemicals

The *Acinetobacter* strains used in this study were the wild-type strain BD413 (Juni 1972) and transformation-deficient strain AAC211 (Palmen et al. 1992). Luria-Bertani medium (LB) and LB-agar were prepared as described previously (Vosman and Hellingwerf 1991). *Acinetobacter* minimal medium and minimal agar, prepared according to Juni (1974), contained 30 mM lactic acid, 11 mM KH_2PO_4 , 95 mM Na_2HPO_4 , 0.81 mM MgSO_4 , 37 mM NH_4Cl , 0.068 mM CaCl_2 , 1.8 μM FeSO_4 . The pH of the medium was 6.8.

DNA isolation

Chromosomal DNA was isolated as described by Vosman and Hellingwerf (1991). Plasmid DNA was isolated according to the method of Ish-Horowicz and Burke (1981).

Transformations

For transformations, a 0.5-ml culture sample was incubated with approximately 2 μg pAVA213-8 DNA for 60 min at 30°C. After incubation, 50 μg of DNaseI (stock solution: 5 mg/ml) was added to prevent further DNA uptake. The transformation mixture was incubated for an additional hour to allow expression of the kanamycin marker. pAVA213-8 is a pUC18-based plasmid containing a 6.4 kb chromosomal DNA fragment from *A. calcoaceticus* in which an *nptII* gene (encoding kanamycin resistance) has been inserted (Palmen et al. 1992). The total size of the plasmid is 10.5 kb. Due to the non-replicative character of pAVA213-8 in *A. calcoaceticus*, transformants are only formed after integration of the kanamycin marker into the recipient's chromosome, which is facilitated by homologous *A. calcoaceticus* sequences (3.5 and 2.9 kb) flanking the kanamycin marker (Palmen et al. 1992). Kanamycin-resistant transformants were selected on media containing 15 μg kanamycin/ml. The viable count was determined on LB-plates in parallel. Colonies were counted after 2 days' incubation at 30°C. The transformation frequency was calculated as the number of transformed cells divided by the total viable count.

Reproducibility

The quantitative reproducibility of the transformation data is affected by (1) the level of competence reached in the separate batches of cells used in different experiments and (2) the scatter in the results obtained with equally treated samples from the same competent culture. To provide some insight to the variation that is

unavoidable between experiments, we calculated the mean and standard deviation of 11 transformations of *A. calcoaceticus* with pAVA213-8 DNA. The observed transformation frequencies ranged between 1.6×10^{-2} and 9.9×10^{-2} , with a mean of 5.6×10^{-2} and a standard deviation of 2.9×10^{-2} . The variation between the transformation frequencies within one competent culture was much smaller. To demonstrate this, we analyzed the transformation frequency from five transformations obtained with the same competent culture. The transformation frequency ranged between 4.3×10^{-3} and 5.6×10^{-3} , with a mean of 5.1×10^{-3} and a standard deviation of 5.4×10^{-4} . Thus, the variability between separate batches of competent cells was much larger than within the same batch of competent cells.

Chemostat experiments

The chemostat media contained the following salts: 11 mM KH_2PO_4 , 95 mM Na_2HPO_4 , 0.81 mM MgSO_4 , 37 mM NH_4Cl , 0.068 mM CaCl_2 , 1.8 μM FeSO_4 and 1 ml trace element solution/l. Trace element solution (pH 6.5) contained the following per l: 50 g EDTA, 5 g $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$, 1.6 g $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$, 5 g $\text{MnCl}_2 \cdot 4\text{H}_2\text{O}$, 1.1 g $(\text{NH}_4)_6\text{Mo}_7\text{O}_{24} \cdot 4\text{H}_2\text{O}$, 2.2 g $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$, 50 mg H_3BO_3 , 10 mg KI, 50 mg $\text{CoCl}_2 \cdot 6\text{H}_2\text{O}$. To obtain a carbon-limited culture, 20 mM lactic acid was added. For nitrogen limitation, only 3 mM NH_4Cl was added together with 60 mM lactic acid. Potassium limitation was obtained by adding only 50 μM KH_2PO_4 and 30 mM Na_2HPO_4 together with 60 mM lactic acid. *A. calcoaceticus* BD413 was grown at 30°C. The chemostat experiments were performed in Bioflo model C30 fermenters (New Brunswick, Edison, NJ, USA). The pH was kept at 7.0 by titration with 1 N HCl or 1 N NaOH, depending on the limitation used. The working volume of the culture vessel was 300 ml. The cultures were aerated at a flow of 1.6 l air/min, with a stirring rate of 400 rpm. Constant values for the optical density and transformation frequency indicated that steady state had been reached. In general, the chemostat features were as described by Veldkamp (1976).

