Determination of C/N Ratios Required for De-Repression of Nitrogenase in *Rhodobacter capsulatus*

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Phototrophic continuous and batch cultures of *Rhodobacter capsulatus* were employed to identify the C/N ratio above which nitrogenase is de-repressed. The cultures were grown with limiting amounts of ammonium as source of bound nitrogen and with L-lactate or L-malate as sources of carbon and reducing equivalents. De-repression of nitrogenase was determined on the basis of the occurrence of dinitrogen fixation, acetylene reduction and *nifH* promoter activities as well as on the basis of hydrogen evolution and nitrogenase polypeptides. In continuous culture, cells started to fix dinitrogen, to reduce acetylene, to activate the *nifH* promoter and to form nitrogenase polypeptides, when consuming lactate per ammonium at a C/N ratio of about 6 (this ratio represents the number of C and N atoms consumed). With malate as carbon source all of the activities became detectable above a C/N ratio of about 8. Essentially the same C/N ratios were determined with batch cultures for the occurrence of N-limitation of growth and hydrogen evolution. The experimentally determined C/N ratios for nitrogenase de-repression essentially agreed with C/N ratio of 5.8 and 7.8 calculated for the assimilation of ammonium and either lactate or malate, into biomass of an elemental composition of $CH_{1.83}N_{0.183}O_{0.5}$. This means that the occurrence of N-limitation and nitrogenase de-repression is defined by a threshold C/N ratio required for biomass production. As experimentally and theoretically shown, this ratio depends on the reduction state of the carbon source. It is concluded that the C/N ratio of nutrient consumption represents an intracellular signal which is directly translated into nitrogenase de-repression.

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