NRC Research Press

NOTE

Succinate, iron chelation, and monovalent cations affect the transformation efficiency of *Acinetobacter baylyi* ATCC 33305 during growth in complex media

Colleen G. Leong, Caroline M. Boyd, Kaleb S. Roush, Ricardo Tenente, Kristine M. Lang, and C. Phoebe Lostroh

Can. J. Microbiol. Downloaded from www.nrcresearchpress.com by COLORADO COLLEGE - TUTT LIBRARY on 01/26/18
For personal use only.

Abstract: Natural transformation is the acquisition of new genetic material via the uptake of exogenous DNA by competent bacteria. *Acinetobacter baylyi* is model for natural transformation. Here we focus on the natural transformation of *A. baylyi* ATCC 33305 grown in complex media and seek environmental conditions that appreciably affect transformation efficiency. We find that the transformation efficiency for *A. baylyi* is a resilient characteristic that remains high under most conditions tested. We do find several distinct conditions that alter natural transformation efficiency including addition of succinate, Fe^{2+} (ferrous) iron chelation, and substitution of sodium ions with potassium ones. These distinct conditions could be useful to fine tune transformation efficiency for researchers using *A. baylyi* as a model organism to study natural transformation.

Key words: natural transformation, *Acinetobacter baylyi*.

Résumé : La transformation naturelle consiste en l'acquisition de nouveau matériel génétique par les bactéries compétentes au moyen de la captation d'ADN exogène. *Acinetobacter baylyi* est un modèle de transformation naturelle. Les auteurs se concentrent ici sur la transformation naturelle de la souche ATCC 33305 de *A. baylyi* cultivée dans des milieux complexes et recherchent les conditions environnementales qui affectent de manière appréciable l'efficacité de transformation. Ils trouvent que l'efficacité de transformation de *A. baylyi* constitue une caractéristique résistante qui demeure élevée sous la plupart des conditions testées. Ils identifient plusieurs conditions distinctes qui modifient l'efficacité de transformation naturelle dont l'ajout de succinate, la chélation de fer ferreux Fe^{2+} et la substitution d'ions sodium par des ions potassium. Ces conditions distinctes pourraient être utiles aux chercheurs qui utilisent *A. baylyi* comme organisme modèle d'étude de la transformation naturelle pour raffiner l'efficacité de transformation. [Traduit par la Rédaction]

Mots-clés : transformation naturelle, *Acinetobacter baylyi*.

Acinetobacter baylyi is a model organism for study of competence because of its high natural transformation efficiency (reviewed in Young et al. 2005). In other organisms, specific environmental conditions such as access to nutrients or cell density induce transient competence (Thomas and Nielsen 2005). In the work reported in this short note, the goal was to discover specific environmental conditions such as broth supplementation or temperature that would allow researchers to dial the transformation efficiency of *A. baylyi* up or down in a simple reproducible manner.

While there are many previous investigations of culture conditions that affect transformation efficiency in *A. baylyi*, the results are difficult to synthesize to create a coherent picture of environmental conditions that induce competence in this species. This difficulty in comparing across studies was the major motivations for the work presented here. Studies of whether growth phase affects transformation efficiency in *A. baylyi* are an illustrative case in point. Juni and Janik (1969) first reported that transformation efficiency peaks when the cells are about to enter stationary phase. But, since that time no other authors have reported a peak in transformation efficiency when the cells are about to enter stationary phase even though they measured transformation efficiency over a growth curve (Cruze et al. 1979; Bergan and Vaksvik 1983; Singer et al. 1986; Palmen et al. 1992, 1993, 1994; Porstendörfer et al. 2000). In direct contradiction of

Received 30 June 2017. Revision received 30 July 2017. Accepted 31 July 2017.

C.G. Leong, C.M. Boyd, R. Tenente, and C.P. Lostroh. Department of Molecular Biology, Colorado College, Colorado Springs, CO 80903, USA.
K.S. Roush and K.M. Lang. Department of Physics, Colorado College, Colorado Springs, CO 80903, USA.
Corresponding author: C. Phoebe Lostroh (email: PLostroh@ColoradoCollege.edu).