Turbidostat experiments

A. calcoaceticus BD413 was grown at 30°C in an aerated culture vessel with a volume of 60 ml and stirred at 200 rpm. The pH was kept at 7.0. The optical density of the culture was measured spectrophotometrically via a high-flow rate bypass at a wavelength of 540 nm in a 1-cm flow cell. The spectrophotometer reading controlled the medium supply rate. The optical density at 540 nm was set at 0.5 or 0.1. The medium contained 60 mM sodium lactate, 40 mM potassium phosphate buffer pH 7.0, 0.81 mM MgSO_4 , 37 mM NH_4Cl , 0.068 mM CaCl_2 , and 1.8 μM FeSO_4 .

Pulse experiments

Cells were grown in a potassium-limited chemostat at a dilution rate of 0.2 h^{-1} , at 30°C and pH 7.0. Steady state cultures were pulsed with excess amounts of KCl (0.5 or 10 mM, final concentration). To pulsed growing cells, the KCl was added just before the medium pump was arrested. To test the effect of growth cessation prior to the nutrient upshift, steady state cells were pulsed after the medium pump had been stopped (with a delay varying between 0 and 45 min).

Results

Competence for natural transformation in *Acinetobacter calcoaceticus* is maximally induced after dilution of an overnight culture into fresh mineral medium (Palmen et al. 1992, 1993). A transformation frequency of 5×10^{-2} is routinely obtained using the integrative plasmid pAVA213-8 as transforming DNA and selecting for kanamycin-re-

Fig. 1 The development of competence for natural transformation of *Acinetobacter calcoaceticus* in batch culture (LB medium) related to growth phase. An overnight culture was diluted 1:25 into fresh LB medium. At various intervals, samples were taken and transformed with a saturating amount of pAVA213-8 plasmid DNA, using a standard transformation protocol (see Materials and methods). Filled circles, growth; open triangles, transformation frequency

sistant transformants. Cultures remain competent during the entire exponential growth phase and lose competence in the stationary phase (Palmen et al. 1993). Cells stationary for approximately 4 days still readily induce competence upon dilution into fresh medium (Fig. 1). Growing *Acinetobacter* in a rich medium (such as LB medium) shows that the transformation frequency already starts to decrease during the exponential growth phase and proceeds during the stationary phase (Fig. 1). The increase in transformation frequency between 30 and 95 h of incubation may be the result of an increased viability of competent cells compared to non-competent cells (the viable count at $t = 95$ h decreased tenfold compared to $t = 24$ h). Alternatively, it may be the result of limited induction of competence due to growth on nutrients liberated by lysed cells.

The decrease in transformation frequency of exponentially growing cells may indicate that the competence level of the culture decreases during exponential growth. To test this hypothesis, *Acinetobacter* was cultured with an extended exponential growth phase via two independent culturing techniques.

Steady-state batch cultures

In the first system, extended exponential growth was obtained by repeatedly diluting an *A. calcoaceticus* BD413 culture into fresh, prewarmed mineral medium as soon as an OD_{540} of 0.1 was reached. Under these conditions the cells grew exponentially in a more or less constant environment for 4 days and the transformation frequency gradually decreased from 5×10^{-2} initially, to 7×10^{-5} ,

Fig. 2 The effect of exponential growth of *Acinetobacter calcoaceticus* BD413 on competence development. BD413 was grown at a constant OD_{540} of 0.5 in a turbidostat using a lactate-based mineral medium. At various intervals, samples were taken and transformed with a saturating amount of pAVA213-8 plasmid DNA, using a standard transformation protocol (see Materials and methods). Filled circles, growth; open triangles, transformation frequency

i.e., almost a 1,000-fold decrease after 4 days of exponential growth at the maximal growth rate (μ_{max}).

