No Correlation between Plasmid Content and Ability to Reduce Nitrate in Wild-Type Strains of *Rhodobacter capsulatus*

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Patterns of endogenous plasmids and nitrate reductase activities were analyzed in the phototrophic bacterium *Rhodobacter (Rb.) capsulatus*. From 10 strains investigated (including a UV-induced plasmidless *nit*$^-$ mutant), 4 were unable to grow photosynthetically with nitrate as N-source and lacked nitrate reductase activity (*nit*$^-$ strains). Irrespective of the *nit* phenotype, all wild-type strains contained at least one large plasmid with a size ranging from 93 to 134 kb. Thus, other than in plasmid-cured mutants (J. C. Willison, FEMS Microbiol. Lett. **66,** 23–28 [1990]), in wild-type strains of *Rb. capsulatus* the *nit*$^-$ character was not related to lack of endogenous plasmids.

Among the purple non-sulfur bacteria, the two *Rhodobacter (Rb.)* species *capsulatus* and *sphaeroides* are unique in that the majority of strains have the ability to reduce nitrate [1]. Depending on the strain and the growth conditions, this reduction process can be classified as respiratory or assimilatory [2, 3]. In several nitrate reductase-positive *Rb. capsulatus* strains (*nit*$^+$ strains) the *nit*$^+$ phenotype was rather unstable. *Nit*$^-$ clones were found to occur at frequencies of about 10^{-5} to 10^{-6} [4]. An extrachromosomal location of *nit* genes could explain the uneven distribution of nitrate reductase-positive characters among *Rb. capsulatus* and the instability of the *nit* phenotype occasionally observed in wild-type strains. Recently, plasmid-cured derivatives of *Rb. capsulatus* AD2 isolated by conjugative introduction of a vector containing a cloned 3.4 kb *Hind*III fragment of its endogenous plasmid were found to have the *nit*$^-$ phenotype [5]. The results of these experiments supported the hypothesis that, by analogy to observations made with the bacterium *Alcaligenes eutrophus* [6], genes required for nitrate reduction in *Rb. capsulatus* may be located on a plasmid. If this were true, one might expect that, in a collection of wild-type strains of *Rb. capsulatus* (comprising *nit*$^+$ and *nit*$^-$ phenotypes as well), a correlation exists between the patterns of endogenous plasmids and the ability to reduce nitrate. Accordingly, we have analyzed plasmid content and ability to reduce nitrate in a collection of wild-type strains and a UV-induced *nit*$^-$ mutant of *Rb. capsulatus*.

The *Rb. capsulatus* strains AD2, A1, BK5, Fc101, Kb1 = DSM 155, R1, R10, DSM 152, DSM 1710, and C2 (= UV-induced *nit*$^-$ mutant of AD2 [4]) are kept in the culture collection of the institute. Plasmid containing *Escherichia coli* strains were obtained from the Central Public Health Laboratory, London. *Rb. capsulatus* strains were grown photosynthetically (30 °C, illumination provided by 60 W tungsten lamps) in screw cap tubes (2.6 to 13 ml) in RCV medium [7] with ammonium sulfate (0.1%) or potassium nitrate (0.1%) as N-source. In the latter case, the phosphate concentration of the medium was increased twofold. *E. coli* strains were grown aerobically at 37 °C in Luria-Bertani medium (LB medium [8]). Bacterial growth was measured by monitoring the turbidity at 660 nm ($O.D._{660}$).

Plasmid contents of bacteria were analyzed by agarose gel electrophoresis [9]. With *Rb. capsulatus* strains, reproducible results were only obtained when using cells from the early stationary phase of photosynthetic cultures (40–45 h incubation up to $O.D._{660}$ values of 1.5 to 2.0). Plasmid contents of *E. coli* strains were analyzed with cells from overnight cultures.

Plasmid sizes were determined by comparing the migration distances of unknown samples with those of marker plasmids in 0.6% agarose gels (18 × 24 cm) run at 100 V for 6 h. A calibration curve was constructed with plasmids of *E. coli* (R300B: 9 kb; R7K: 31.2 kb; Tp209: 43.7 kb; R1460: 53.3 kb; R778b: 60.8 kb; pIE509: 78 kb; R714b: 126 kb; R27: 174.7 kb) by plotting log(kb) against the migration distance.

Nitrate reductase activities of intact cells were assayed in a reaction mixture with dithionite-reduced benzylviologen as electron donor [10]. Protein content of whole cells was determined by a biuret method. Enzyme activities are expressed as nmol/min · mg protein.

Table I summarizes the experimental results of this study. Irrespective of the *nit* phenotype, all *Rb.*

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Table I. Nitrate reductase activities and plasmid profiles in wild-type strains and a UV-induced *nit*$^-$ mutant (C2) of the phototrophic bacterium *Rhodobacter capsulatus*.

Strain	Growth with NO_3^- as N-source	NO_3^- reductase activity [units/mg prot.]*	Type(s) of plasmid present
AD2	++	50–80	115 kb
A1	(+)	(5–10)	134 kb
BK5	++	140–180	134, 115 kb
Kb1	++	5–10	115 kb
R10	+	5–10	106 kb
DSM 1710	++	15–30	99 kb
B10	–	n.d.	134 kb**
Fc101	–	n.d.	115, 32, 3 kb
R1	–	n.d.	93 kb
DSM 152	–	n.d.	99 kb
C2	–	n.d.	n.d.

Rb. capsulatus strains were grown photosynthetically in a malate-minimal medium [7] supplemented with 0.1% KNO_3 or 0.1% ammonium sulfate. Nitrate reductase activities were measured according to [10] and plasmid patterns were analyzed by agarose gel electrophoresis [9]. + = weak growth; ++ = moderate and good growth; values in parentheses refer to variable results. n.d. = not detected. * unit = nmol/min·mg protein. ** Data of ref. [12].

capsulatus strains examined (with the exception of strain C2, a UV-induced *nit*$^-$ mutant of AD2 [4]) contained at least one large plasmid with a size ranging from 93 to 134 kb. The nitrate reductase-negative strain Fc101 harboured three plasmids of 3, 32 and 115 kb, and the *nit*$^+$ strain BK5 contained two plasmids of 115 and 134 kb, respectively.

If the *nit* genes of *Rb. capsulatus* were located on a large endogenous plasmid one might expect that *nit*$^-$ wild-type strains would lack this specific plasmid. However, the results with the *nit*$^-$ wild-type strain Fc101 and the *nit*$^-$ mutant C2 (a derivative of strain AD2) clearly show that a simple relation of this kind does not exist. The molecular cause(s) of the missing nitrate reductase activity in mutant C2 must be more complex. It is very likely that the UV treatment having been used to isolate this mutant [4] has not only inactivated the *nit* gene(s) but possibly also the mechanism responsible for the replication of the endogenous plasmid. It may be noted in this connection that cell morphology and growth behaviour of mutant C2 differ from those seen in cultures of the parent strain AD2.

Our results show that, in *Rb. capsulatus* wild-type strains, a simple relation between *nit* phenotypes and the presence or absence of large endogenous plasmids does not exist. The observation that plasmid-curing of *Rb. capsulatus* AD2 leads to the loss of the capacity to reduce nitrate [5] could also be explained by assuming that a regulatory gene (comparable to the *nar*L gene of *E. coli* whose product activates the nitrate reductase operon [11]) is located on the endogenous plasmid. Further experiments must show whether strains unable to reduce nitrate have lost the nitrate (nitrite) reductase genes (independent of whether the latter are located on the chromosome or a plasmid) or lack an activating factor required for expression of the latter genes.

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