Conjugative Transfer and Expression of Genes Coding for Periplasmic Nitrate Reductase in the Purple Bacterium Rhodospirillum rubrum

Jobst-Heinrich Klemme, Annette Kaminski and Angela Hougardy

Institut für Mikrobiologie & Biotechnologie der Universität Bonn, Mecklenheimer Allee 168, D-53115 Bonn

Z. Naturforsch. 51c, 900–902 (1996); received October 22/November 4, 1996

Conjugation, Nitrate Reductase, nap Genes, Rhodobacter, Rhodospirillum

Plasmid pFR400, a derivative of the vector plasmid pPHU231 containing the structural genes of the periplasmic nitrate reductase (nap genes) of Rhodobacter sphaeroides DSM158 (F. Reyes et al., Molec. Microbiol. 19, 1307–1318 [1996]) was transferred by conjugative mating to a streptomycin-resistant strain of the nitrate reductase-negative nonsulfur purple bacterium Rhodospirillum rubrum S1. Transconjugant cells of the latter bacterium, identified by their resistance to streptomycin and tetracycline and by their plasmid pattern, contained an active nitrate reductase which, like other periplasmic bacterial nitrate reductases, was not repressed by ammonium.

Introduction

Among the nonsulfur purple bacteria, nitrate reducing strains are mainly found within the genera Rhodobacter and Rhodopseudomonas (Klemme, 1979; Klemme et al., 1980; McEwan et al., 1984; Martinez-Luque et al., 1991). The few reports of nitrate reduction in members of the genus Rhodospirillum (Katoh [1963a, b]; Brown and Herbert [1977]) were either not confirmed by other investigators or were based only on incidental growth experiments without demonstration of nitrate reductase activity in vitro. According to our own experience, none of 5 wild type strains of R. rubrum is able to reduce nitrate. In view of the fact that, in some Rhodobacter capsulatus strains, nitrate reductase genes are located on endogenous plasmids (Willison, 1990; Koch and Klemme, 1994) it was of interest to see whether R. rubrum cells can be transformed to a NO₃⁻ reduction-positive state by conjugal transfer of nitrate reductase genes.

Materials and Methods

R. rubrum S1 (DSM 467); R. rubrum K100, a Sm-resistant spontaneous mutant of strain S1; E. coli C600(RP4) and E. coli SM10 (a genetically modified strain bearing the trc genes of the broad host range plasmid RP4 in its chromosomal DNA) are kept in the culture collection of the institute. Plasmid pFR400 (a gift of Dr. C. Moreno-Vivian, University of Cordoba, Spain) is a derivative of the mobilizable cloning vector pPHU231 containing a 6.8 kb PsI-fragment of the DNA of Rhodobacter sphaeroides DSM158 with the structural genes (nap genes) of the periplasmic nitrate reductase (Reyes et al., 1996). pFR400 contains a Tc-resistance site as a selection marker.

If not otherwise stated, phototrophic bacteria were grown photosynthetically at 30 °C in a malt-ammoniumsulfate medium supplemented with 0.05% [w/v] yeast extract (Kern et al., 1994). Agar plates were incubated aerobically in the dark or anaerobically in the light by using a GasPak jar (BBL, Microbiology Systems, Beeton Dickinson, Heidelberg). Antibiotic resistances were selected by using PY medium (see Kern et al., 1994) supplemented with the appropriate antibiotics (Km: 50 µg/ml; Sm: 200 µg/ml; Tc: 5 µg/ml). E. coli strains were cultivated aerobically at 37 °C in LB medium (see Kern et al., 1994). Protein assays and tests for nitrate reduction were performed as described by Witt and Klemme, 1991). Plasmid contents of bacterial strains were analyzed by agarose gel electrophoresis (Eckhardt, 1978).

Results and Discussion

To test the ability of R. rubrum K100(SmR) to act as a recipient strain, conjugation experiments with E. coli C600 harboring the broad host range plasmid RP4 were conducted. By using a filter mating technique (Kern et al., 1994). R. rubrum
K100(RP4)-transconjugants (selected by their resistance to Km) were obtained at frequencies of $10^{-3}$ to $10^{-4}$ per recipient cell. Plasmid pFR400 (with a 6.8 kb PstI-fragment of the DNA of Rhodobacter sphaeroides DSM 158 containing the genes of the periplasmic nitrate reductase; Reyes et al., 1996) was transformed to E. coli SM10. Due to integration of tra genes of the broad host range plasmid RP4 within its chromosomal DNA, the latter strain acts as host for mobilizable plasmids such as pFR400. E. coli SM10(pFR400)-transformants were now used as donor cells for matings with R. rubrum K100. R. rubrum colonies growing on selective agar plates (Sm: 200 µg/ml; Tc: 5 µg/ml) were picked as presumptive pFR400-transconjugants and subjected to agarose gel electrophoresis. Contrary to wild type strains of R. rubrum (including strain K100) containing only one endogenous 55 kb plasmid (Kuhl et al., 1983; Kawamukai et al., 1990), transconjugant strains showed two plasmid bands the upper one corresponding to the endogenous 55 kb plasmid, and the lower one corresponding to pFR400 (Fig. 1).