Turbidostat cultures

A second system that allows *A. calcoaceticus* to grow exponentially at μ_{max} for an extended period is the turbidostat. In a turbidostat the optical density is kept constant by control of the medium flow through the culture vessel, allowing the organism to grow virtually at its maximum growth rate. During the batch period in the turbidostat, *Acinetobacter* displayed the characteristic high transformation frequency (Fig. 2). After the OD reached the set-point, fresh medium was supplied and the culture was maintained at a constant optical density. During the resulting prolonged exponential growth phase, at a growth rate of more than 95% of μ_{max} , the transformation frequency decreased from 10^{-1} to 10^{-3} , which is a 100-fold decrease in transformation frequency after 3 days of exponential growth. It has not been possible to grow *Acinetobacter* in the turbidostat for a longer period due to technical problems with cell aggregation. Decreasing the set-point for the optical density from 0.5 to 0.1 did not solve this aggregation problem. Nevertheless, this experiment confirms that exponential growth at rates near μ_{max} results in a gradual decrease of competence. The conclusion from the steady-state batch and turbidostat experiments must be that the level of competence is strongly reduced in exponentially growing *A. calcoaceticus* cultures.

Chemostat cultures

For a better understanding of the physiological regulation of competence induction, the effect of different growth limitations on the expression of competence was studied in the chemostat. Again, the level of competence ex-

Fig. 3 Dependence of the transformation frequency of *Acinetobacter calcoaceticus* BD413 upon the dilution rate at different growth limitations. Filled circles, carbon limitation; open triangles, nitrogen limitation; filled inverted triangles, potassium limitation; open squares, phosphate limitation. At steady state, samples were taken and transformed with a saturating amount of pAVA213-8 plasmid DNA, using a standard transformation protocol (see Materials and methods)

pressed was estimated from the number of transformants formed, using the standard transformation assay (see Materials and methods). Chemostat studies on two other naturally transformable organisms, *Azotobacter vinelandii* and *Bacillus subtilis*, showed a dilution rate-dependent (i.e. growth rate-dependent) induction of competence. Both organisms induced competence maximally at a dilution rate equal to approximately 20–25% of their maximum growth rate (data not shown): *A. vinelandii* when limited for iron and *B. subtilis* when limited for an anabolic substrate. Therefore, competence induction in *A. calcoaceticus* BD413 was also characterized with respect to the effect of variation of the dilution rate. The cells were grown under nitrogen, carbon, phosphate, or potassium limitation to determine also the effect of different growth limitations on the expression of competence. The results of these experiments are summarized in Fig. 3. The transformation frequency of an *A. calcoaceticus* culture was low (10^{-7} – 10^{-6}) at low dilution rates when grown under nitrogen, carbon, or phosphate limitation. The response of nitrogen-, carbon- and phosphate-limited cultures to an increase in dilution rate appeared to be very similar. The transformation frequency increased exponentially with the dilution rate up to about 10^{-4} at a dilution rate of 0.6–0.7 h^{-1} . However, when *A. calcoaceticus* was grown under potassium limitation, the transformation frequency was independent of the dilution rate. Under this limitation, the mean transformation frequency equalled 2.2×10^{-4} (SD = 1.1×10^{-4} ; $n = 10$) overall.

Elsewhere, the idea has been brought forward that the biological function of the natural transformation process is to provide the cell with nutrients (Stewart and Carlson 1986). In the chemostat, the specific growth rate of the cells is dictated by the supply rate of a growth-limiting nutrient and at lower dilution rates the limitation becomes more and more stringent. It was of interest to know if *Acinetobacter* is able to use internalized DNA as a source