Copyright remains with the author(s) or their institution(s). Permission for reuse (free in most cases) can be obtained from RightsLink.

 Published at www.nrcresearchpress.com/cjm on 3 August 2017.

Can. J. Microbiol. Downloaded from www.nrcresearchpress.com by COLORADO COLLEGE - TUTT LIBRARY on 01/26/18
For personal use only.

Juni and Janik (1969), these authors instead report highest transformation efficiency during exponential phase. But even though these authors report highest transformation efficiency during exponential phase, the pattern of transformation efficiency was not constant across studies. For example, some observe a peak so that transformation efficiency is not at its highest immediately after dilution into fresh broth but instead maxes out some time later during exponential phase (Cruze et al. 1979; Porstendörfer et al. 2000). In contrast, others report the highest transformation efficiency upon dilution to begin the growth curve, with continuously falling transformation efficiency during the rest of exponential phase (Bergan and Vaksvik 1983; Singer et al. 1986; Palmen et al. 1993, 1994). Still others have observed the exact opposite, namely a dramatic increase in transformation efficiency over the course of exponential phase, reaching saturation during exponential phase and remaining high for the duration of stationary phase (Palmen et al. 1992). More unusual temporal patterns have been observed, such as finding the highest transformation efficiency at 1–2 h postdilution and again at 5–7 h postdilution with a dip (rather than a peak) that bottoms out at 4 h postdilution; in this experiment the cells were in exponential phase from 1 to 5 h (Palmen et al. 1993). Finally, an examination of transformation efficiency over an extended growth curve (>100 h of incubation) found that transformation efficiency was high in exponential phase, fell to a low in stationary phase at 30 h postinoculation, but then dramatically rebounded back to the same level observed in exponential phase when the culture was sampled after 95 h of incubation (Palmen et al. 1994). High transformation efficiency after 90 h is consistent with induced expression of *A. baylyi com* genes during long-term stationary phase (Lostroh and Voyles 2010).

The timing of the maximum transformation efficiency is not the only difference among these observations. For example, the magnitude of the changes in transformation efficiency is also very different, with some authors reporting less than 10-fold differences in maximum and minimum transformation efficiency during exponential phase (Cruze et al. 1979; Bergan and Vaksvik 1983; Palmen et al. 1994) and others finding differences of 2 or more logs (Palmen et al. 1992; Porstendörfer et al. 2000). In the single study that examined transformation efficiency over the 100+ h growth curve, the difference in the maximum and minimum transformation efficiency observed was more than 10 000 (Palmen et al. 1994). Another confounding factor is that none of the papers summarized in this introduction report the statistical significance of any differences in transformation efficiency and some do not specify the number of independent trials performed. None of the figures includes any indication of variation, such as error bars, for any time point.

These findings, many of which are explicitly contradictory, make it very difficult to know exactly how growth phase affects transformation efficiency in *A. baylyi*. Perhaps the differences among these results are due to the use of different kinds of transforming DNA; some authors used auxotrophy and chromosomal DNA from a prototroph for transformation, while others used either chromosomally encoded antibiotic resistance markers or antibiotic resistance markers carried on plasmids. Some used purified DNA while others used cell lysates. The markers vary in traits such as how many selectable markers there were per milligram of DNA, how long the markers were, and how long it might take for the phenotype encoded by the marker could be expressed. But it is not obvious why different kinds of DNA would lead to such contradictory results as transformation efficiency increasing steadily during exponential phase versus transformation efficiency decreasing steadily during exponential phase. Other variables include the type of broth used during cultivation, the temperature of cell cultivation and (or) during transformation, and even the transformation procedure itself. Just one example of this variation is that one group took a sample from the population and diluted it into buffer or broth and added DNA to the diluted cells (Cruze et al. 1979), while the others took a sample from the growth curve and added DNA directly to the sample.

A similarly bewildering group of observations confronts anyone who would like to know how other seemingly simple environmental parameters such as carbon source, cations, and temperature affect the transformation efficiency of *A. baylyi* (Cruze et al. 1979; Singer et al. 1986; Lorenz et al. 1992; Palmen et al. 1993).

