

JS1-2:

Regulation of N₂O reductase genes by the two-component system NasST in *Bradyrhizobium diazoefficiens*

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The greenhouse gas nitrous oxide (N₂O) is emitted from soybean rhizosphere through the degradation of the root nodules at the late growth period. Soybean hosts endosymbiotic N₂-fixing soil bacteria (rhizobia) that reduce N₂O through N₂O reductase (Nos). Previous work has shown that inoculation of *Bradyrhizobium diazoefficiens* strains with induced Nos activity can mitigate N₂O emission from soybean fields. In *B. diazoefficiens* USDA110, full expression of nos genes requires oxygen limitation, the presence of a nitrogen oxide and the FixLJ-FixK2 regulatory cascade.

Here, I will present the recently discovered regulation of nos genes through the two-component system NasST. NasS is a nitrate sensor and NasT is a transcription antiterminator. Mutation of nasS induced both N₂O reductase activity and transcription of nos genes (nosRZD), in cells of *B. diazoefficiens* incubated in the absence of nitrate. The NasS_NasT protein complex was dissociated in vitro by the addition of nitrate, suggesting the release of NasT, which is known to bind the leader RNA of the target gene, thereby preventing hairpin formation and allowing complete transcription. Disruption of nasT led to a marked decrease in nos transcription in *B. diazoefficiens* cells incubated with nitrate, indicating that NasST system regulates nos transcription in response to nitrate. Although analysis of the region upstream nosR and nosZ genes revealed no regulatory hairpin structures similar to those present in the leader RNA of other genes regulated by NasT, we could confirm binding of purified NasT with nosR RNA, but not with nosZ RNA.

These results suggest that NasST mediates the induction of nos genes by nitrate in two steps: (i) the presence of nitrate promotes the dissociation of NasS_NasT protein complex and, (ii) released NasT activates the transcription of nos gene cluster by binding nosR RNA. The ecological implications of this regulation remain unclear but several features will be discussed.

keywords: Nitrous oxide reductase, *Bradyrhizobium diazoefficiens*, nos, NasST, Nitrate