of carbon, nitrogen, or phosphate for growth. To determine whether or not *A. calcoaceticus* is able to use DNA as a nutrient source, strain BD413 and transformation deficient mutant AAC211 (Palmen et al. 1992) were incubated in media in which growth is limited by the carbon, nitrogen, or phosphate source. The addition of either a fivefold saturating amount of DNA (approximately 5 $\mu\text{g}/\text{ml}$) or the same amount of DNA after digestion with DNase to these cultures did not result in growth under carbon and nitrogen limitation. Although the nitrogen-limited culture did not grow after addition of DNA, it did take up DNA as was concluded from the (low) number of transformants observed. The phosphate-limited cultures did grow after addition of DNA, but also after addition of the same amount of DNA hydrolyzed into nucleotides. Also, the phosphate-limited culture took up DNA, as indicated by the formation of transformants. To test whether DNA uptake is necessary for growth under phosphate-limited conditions, the transformation deficient strain AAC211 was used to assay the effect of DNA addition. After dilution of a phosphate-limited culture into medium without added phosphate, the cells still grew at a low rate, probably due to contamination of the medium with residual phosphate. Addition of DNA or nucleotides to fresh medium at the time of dilution did not affect the growth rate. It did, however, affect the final OD of the culture. When the phosphate-limited culture became fully depleted for phosphate and stopped growing, the phosphate limited cultures with added DNA or nucleotides continued to grow. From the transformation assay it was concluded that DNA is not taken up by AAC211 [as was expected since this strain is fully transformation-deficient and does not take up radioactively labelled DNA (unpublished results)]. The addition of DNA or nucleotides resulted in an increased availability of phosphate that facilitated further growth, although without taking up DNA. This means that the added DNA and the nucleotides served as an external source of phosphate. Phosphate may be liberated by the action of phosphatases. The amount of DNA added (5 $\mu\text{g}/\text{ml}$ cells) contains only a limited amount of carbon and nitrogen compared to the requirement of the organism and therefore will not result in visible growth of the culture in the case of the carbon- and nitrogen-limited cultures. However, the DNA was added at a fivefold saturating concentration, which means that the DNA that is taken up will not be sufficient to provide the organism with carbon and nitrogen in amounts necessary to support significant growth. The conclusion from these experiments therefore must be that DNA does not provide *Acinetobacter* with carbon, nitrogen, or phosphate as a sole source for growth.

Pulse experiments

Dilution of an overnight culture into fresh medium triggers competence induction. Upon dilution, cells encounter high substrate levels that allow the stationary culture to resume growth at a maximum rate. To test whether the re-

Fig. 4A,B Effect of a K^+ -pulse on induction of competence by K^+ -limited steady state *Acinetobacter calcoaceticus* cells. K^+ -limited chemostat cells were grown at 30°C, pH 7.0 and a dilution rate of 0.2 h^{-1} . At various intervals, samples were taken and transformed with a saturating amount of pAVA213-8 plasmid DNA using a standard transformation protocol (see Materials and methods). **A** A pulse of 10 mM KCl (final concentration) was applied just before arresting the medium supply. **B** A pulse of 10 mM KCl (final concentration) was added 30 min after arresting the medium supply. Open circles growth; filled inverted triangles transformation frequency

lie of a growth limitation induces competence, cells were grown in a potassium-limited chemostat at a dilution rate of 0.2 h^{-1} and after steady state was reached, the culture was pulsed with excess potassium, allowing the cells to resume unlimited growth at a maximal rate. The result of a typical experiment is shown in Fig. 4A. Potassium was added to growing cells just before interruption of the medium supply to a final concentration of 0.5 or 10 mM. Both concentrations yielded similar results. After addition of potassium, the transformation frequency increased during the first 30 min. Subsequently, it decreased again, probably due to cell growth without further expression of competence. The increase of the transformation frequency after pulsing with potassium was in the order of three- to fourfold. Thus, only a small increase in transformation frequency was observed after transfer of slowly growing, nutrient-limited cells to conditions with excess nutrient allowing growth at a maximal rate. Still, an overnight culture transferred into fresh medium transformed at a much higher frequency. Therefore, it was tested whether growth cessation is necessary before maximal levels of compe-

Fig. 5 Effect of the time interval between interruption of the medium supply and the application of the K^+ -pulse (10 mM final concentration) on the transformation frequency of K^+ -limited steady-state chemostat *Acinetobacter calcoaceticus* cells. At various intervals, samples were taken and transformed with a saturating amount of pAVA213-8 plasmid DNA, using a standard transformation protocol (see Materials and methods). Filled circles, transformation frequency of the chemostat culture before sampling and pulsing