Another challenge in using the existing literature to optimize the transformation efficiency of *A. baylyi* is that the standard wild-type strains used in Europe and the US are probably not the same. Many European investigators use strain BD413, while many US authors use the related ADP1 strain. Although these are both descended from Juni's microencapsulated mutant BD413 (Taylor and Juni 1961), they evolved separately for many decades in laboratories on the 2 continents. Furthermore, transformation efficiency is a trait that is known to be affected by laboratory culture (Bacher et al. 2006). Use of minimally passaged standard wild-type cells that everyone can obtain was thus another motivation for the studies reported in this short-form note. Throughout this work we used the wild-type *A. baylyi* strain from the American Type Culture Collection (ATCC; strain number 33305), which they refer to as synonymous with strains BD413 and ADP1. Their history on the organism does not report the date on which the strain was deposited nor the laboratory from which the strain was obtained (www.atcc.org/products/all/33305, accessed 29 June 2017), but a benefit of using this strain is that anyone can obtain it in lyophilized form.

Can. J. Microbiol. Downloaded from www.nrcresearchpress.com by COLORADO COLLEGE - TUTT LIBRARY on 01/26/18
For personal use only.

Here we present a study comparing transformation efficiency under a variety of conditions for *A. baylyi* ATCC 33305 grown in complex broth with a standard recipe of 5 g of tryptone, 10 g of yeast extract, 10 g NaCl/L (lysogeny broth (LB) (Bertani 1951)). Our purpose is to find environmental conditions that are simple to achieve in the laboratory and that appreciably affect transformation efficiency in a reproducible manner. We first look at competence over a growth curve, as have others (Juni and Janik 1969; Cruze et al. 1979; Bergan and Vaksvik 1983; Singer et al. 1986; Palmen et al. 1992, 1993, 1994; Porstendörfer et al. 2000). After finding high transformation efficiency throughout exponential phase, we next use exponential phase cells to test alterations of the LB recipe. These include addition of succinate, removal of ferrous iron, addition of divalent cations magnesium and calcium, and replacement of sodium ions with potassium ones. The variations tested were selected because they are relevant in the organism's natural soil habitat (Paul 2015). Finally, we measure competence of *A. baylyi* as a function of incubation temperature, which also varies in soils, and because different laboratories use different incubation temperatures for routine cultivation of *A. baylyi*.

Unless otherwise noted, we grew the cells at 37 °C with high aeration (Kok et al. 1999). To test a condition, we used independent overnight cultures in LB to inoculate an experimental broth and subculture it according to the condition to be tested; each overnight culture originated from a separate colony and was used only once to create independent samples. To measure the transformation efficiency, 0.5 mL of cells were incubated with 5 μg of chromosomal DNA from a streptomycin-resistant donor for 30 min (for the growth curve) or 60 min (for all other experiments). Then extracellular DNA was degraded with DNase I (0.5 mg/mL, 37 °C for 15 min (growth curve) or 30 min (all other experiments); New England Biolabs) and the cells were diluted and plated to determine transformation efficiency (Palmen et al. 1992). We calculate the transformation efficiency as the colony-forming units per millilitre on streptomycin plates (20 μg/mL) divided by the colony-forming units per millilitre on nonselective plates. We never observed any spontaneous streptomycin-resistant colonies in control transformations lacking exogenous DNA, and we always verified that the donor DNA was sterile. Using BD413 cells in the 1990s, transformation efficiencies as high as $10^{-0.6}$ were observed and the entire population was therefore inferred to be competent because of the many additional steps besides DNA import that are needed to acquire a new DNA segment (Palmen et al. 1993). In our case, we selected 5 μg of transforming DNA because the resulting transformation efficiency was 2 orders of magnitude lower than $10^{-0.6}$, which might make it possible to detect environmental conditions that increase the transformation efficiency.