When transconjugant clones were grown in the absence of Tc as selective agent, plasmid pFR400 was not stably maintained in the cell population.

Table 1. Nitrite accumulation and cellular nitrate reductase activities in photosynthetic cultures of Rhodospirillum rubrum wild type K100 and a K100-transconjugant constructed by conjugational insertion of pFR400, a plasmid containing the structural genes (nap genes) of the periplasmic nitrate reductase of Rhodobacter sphaeroides DSM158.

<table>
<thead>
<tr>
<th></th>
<th>Wild type K100</th>
<th>K100-pFR400-transconjugant</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nitrate reductase activity in basal medium (A)</td>
<td>0</td>
<td>12–18</td>
</tr>
<tr>
<td>basal medium plus 10 mM KNO$_3$ (B)</td>
<td>0</td>
<td>30–40</td>
</tr>
<tr>
<td>basal medium plus 12.5 mM NH$_4$NO$_3$ (C)</td>
<td>0</td>
<td>40–60</td>
</tr>
<tr>
<td>Nitrite accumulation (µM) in medium C</td>
<td>0</td>
<td>60–110</td>
</tr>
</tbody>
</table>

For preparation of basal medium, the standard malate-ammoniumsulfate medium (Kern et al., 1994) was modified by omitting ammoniumsulfate and adding 0.1% (w/v) yeast extract. Nitrate reductase activities (nmol/min/g protein) were assayed at 30 °C as described by Witt and Klemme (1991). Data were obtained in three different experiments.

After 20 cultural transfers, less than 3% of the cells retained the Tc-resistance.

For analysis of nitrate reducing capacity, one R. rubrum transconjugant was chosen at random. To be sure that any nitrate reducing activity measured in cell suspensions of transconjugants was not due to contamination with other bacteria, the purity of cultures was carefully checked by streaking of samples on PY-agar plates. Contrary to the wild type strain K100, the transconjugant cells contained nitrate reductase activity up to 60 nmol/min g protein and excreted nitrite in nitrate-containing culture media up to growth inhibitory concentrations of about 100 µM NO$_2^-$ and more (Table I). As shown for other bacterial periplasmic nitrate reductases (Reyes et al., 1996), the nitrate reductase activity in transconjugant R. rubrum cells was not repressed by ammonium. The successful transfer and expression in R. rubrum of nap genes originating from another photosynthetic bacterium shows that the lack of nitrate reducing activity in wild type strains of the former organism is most probably due to the absence of nitrate reductase structural genes rather than to the absence of enzyme systems responsible for biosynthesis of the molybdenum cofactor and/or for export of nitrate reductase subunits to the periplasmic space. If R. rubrum did not contain a molybdenum cofactor processing system, one would expect the organism to be devoid of active molybdoenzymes (DMSO-reductase a.o.). However, wild-type
strains of this bacterium do contain a periplasmic DMSO-reductase (Sajitz et al., 1993).

In view of the fact that possession of an active nitrate reductase (NIT+ character) in purple bacteria, is normally found only in a fraction of newly isolated strains of a given species (for example, only about 40% of new isolates of *Rb. capsulatus* are NIT+), it may be asked if this variability is due to gain and loss of nitrate reductase genes by horizontal gene transfer mediated by plasmids. Note in this connection that (i) the endogenous plasmid of *Rb. capsulatus* AD2 harbouring the *nap* genes of this organism can be transferred via conjugative mating to other purple bacteria (Koch and Klemme, 1994) and that (ii) even for photosynthetic species, the possibility of horizontal gene transfer between different species of purple bacteria is being discussed (Nagashima et al., 1993). The ecological significance of conjugative gene transfer processes mediated by endogenous plasmids between different species of purple nonsulfur bacteria is now investigated in our laboratory.

Acknowledgements

The authors thank Dr. C. Moreno-Vivian (University of Cordoba, Spain) for his gift of plasmid pFR400.


Willison J. (1990), Derivatives of *Rhodobacter capsulatus* strain AD2 cured of their endogenous plasmid are unable to utilize nitrate. FEMS Microbiol. Lett. 66, 23–28.