tence can be induced. Again, this hypothesis was examined via a pulse experiment, but now potassium was added 30 min after interruption of the medium supply. Under these conditions, competence was expressed maximally after 60 min (Fig. 4B). The transformation frequency transiently increased from 7.3×10^{-6} to 2.2×10^{-4} (i.e., a 30-fold increase). Pulsing 45 min after interruption of the medium supply led to an increase in transformation frequency from 1.1×10^{-5} to 1.2×10^{-4} , corresponding to a tenfold increase. The observed initial value of the transformation frequency in these pulse experiments varied between 7×10^{-6} and 1×10^{-4} . This kind of variation between different experiments is frequently observed. However, the variation within an experiment is much lower (Palmen et al. 1993). This means that comparison of the absolute transformation frequencies between separate experiments is difficult, but the differences observed within an experiment are significant. When cells of a potassium-limited chemostat culture were diluted 1:25 into fresh mineral or LB medium and were incubated for 3.5 h, allowing competence to be induced as in the standard procedure for competence induction, the transformation frequency increased 20- and 30-fold, respectively. This indicates that maximal induction of competence in potassium-limited chemostat cells is only 20- to 30-fold, as observed in the pump-stop-pulse experiment. In a batch culture in a single step, the transformation frequency can easily increase 10,000-fold or more (Palmen et al. 1993).

To examine the effect of variation of the length of interruption of medium supply, samples (5 ml) of a potassium-limited chemostat culture were incubated in an Erlenmeyer flask (100 ml) and pulsed with 10 mM KCl at different times after sampling (interruption of the potassium supply). The minimal time between pulsing and taking the sample at $t = 0$ was in the order of 2 to 5 min. This short interval between interruption of potassium supply and application of the pulse already resulted in maximum induction of competence (Fig. 5).

Discussion

Our results (and those of Cruze et al. 1979) indicate that competence in *Acinetobacter calcoaceticus* is optimally induced after an increase in nutrient availability, preceded by cessation of growth. This contrasts with previous reports that suggested that competence for natural transformation in *Acinetobacter* was induced in the transition to the stationary phase (Juni 1978). Induction of competence in *Acinetobacter* may well be quite similar to competence induction in *Neisseria gonorrhoeae*, *Deinococcus radiodurans*, *Synechococcus*, and *Chlorobium* (see Introduction).

The turbidostat and steady-state batch cultures showed that competence is continuously expressed, but that the level of competence gradually decreases in exponentially growing cultures. From this, it was expected that chemostat cultures could display a transformation frequency 1,000-fold lower than observed in batch cultures. The final level of competence of cells in the chemostat strongly depended on the growth conditions. First, the nature of the limitation was important. When *Acinetobacter* was grown under carbon, nitrogen, or phosphate limitation, the nutrient availability controlled the transformation frequency. The effect of the dilution rate was somewhat obscured by the occurrence of string formation at dilution rates higher than 0.4 h^{-1} , leading to an overestimation of the transformation frequency above these rates. At a dilution rate of 0.6 h^{-1} , 50% of the colony forming units are present as a string (with a mean size of 10 cells per string). Nevertheless, string formation cannot explain the effect of the dilution rate on the competence level. After maximal correction for string formation there still was an increase in the transformation frequency by a factor of 20 between a dilution rate of 0.1 h^{-1} and 0.6 h^{-1} .

A disadvantage of the use of the transformation frequency to assay the level of competence is that a transformant is the result of not only expression of competence, but also of DNA uptake and integration – processes that might be affected by growth conditions. Therefore, it cannot be excluded that the observed decrease in transformation frequency in response to a decrease in dilution rate of the carbon-, nitrogen- and phosphate-limited cultures is not the result of a decrease in level of competence of the culture, but is a result of an increased use of internalized DNA as building blocks in the organism's metabolism, leaving less DNA available for incorporation into the recipient chromosome. Although the DNA taken up cannot be used as a sole source for carbon, nitrogen, or phosphate, it may be used as additional nucleotide source for DNA synthesis, thereby relieving the nutrient flow towards nucleotide synthesis. However, the cells require unequal amounts of carbon, nitrogen, and phosphate for growth. Furthermore, the C/N/P ratio of DNA is very different from the corresponding ratio of cell material. Therefore, the more or less identical response of the transformation frequency toward the dilution rate in chemostat cultures grown under such diverse limitations as for car-