Fig. 1. *Acinetobacter baylyi* ATCC 33305 transformation efficiency versus time over a growth curve. Cells were grown at 37 °C in LB broth with high aeration. The results include 3 independent time series. The transformation efficiency (circles; left axis) was calculated as (streptomycin-resistant CFU/mL)/(total CFU/mL). Errors bars denote standard deviation, which for this log plot are given by $\Delta y = 0.434\frac{\Delta TE}{\langle TE \rangle}$, where $y = \log\langle TE \rangle$ and ΔTE is defined as the standard deviation from the mean transformation efficiency, <TE>. For the 0.5, 1, 1.5, 2.5, and 6 h time points $n = 2$ while for the other time points $n = 3$. The growth curve (squares) is plotted on the right axis. None of the differences among average transformation efficiencies are statistically significant.

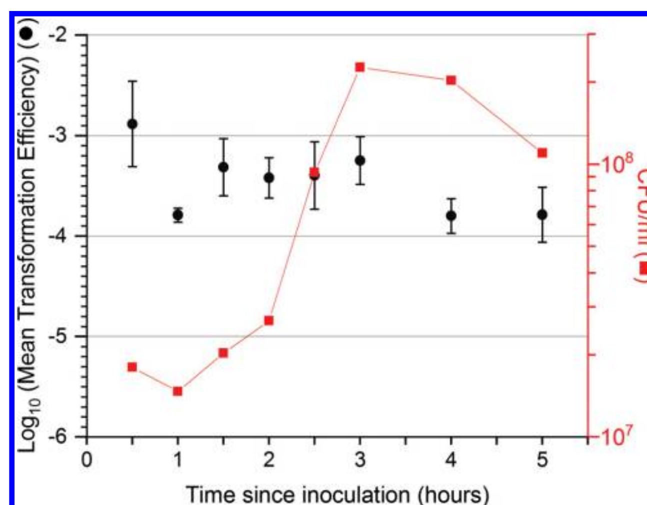


Figure 1 presents the transformation efficiency over a growth curve of *A. baylyi* ATCC 33305 growing in LB at 37 °C with high aeration. We ran 3 independent trials and report the average and standard deviation at each time point. The transformation efficiency was highest ($10^{-2.9}$) at 30 min after dilution. We did not observe a spike in transformation efficiency any time during exponential phase. Transformation efficiency was slightly lower later during exponential phase, ranging from $10^{-3.8}$ at 1 h postinoculation to $10^{-3.2}$ at 3 h postinoculation. Thus, the ATCC 33305 strain remains competent throughout exponential growth in LB, varying in transformation efficiency less than 10-fold. After the onset of stationary phase, transformation efficiency dropped a small amount to an average of $10^{-3.8}$. We used an ANOVA to test whether any of the time points were significantly different from any of the others and found no difference ($p > 0.4$). The ANOVA test assumes that the data are normally distributed, which is usually the case for biological traits but in the unlikely case that this assumption is violated, we also applied the Kruskal–Wallis test which can be applied to data with any distribution (normal or nonparametric). Again, there was no statistically significant difference between any of the transformation efficiencies at the different time points ($p > 0.8$).

Can. J. Microbiol. Downloaded from www.nrcresearchpress.com by COLORADO COLLEGE - TUTT LIBRARY on 01/26/18
For personal use only.

Table 1. OD_{600} at time of sampling for supplementation studies.

Condition	Sampling time (hours postinoculation)	Average OD_{600} at sampling time
LB control	3	0.36
LB + succinate	2.75	0.47
LB + succinate + divalent cations	2.75	0.38
LB + iron chelator	3.5	0.43
LBK	3	0.25
LBK + iron chelator	3.5	0.35

Note: LB, lysogeny broth (5 g of tryptone, 10 g of yeast extract, 10 g NaCl/L); LBK, LB in which an equimolar amount of potassium chloride (7.4 g/L KCl) is substituted for the sodium chloride.

Based on these results, we selected exponential phase for sampling cultures to compare parameters other than growth phase (Table 1) and we used the standard procedure of monitoring the OD_{600} of test cultures to sample them during exponential phase. For the rest of the experiments documented in this short-form note, including those reported in Figs. 2 and 3, we sampled cultures between an OD_{600} of 0.2 and 0.5.

