Effect of Copper on Expression of Functional Genes and Proteins Associated with Bradyrhizobium diazoefficiens Denitrification

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Abstract

https://github.com/pachecopedrojose025/proyecto$_{f}inal$

Nitrous oxide (N2O) is a powerful greenhouse gas that contributes to climate change. Denitrification is one of the largest sources of N2O in soils. The soybean endosymbiont Bradyrhizobium diazoefficiens is a model for rhizobial denitrification studies since, in addition to fixing N2, it has the ability to grow anaerobically under free-living conditions by reducing nitrate from the medium through the complete denitrification pathway. This bacterium contains a periplasmic nitrate reductase (Nap), a copper (Cu)-containing nitrite reductase (NirK), a c-type nitric oxide reductase (cNor), and a Cu-dependent nitrous oxide reductase (Nos) encoded by the napEDABC, nirK, norCBQD and nosRZDFYLX genes, respectively. In this work, an integrated study of the role of Cu in B. diazoefficiens denitrification has been performed. A notable reduction in nirK, nor, and nos gene expression observed under Cu limitation was correlated with a significant decrease in NirK, NorC and NosZ protein levels and activities. Meanwhile, nap expression was not affected by Cu, but a remarkable depletion in Nap activity was found, presumably due to an inhibitory effect of nitrite accumulated under Cu-limiting conditions. Interestingly, a post-transcriptional regulation by increasing Nap and NirK activities, as well as NorC and NosZ protein levels, was observed in response to high Cu. Our results demonstrate, for the first time, the role of Cu in transcriptional and post-transcriptional control of B. diazoefficiens denitrification. Thus, this study will contribute by proposing useful strategies for reducing N2O emissions from agricultural soils.

Keywords

Cu-containing nitrite reductase; enzymatic activity; gene expression; nitric oxide reductase; nitrous oxide reductase; periplasmic nitrate reductase

1 Introduction

With a 300-fold greater global warming potential than carbon dioxide (CO2), nitrous oxide (N2O) is one of the main biogenic greenhouse gases (GHG), and has also been described as the biggest single cause of ozone depletion [1] [20]. N2O emissions from human activities, fundamentally Agriculture, Forestry and Other Land Use (AFOLU), have notably increased since the Green Revolution in the early 60s. During the period 2007–2016, these activities represented 81% of the anthropogenic emissions of N2O, according to the last special report by the Intergovernmental Panel on Climate Change [2] [24]. In particular, agriculture has become the major source of N2O emissions, accounting for approximately 78% of the anthropogenic N2O sources [2] [24] because of a global agricultural

intensification and a great increase in the non-synchronized use of synthetic nitrogen fertilisers [3-5] [11][21][26]. Several biological pathways occurring in agricultural soils are involved in N2O emissions. Among all of them, nitrification and denitrification are the main microbial N2O sources directly affected by soil nitrogen fertilisation, but only denitrification is known to be the largest source of N2O [6] [27].

Apart from other organisms, such as archaea and fungi, some facultative bacteria possess the ability to adapt their metabolism to an oxygen-depleted environment in the presence of nitrate as a respiratory substrate through the activation of denitrification. This pathway consists of the dissimilatory reduction of nitrate or nitrite to dinitrogen (N2) via the gaseous intermediates nitric oxide (NO) and nitrous oxide (N2O). In this process, specific metalloenzymes are sequentially involved: periplasmic (Nap) or membrane-bound (Nar) nitrate reductases, copper (Cu)-containing (NirK) or cytochrome cd1-containing (NirS) nitrite reductases, nitric oxide reductases (cNor, qNor or CuANor), and nitrous oxide reductase (Nos). The majority of denitrifiers are found in the phylum Proteobacteria, within the domain Bacteria. The alpha-proteobacterium Paracoccus denitrificans and the gamma-proteobacteria Pseudomonas stutzeri and Pseudomonas aeruginosa are the first model organisms where denitrification were widely studied. Reviews covering the physiology, biochemistry and molecular genetics of denitrification have been published elsewhere [8–11,13,14] [34][30][31][13][5][29].Over recent years, several reports about denitrification in plant endosymbiotic bacteria emerged [15–17] [3][2][22]. Thanks to their capacity to establish an N2-fixing symbiotic relationship with plants, these bacteria can contribute to natural N soil enrichment, while reducing the need for chemical fertilisation. Therefore, symbiotic N2 fixation is considered a process with economic, ecological and agricultural importance. In this process, a mutualist association between soil bacteria, commonly known as rhizobia, and plants of the Fabaceae family is established. Rhizobia may induce the formation of nodules in the legume roots and on the stems of some aquatic legumes; nodules are specialized structures where N2 fixation takes place [18] [19].