bon, nitrogen, and phosphate is not expected. In the future, parallel determination of the transformation frequency and the level of expression of competence, via a reporter-gene fusion, should reveal whether or not the transformation frequency is a reliable measure for competence. Such *Acinetobacter* strains are not available yet. Experiments with a *Bacillus subtilis* strain carrying a wild-type *comK* and an additional *comK::lacZ* fusion integrated in the chromosome at the *amyE* locus have shown a good correlation between the transformation frequency and expression of competence determined via β -galactosidase activity (Palmen et al. 1993; data not shown). Therefore, we interpret the observed decrease of the transformation frequency with decreasing dilution rate in the carbon-, nitrogen- and phosphate-limited chemostat cultures as a result of the nutrient availability on the expression of competence.

Potassium limitation imposes a severe drain on the energy supply in enterobacteria and forces organisms to maintain high respiration levels (Huetting et al. 1979; Crabbendam et al. 1985; Mulder et al. 1986; Pennock and Tempest 1988). This may result in high levels of the free energy intermediates in the cell. *A. calcoaceticus* has potassium-uptake systems quite similar to those of enterobacteria (Siebers and Altendorf 1993). Therefore, the chemostat data suggest that the transformation frequency of cells grown under potassium limitation may remain high – independent of the dilution rate – due to a high free energy load of the cells.

Interruption of the potassium supply of potassium-limited chemostat cells led to inhibition of growth and a subsequent potassium pulse resulted in an increased induction of competence. The minimal time interval required between holding the medium supply and addition of the substrate pulse leading to the increased competence was shorter than 2–5 min. It is not yet clear whether this time interval is long enough to stop growth completely. Studies using *Escherichia coli* have shown that growth of a potassium-limited chemostat culture does not stop immediately after interruption of the potassium supply (Mulder et al. 1988). Rather, the growth rate of such an *E. coli* culture gradually decreases due to a reduced need for potassium at lower growth rates and a redistribution of potassium among daughter cells (Mulder et al. 1988). However, the decrease in growth rate is only detectable at high growth rates, whereas our pulse studies were done with cells grown at low growth rates.

The observed regulation of competence induction may be interpreted as a consequence of the involvement of a promoter(s), which (1) reacts to nutrient upshifts and nutrient availability, (2) increases its response after cessation of growth, and (3) seems to be affected by the free-energy load of the cell. Surprisingly, competence regulated this way parallels the regulation of the *E. coli fis* promoter (Ball et al. 1992). Fis is a DNA binding protein and is involved in regulation of site-specific recombination, transcription of rRNA and tRNA operons, and DNA replication (Ball et al. 1992). The *fis* promoter is activated upon a nutrient upshift in the lag phase of a batch culture and

seems to act as an activator for growth when the cell encounters excess nutrient conditions (Augustin et al. 1994). *fis* expression is repressed via autoregulation during exponential growth. In addition, the *fis* promoter is an example of a stringent promoter, showing decreased activity upon a decrease in growth rate (Vicente et al 1991; Ball et al. 1992). Thus, not only the effect of a decrease in competence during exponential growth, but also the dilution rate dependence of competence in the carbon-, nitrogen- and phosphate-limited chemostat cultures parallels *fis* promoter activity. The effect of a potassium limitation on *Fis* expression in *E. coli* has not yet been studied.

Because of the observed analogies, it would be interesting to study the regulation of *fis* expression in *E. coli* in a potassium-limited chemostat. Also, it would be relevant to know whether *A. calcoaceticus* contains a *fis*-like gene.

Comparing the chemostat results of *Acinetobacter* with those we obtained with *Azotobacter vinelandii* and *Bacillus subtilis* (unpublished results), a large difference in expression of competence in response to the dilution rate was observed. *A. vinelandii* induced competence only in an iron-limited chemostat at dilution rates below 0.075 h^{-1} ($\mu_{\text{max}} \sim 0.25\text{ h}^{-1}$), whereas *B. subtilis* induced competence under an anabolic limitation at a dilution rate of 0.2 h^{-1} ($\mu_{\text{max}} \sim 0.75\text{ h}^{-1}$). In batch culture, both organisms induced competence in the transition from the exponential phase to the stationary phase upon nutrient limitation, which may explain the observed induction of competence of these organisms only at low dilution rates in the chemostat. Competence induction in *B. subtilis* is regulated via a complex phosphorylation cascade, which also has a connection to other cellular responses such as secondary metabolite formation and sporulation (Dubnau 1991). From the data obtained so far, we do not think that such a complex regulatory pathway is present in *A. calcoaceticus*.