We investigated the effects of succinate supplementation because succinate is a preferred growth substrate for *A. baylyi* and is present in the rhizosphere (Nielsen and Van Elsas 2001; Barbe et al. 2004; de Berardinis et al. 2008; Vallenet et al. 2008). Succinate arises in soils from degradation of the waxy plant root substance suberin. Strain ATCC 33305 grown in LB + succinate (0.02% *m*/*v*) was on average 2.5 times more competent than cells grown in unsupplemented LB ($p < 0.01$ Mann–Whitney *U* test; Fig. 2). These results are reminiscent of the effect of succinate on BD413 cells in a high-phosphate buffer in sterile soil (Nielsen and Van Elsas 2001).

Because others have found that the transformation efficiency of *A. baylyi* is stimulated by cations when the cells are in buffer lacking a carbon source (Lorenz et al. 1992; Palmen et al. 1993), we next investigated Mg^{2+} and Ca^{2+}. We supplemented LB + succinate with 2 mmol/L $MgSO_4$ and 100 μmol/L $CaCl_2$, which are typical concentrations used in minimal media. The transformation efficiency, however, was not higher than that of cells grown in LB + succinate (Fig. 2). It is possible that higher concentrations of these cations would have had an effect, but we did not pursue this line of investigation further.

We turned instead to the effects of ferrous iron because forms of iron vary in soils depending on many parameters (Colombo et al. 2014), and so *A. baylyi* may encounter fluctuating levels of Fe^{2+} in its natural habitat. We investigated the effect of removing ferrous iron from LB using a chelator specific for Fe^{2+}, namely 2,2′-dipyridyl at a concentration (20 μmol/L) unlikely to affect growth rate (Eijkelkamp et al. 2011). Cells grown in LB + iron chelator had a transformation efficiency 1.6 times

Fig. 2. Effects of LB broth supplementation on transformation efficiency for *Acinetobacter baylyi* ATCC 33305. Cells were grown at 37 °C in LB broth with high aeration and sampled during exponential phase. Errors bars denote standard deviation, which for this log plot are given by $\Delta y = 0.434\frac{\Delta TE}{<TE>}$ where $y = \log<TE>$ and ΔTE is defined as the standard deviation from the mean transformation efficiency, <TE>. For the control, $n = 16$; for the other conditions $n = 4$. *, $p < 0.01$ compared with control; **, $p < 0.05$ compared to control; Mann–Whitney *U* test.

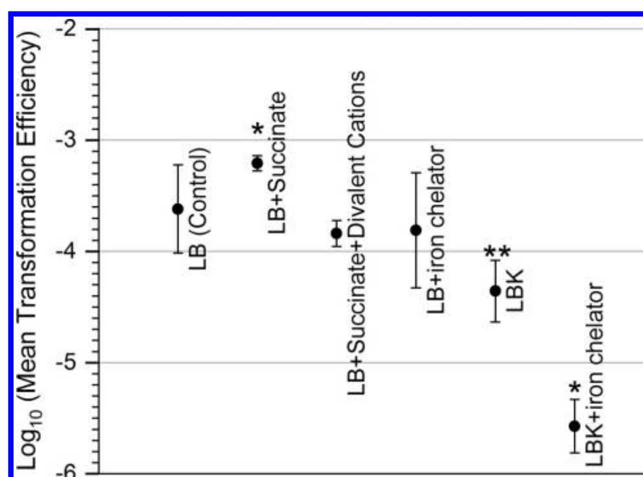
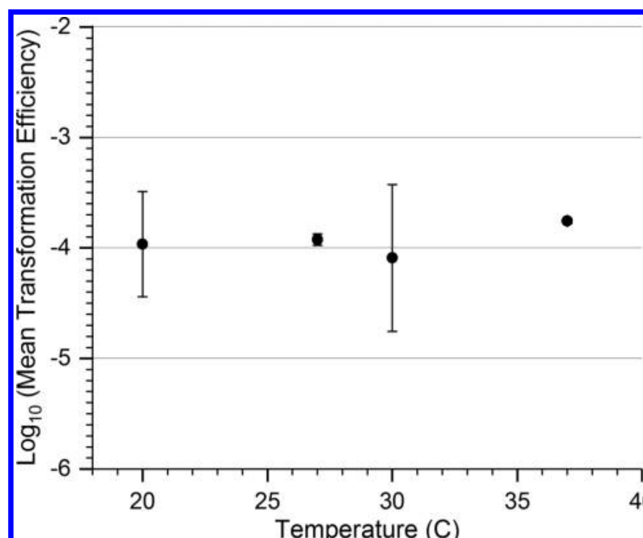