Bradyrhizobium diazoefficiens, which establishes nitrogen-fixation symbiosis with soybean (Glycine max), is considered a model organism in the study of denitrification in rhizobia because it is the only known rhizobia species able to grow under oxygen-limiting conditions with nitrate as sole electron acceptor and, also, to perform the complete denitrification pathway under both free-living and symbiotic conditions [15] [3]. Denitrification in B. diazoefficiens is carried out by a periplasmic nitrate reductase (Nap), encoded by the napEDABC operon [19] [9], a Cu-containing nitrite reductase (NirK), encoded by the nirK gene [20] [32], a cytochrome c-type nitric oxide reductase (cNor), encoded by the norCBQD operon [21] [17], and a Cu-dependent nitrous oxide reductase (Nos), encoded by the nosRZDFYLX genes [22] [33]. Nap is a functional heterodimer comprising the catalytic subunit NapA of about 90 kDa that contains a bis molybdopterin guanine dinucleotide (Mo[MGD]2) cofactor and a [4Fe-4S] centre, and NapB (15 kDa) that contains 2 heme c groups and receives electrons from the membrane-bound NapC (25 kDa) which binds 4 heme c groups. NirK is a homotrimer with a predicted molecular mass of about 35 kDa per monomer that contains type 1 and type 2 Cu centres. The catalytic subunit of cNor, NorB, contains heme b and a binuclear active centre (heme b3 and FeB). NorC is a membrane-anchored protein (16 kDa) that contains heme c. Finally, the catalytic subunit of Nos, NosZ (120–160 kDa), is a homodimer Cu-containing enzyme with two distinct Cu centres (CuA and CuZ).

Similarly to many other denitrifiers, expression of denitrification genes in B. diazoefficiens requires both oxygen limitation and the presence of nitrate or a derived nitrogen oxide (NOx), this control being mediated by the FixLJ-FixK2-NnrR regulatory cascade [23–25] [15][16][6]. In fact, the expression of napEDABC, nirK and nosRZDFYLX genes requires microoxic conditions and directly depends on the transcriptional regulator FixK2 [25,26] [6][28], while expression of norCBQD genes

relies on NO, being NnrR the transcriptional regulator which directly interacts with the norCBQD promoter [25,27] [6][12]. In this context, the molecular discriminatory determinants for selective FixK2 recognition and target activation were recently unveiled [28][7].

Besides being a source of N2O, the ecological and environmental importance of denitrification lies in the fact that Nos is the only known enzyme able to remove N2O from ecosystems [4] [21], the expression and activity of this enzyme becoming a natural target to effectively reduce N2O emissions from agricultural soils. Increasing knowledge of the regulation and biochemistry of N2O metabolism in rhizobia will raise opportunities for the design of effective mitigation strategies to reduce N2O emissions from legume crops [1].

Nowadays, new environmental factors are emerging as candidates for controlling denitrification, such as pH [30,31] [8][18] or Cu [32] [4]. In the case of Cu, it is an essential cofactor in critical enzymes, such as multicopper oxidases, as well as the Nos and NirK denitrification enzymes. The role of this metal in denitrification has been studied in a wide range of non-symbiotic microorganisms, such as Pseudomonas perfectomarinus [33] [14], P. stutzeri [32] [4], P. denitrificans [34,35] [10][25] and Achromobacter xylosoxidans [34] [10]. Regarding rhizobia, Serventi et al. (2012) [36] [23] investigated the role of Cu in cytochrome oxidase biogenesis in B. diazoefficiens. Nevertheless, studies covering Cu influence on the denitrification pathway in rhizobia are scarce. This study provides an integral view of the involvement of Cu in B. diazoefficiens denitrification, analysing the effect of different Cu regimes on gene expression, as well as on the protein levels and activity of the denitrification enzymes in free-living cultures.

2 Materials and Methods

3 Results

3.1 Copper effect on B. diazoefficiens 110spc4 growth under different oxygen conditions

3.2 Disparate response of denitrification gene expression to copper

3.3 Influence of copper on expression and activity of denitrification enzymes

4 Discussion

5 Conclusions

Acknowledgements

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