The biological function of natural transformation in *A. calcoaceticus* is not to provide the cell with nutrients. First, DNA uptake did not provide *A. calcoaceticus* with carbon, nitrogen, or phosphate, at least not as a significant sole nutrient source for growth. Second, the regulation of competence development as a function of the stringency of carbon, nitrogen, and phosphate limitation was just opposite to what one would expect if the "nutrient supply hypothesis" would apply. The level of competence in these cultures decreased with decreasing nutrient availability. This means that natural transformation has a more genetic function in *Acinetobacter*. The DNA taken up may be used as a matrix to repair DNA damage, or natural transformation may be used as a means to exchange genetic information in horizontal gene transfer. In *Neisseria gonorrhoeae*, antigenic and phase variation are mediated by exchange of DNA via natural transformation (Gibbs et al. 1989).

References

- Ahlquist EF, Fewson CA, Ritchie DA, Podmore J, Rowell V (1980) Competence for genetic transformation in *Acinetobacter calcoaceticus* NCIB8250. FEMS Microbiol Lett 7: 107–109
- Augustin LB, Jacobson BA, Fuchs JA (1994) *Escherichia coli* *Fis* and *DnaA* proteins bind specifically to the *nrd* promoter region and affect expression of an *nrd-lac* fusion. J Bacteriol 176: 378–387
- Avery OT, Mcleod CM, McCarthy M (1944) Studies on the chemical nature of the substance inducing transformation of pneumococcal types. J Exp Med 9: 137–158
- Ball CA, Osuna R, Ferguson KC, Johnson RC (1992) Dramatic changes in *Fis* levels upon nutrient upshift in *Escherichia coli*. J Bacteriol 174: 8043–8056
- Bernstein H, Byers GS, Michod RE (1981) Evolution of sexual reproduction: importance of DNA repair, complementation and variation. Am Naturalist 117: 537–549
- Bertani G, Baresi L (1987) Genetic transformation in the methanogen *Methanococcus voltae* PS. J Bacteriol 169: 2730–2738
- Biswas GD, Sox T, Blackman E, Sparling PF (1977) Factors affecting genetic transformation of *Neisseria gonorrhoeae*. J Bacteriol 129: 983–992
- Biswas GD, Thompson SA, Sparling PF (1989) Gene transfer in *Neisseria gonorrhoeae*. Clin Microbiol Rev 2S: 24–28
- Crabbendam PM, Neijssel OM, Tempest DW (1985) Metabolic and energetic aspects of the growth of *Clostridium butyricum* on glucose in chemostat culture. Arch Microbiol 142: 375–382
- Cruze JA, Singer JT, Finnerty WR (1979) Conditions for quantitative transformation in *Acinetobacter calcoaceticus*. Curr Microbiol 3: 129–132
- Dubnau D (1991) Genetic competence in *Bacillus subtilis*. Microbiol Rev 55: 395–424
- Elgar MA, Crozier RH (1988) Sex with dead cells may be better than no sex at all. Trends Ecol Evol 3: 249
- Essich E, Stevens E Jr, Porter RD (1990) Chromosomal transformation in the cyanobacterium *Agmenellum quadruplicatum*. J Bacteriol 172: 1916–1922
- Gibbs CP, Reimann B-Y, Schulz E, Kaufmann A, Haas R, Meyer TF (1989) Reassortment of pilin genes in *Neisseria gonorrhoeae* occurs by two distinct mechanisms. Nature 338: 651–652
- Huetting S, de Lange T, Tempest DW (1979) Energy requirement for maintenance of the transmembrane potassium gradient in *Klebsiella aerogenes* NCTC 418: a continuous culture study. Arch Microbiol 123: 183–188
- Ish-Horowicz D, Burke FJ (1981) Rapid and efficient cosmid cloning. Nucleic Acids Res 9: 2989–2999
- Juni E (1972) Interspecies transformation of *Acinetobacter*: genetic evidence for a ubiquitous genus. J Bacteriol 112: 917–931
- Juni E (1974) Simple genetic transformation assay for rapid diagnosis of *Moraxella osloensis*. Appl Microbiol 27: 16–24
- Juni E (1978) Genetics and physiology of *Acinetobacter*. Ann Rev Microbiol 32: 349–371
- Juni E, Janik A (1969) Transformation of *Acinetobacter calcoaceticus* (*Bacterium anitratum*). J Bacteriol 98: 281–288
- Michod RE, Wojciechowski MF, Hoelzer MA (1988) DNA repair and the evolution of transformation in the bacterium *Bacillus subtilis*. Genetics 118: 31–39
- Mulder MM, Mattos MJT de, Postma PW, Dam K van (1986) Energetic consequences of multiple K^+ uptake systems. Biochim Biophys Acta 851: 223–228
- Mulder MM, Gulden HML van der, Postma PW, Dam K van (1988) Continued growth of *Escherichia coli* after stopping medium addition to a potassium-limited chemostat culture. J Gen Microbiol 134: 777–783
- Ormerod JG (1988) Natural genetic transformation in *Chlorobium*. In: Olson JM, Ormerod JG, Ames J (eds) Green photosynthetic bacteria. Plenum Press, New York
- Page WJ, Tigerstrom M von (1979) Optimal conditions for transformation of *Azotobacter vinelandii*. J Bacteriol 139: 1058–1061