Fig. 3. Effect of temperature on transformation efficiency in LB + succinate for *Acinetobacter baylyi*. Cells were grown at 37 °C in LB + succinate (0.02%) broth with high aeration and sampled during exponential phase. Each data point, including the control, contains 4 individual measurements of transformation efficiency. Errors bars denote standard deviation, which for this log plot are given by $\Delta y = 0.434\frac{\Delta TE}{<TE>}$, where $y = \log<TE>$ and ΔTE is defined as the standard deviation from the mean transformation efficiency, <TE>. The transformation efficiencies are not significantly different from each other.



Can. J. Microbiol. Downloaded from www.nrcresearchpress.com by COLORADO COLLEGE - TUTT LIBRARY on 01/26/18
For personal use only.

less competent than cells grown under LB control conditions (Fig. 2), but this difference is not statistically significant. In the related species *A. baumannii*, competence genes are mildly downregulated by chelation of ferrous iron, but it is not known how large a change in gene expression would be needed to affect transformation efficiency nor whether chelation of ferrous iron affects *com* gene expression in *A. baylyi* (Eijkelkamp et al. 2011).

Still seeking an alteration to LB that might enhance natural transformation, we investigated potassium. *Acinetobacter baylyi* is likely exposed to fluctuating levels of potassium in its natural soil habitat. To investigate potassium, we cultured the cells in LBK, defined as LB in which we substituted equimolar amounts of potassium chloride for the sodium chloride (7.4 g/L KCl). This experimental design maintained the osmolarity of the medium. Cells grown in LBK were about 6 times less competent than the cells grown in LB ($p < 0.05$; Fig. 2).

Individually, LBK and iron deficiency each decrease the natural transformation efficiency relative to the control LB condition. We next tested whether combining the 2 alterations would result in a net decrease in transformation efficiency. As predicted, the negative effects of LBK and iron deficiency combined result in a constructive net negative effect on competence greater than that of either LBK or iron deficiency alone ($p < 0.01$; Fig. 2). The transformation efficiency in LBK + iron chelator is 250 times lower than that in LB + succinate.

It is possible that small perturbations in growth rate could contribute to some of the differences in transformation efficiency that we observed when cells were supplemented with succinate, grown in LBK, or grown in LBK + iron chelator. But, we do not think that this is likely to be the case because of the following observations. Using our standard procedure of 1:100 dilution into control LB, the 16 control cultures reached an OD_{600} between 0.2 and 0.5 at 3 h postinoculation, with an average of 0.36 (Table 1). The cells growing in LB with the various additives or substitutions always achieved a similar OD_{600} between 2.75 and 3.5 h postinoculation, indicating that they grew at about the same rate as cells in the control condition. To discuss one specific example in more detail, the growth of cells in LBK + iron chelator was almost the same as that in control LB. While cultures in control broth achieved an OD of 0.36 after 3 h of aeration, the LBK + iron chelation cultures reached a density of 0.35 at 3.5 h postinoculation. Because the control cultures were sometimes as low as 0.2 at 3 h postinoculation, an OD_{600} of 0.35 at 3.5 h postinoculation is within the normal variation of growth of *A. baylyi* ATCC 33305 in LB. In every case, the growth of cells in the test broths was within the normal variation observed for the same cells growing in control LB.

Having completed our investigation of LB additives, we turned to consider the effects of temperature by varying incubation conditions from 20 to 37 °C, a range that encompasses both its natural soil habitat and usual lab propagation temperatures. At all temperatures, we grew *A. baylyi* in LB + succinate, since that was the broth yielding the maximum transformation efficiency (Fig. 2). As shown in Fig. 3, for strain ATCC 33305 grown in LB, transformation efficiency does not vary with temperature.

Together, the results show that competence in *A. baylyi* ATCC 33305 is a resilient characteristic that remains constant in many environmental conditions. Looking at Figs. 1, 2, and 3 together, the transformation efficiency remained remarkably consistent despite varying growth curve stage, broth composition, and growth temperature. Although competence in *A. baylyi* is robust, there are several distinct conditions that do affect transformation efficiency: addition of succinate has a positive effect ($p < 0.01$), replacement of sodium by potassium and removal of ferrous iron (Fe^{2+}) from the growth medium each has a negative effect ($p < 0.05$), and when combined these last 2 conditions have a strong negative effect ($p < 0.01$). Such methods to fine-tube transformation efficiency will be useful role in future investigations of natural transformation in *A. baylyi* ATCC 33305.

Acknowledgements

This project was funded by the US National Science Foundation (MCB–1330511) and by internal funds at Colorado College (Keller Family Venture Grant, Tashjian-Crecelius Family Prize for Women and Science, Alfred W. Alberts Summer Research Prize, Faculty-Student Collaborative Research Grant, Taber Fund, Natural Science Executive Committee Funds). The funders had no role in study design, data collection and interpretation, or the decision to submit the work for publication. We thank Delaine Winkelblech, Laboratory Coordinator for the Molecular Biology Department.

References

Bacher, J.M., Metzgar, D., and de Crécy-Lagard, V. 2006. Rapid evolution of diminished transformability in *Acinetobacter baylyi*. J. Bacteriol. **188**(24): 8534–8542. doi:10.1128/JB.00846-06. PMID:17028281.

Barbe, V., Vallenet, D., Fonknechten, N., Kreimeyer, A., Oztas, S., Labarre, L., et al. 2004. Unique features revealed by the genome sequence of *Acinetobacter* sp. ADP1, a versatile and naturally transformation competent bacterium. Nucleic Acids Res. **32**(19): 5766–5779. PMID:15514110.

Bergan, T., and Vaksvik, A.K. 1983. Factors affecting quantitative transformation of streptomycin resistance markers in *Acinetobacter calcoaceticus*. Zentralbl. Bakteriol., Mikrobiol. Hyg., Ser. A, **254**(2): 197–213. doi:10.1016/S0174-3031(83)80146-2.

Bertani, G. 1951. Studies on lysogenesis. I. The mode of phage liberation by lysogenic *Escherichia coli*. J. Bacteriol. **62**(3): 293–300. PMID:14888646.

Colombo, C., Palumbo, G., He, J., Pinton, R., and Cesco, S. 2014. Review on iron availability in soil: interaction of Fe minerals, plants, and microbes. J. Soils Sediments, **14**(3): 538–548. doi:10.1007/s11368-013-0814-z.

Cruze, J.A., Singer, J.T., and Finnerty, W.R. 1979. Conditions for quantitative transformation in *Acinetobacter calcoaceticus*. Curr. Microbiol. **3**(3): 129–132. doi:10.1007/BF02601853.

de Berardinis, V., Vallenet, D., Castelli, V., Besnard, M., Pinet, A.,

Cruaud, C., et al. 2008. A complete collection of single-gene deletion mutants of *Acinetobacter baylyi* ADP1. Mol. Syst. Biol. **4**: 174. PMID:18319726.

Eijkelkamp, B.A., Hassan, K.A., Paulsen, I.T., and Brown, M.H. 2011. Investigation of the human pathogen *Acinetobacter baumannii* under iron limiting conditions. BMC Genomics, **12**: 126. doi:10.1186/1471-2164-12-126. PMID:21342532.

Juni, E., and Janik, A. 1969. Transformation of *Acinetobacter calco-aceticus* (Bacterium anitratum). J. Bacteriol. **98**(1): 281–288. PMID:5781579.

Kok, R.G., Young, D.M., and Ornston, L.N. 1999. Phenotypic expression of PCR-generated random mutations in a *Pseudomonas putida* gene after its introduction into an *Acinetobacter* chromosome by natural transformation. Appl. Environ. Microbiol. **65**(4): 1675–1680. PMID:10103267.

Lorenz, M.G., Reipschläger, K., and Wackernagel, W. 1992. Plasmid transformation of naturally competent *Acinetobacter calcoaceticus* in non-sterile soil extract and groundwater. Arch. Microbiol. **157**(4): 355–360. doi:10.1007/BF00248681. PMID: 1590708.