- Palmen R, Vosman B, Kok R, Zee JR van der, Hellingwerf KJ (1992) Characterization of transformation-deficient mutants of *Acinetobacter calcoaceticus*. *Mol Microbiol* 6: 1747–1754
- Palmen R, Vosman B, Buijsman P, Breek CKD, Hellingwerf KJ (1993) Physiological characterization of natural transformation in *Acinetobacter calcoaceticus*. *J Gen Microbiol* 139: 295–305
- Pennock J, Tempest DW (1988) Metabolic and energetic aspects of the growth of *Bacillus stearothermophilus* in glucose-limited and glucose-sufficient chemostat culture. *Arch Microbiol* 150: 452–459
- Redfield RJ (1988) Evolution of bacterial transformation: is sex with dead cells ever better than no sex at all? *Genetics* 119: 213–221
- Sawula RV, Crawford IP (1972) Mapping of the tryptophan genes of *Acinetobacter calcoaceticus* by transformation. *J Bacteriol* 112: 797–805
- Siebers A, Altendorf K (1993) K⁺-translocating Kdp-ATPases and other bacterial P-type ATPases. In: Bakker E (ed) *Alkali cation transport systems in prokaryotes*. CRC Press, Ann Arbor, pp 225–252
- Stewart GJ, Carlson CA (1986) The biology of natural transformation. *Ann Rev Microbiol* 40: 211–235
- Tirgari S, Moseley BEB (1980) Transformation in *Micrococcus radiodurans*: measurement of various parameters and evidence for multiple, independently segregating genomes per cell. *J Gen Microbiol* 119: 287–296
- Trombe M-C, Clavé C, Manias J-M (1992) Calcium regulation of growth and differentiation in *Streptococcus pneumoniae*. *J Gen Microbiol* 138: 77–84
- Veldkamp H (1976) *Continuous culture in microbial physiology and ecology*. Meadowfield Press, Durham
- Vicente M, Kushner SR, Garrido T, Aldea M (1991) The role of the 'gearbox' in the transcription of essential genes. *Mol Microbiol* 5: 2085–2091
- Vosman B, Hellingwerf KJ (1991) Molecular cloning and functional characterization of the *recA* analog from *Pseudomonas stutzeri* and construction of a *P. stutzeri recA* mutant. *Antonie Van Leeuwenhoek* 59: 115–123
- Wahlund TM, Madigan MT (1991) Nitrogen fixation and genetic transformation in *Chlorobium tepidum* (abstract). Seventh international symposium on photosynthetic prokaryotes, Amherst, Massachusetts, USA, p 138 (abstr A)
- Worrell VE, Nagle DP Jr, McCarthy D, Eisenbraun A (1988) Genetic transformation system in the archaeobacterium *Methanobacterium thermoautotrophicum* Marburg. *J Bacteriol* 170: 653–656