Lostroh, C.P., and Voyles, B.A. 2010. *Acinetobacter baylyi* starvation-induced genes identified through incubation in long-term stationary phase. Appl. Environ. Microbiol. **76**(14): 4905–4908. doi:10.1128/AEM.01806-09. PMID:20511417.

Nielsen, K.M., and Van Elsas, J.D. 2001. Stimulatory effects of compounds present in the rhizosphere on natural transformation of *Acinetobacter* sp. BD413 in soil. Soil Biol. Biochem. **33**: 345–357. doi:10.1016/S0038-0717(00)00147-4.

Palmen, R., Vosman, B., Kok, R., van der Zee, J.R., and Hellingwerf, K.J. 1992. Characterization of transformation-deficient mutants of *Acinetobacter calcoaceticus*. Mol. Microbiol. **6**(13): 1747–1754. doi:10.1111/j.1365-2958.1992.tb01347.x. PMID: 1630314.

Palmen, R., Vosman, B., Buijsman, P., Breek, C.K., and Hellingwerf, K.J. 1993. Physiological characterization of natural transformation in *Acinetobacter calcoaceticus*. J. Gen. Microbiol. **139**(2): 295–305. doi:10.1099/00221287-139-2-295. PMID:8436948.

Palmen, R., Buijsman, P., and Hellingwerf, K.J. 1994. Physiological regulation of competence induction for natural transformation in *Acinetobacter calcoaceticus*. Arch. Microbiol. **162**: 344–351. doi:10.1007/BF00263782.

Paul, E.A. 2015. Soil microbiology, ecology, and biochemistry. 4th ed. Elsevier, New York.

Porstendörfer, D., Gohl, O., Mayer, F., and Averhoff, B. 2000. ComP, a pilin-like protein essential for natural competence in *Acinetobacter* sp. strain BD413: regulation, modification, and cellular localization. J. Bacteriol. **182**(13): 3673–3680. doi:10.1128/JB.182.13.3673-3680.2000. PMID:10850981.

Singer, J.T., van Tuijl, J.J., and Finnerty, W.R. 1986. Transformation and mobilization of cloning vectors in *Acinetobacter* spp. J. Bacteriol. **165**(1): 301–303. doi:10.1128/jb.165.1.301-303.1986. PMID:3941044.

Taylor, W.H., and Juni, E. 1961. Pathways for biosynthesis of a bacterial capsular polysaccharide. I. Characterization of the organism and polysaccharide. J. Bacteriol. **81**: 688–693. PMID:13775654.

Thomas, C.M., and Nielsen, K.M. 2005. Mechanisms of, and barriers to, horizontal gene transfer between bacteria. Nat. Rev. Microbiol. **3**(9): 711–721. doi:10.1038/nrmicro1234. PMID: 16138099.

Vallenet, D., Nordmann, P., Barbe, V., Poirel, L., Mangenot, S., Bataille, E., et al. 2008. Comparative analysis of acinetobacters: three genomes for three lifestyles. PLoS One, **3**(3): e1805. doi:10.1371/journal.pone.0001805. PMID:18350144.

Young, D.M., Parke, D., and Ornston, L.N. 2005. Opportunities for genetic investigation afforded by *Acinetobacter baylyi*, a nutritionally versatile bacterial species that is highly competent for natural transformation. Annu. Rev. Microbiol. **59**: 519–551. doi:10.1146/annurev.micro.59.051905.105823. PMID: 16153178.

Can. J. Microbiol. Downloaded from www.nrcresearchpress.com by COLORADO COLLEGE - TUTT LIBRARY on 01/26/18
For personal use only.

This article has been cited by:

1. Peter David Ringel, Di Hu, Marek Basler. 2017. The Role of Type VI Secretion System Effectors in Target Cell Lysis and Subsequent Horizontal Gene Transfer. *Cell Reports* **21**:13, 3927-3940. [Crossref]

Can. J. Microbiol. Downloaded from www.nrcresearchpress.com by COLORADO COLLEGE - TUTT LIBRARY on 01/26/18
For personal use only.