Ro *et al. Applied Biological Chemistry* (2025) 68:29
https://doi.org/10.1186/s13765-025-00999-7

KSABC
The Korean Society for Applied Biological Chemistry

NOTE

Open Access

Regional and strain-level prevalence of nitrogen-fixing *Bradyrhizobium* with potential N_2O reduction in South Korea

Jaeyoung Ro[1], Hor-Gil Hur[1] and Sujin Lee[1*]

Abstract
Agricultural practices are the largest anthropogenic source of nitrous oxide (N_2O), a potent greenhouse gas contributing to global climate change. Applying symbiotic microbial inoculants capable of complete denitrification offers a promising strategy to mitigate N_2O emissions from agricultural fields. This study reports the strain-level diversity and geographical distribution of soybean symbiont bacteria *Bradyrhizobium* species carrying the *nosZ* gene, which encodes nitrous oxide reductase. Of 227 indigenous *Bradyrhizobium* isolates from soybean root nodules across South Korea, 162 were found to possess the *nosZ* gene, indicating their potential for N_2O reduction. The majority of the most prevalent species, *Bradyrhizobium diazoefficiens*, harbor the *nosZ* gene, contributing to the overall high frequency of *nosZ*-positive genotypes nationwide. In contrast, no evidence of the *nosZ* gene was detected in the second most abundant species, *Bradyrhizobium elkanii*, which was predominantly isolated from the southwestern regions, raising the possibility of elevated N_2O emissions in these areas. The presence of the *nosZ* gene varies substantially even within the same species, highlighting the importance of understanding strain-level genetic and functional diversity to develop *Bradyrhizobium* inoculants optimized for both nitrogen fixation and denitrification.

Keywords *Bradyrhizobium*, *nosZ*, Symbiosis, Nitrogen fixation, Denitrification, Nitrous oxide (N_2O), Microbial inoculants, Soybean rhizosphere, Biofertilizer

Introduction

Nitrogen is a vital element for the survival of all living organisms. Plants such as legumes form root nodules that host nitrogen-fixing bacteria and benefit from symbiotic nitrogen fixation. The bacteria colonize the rhizosphere and make nitrogen available to plants by enzymatically converting dinitrogen gas (N_2) to ammonia (NH_3) using nitrogenase activity [1, 2]. Nitrogen fixation dependent on this mutualistic symbiosis drives the global nitrogen cycle, which involves two key biochemical processes: nitrification and denitrification. During microbial nitrification, ammonia is first oxidized to nitrite (NO_2^-) by two enzymes, ammonia monooxygenase (AMO) and hydroxylamine oxidoreductase (HAO), and then to nitrate (NO_3^-) by nitrite oxidoreductase (NXR) under aerobic conditions [1, 3]. The microbial denitrification pathway is achieved by the stepwise reduction of nitrate to dinitrogen gas ($NO_3^-\rightarrow NO_2^-\rightarrow NO\rightarrow N_2O\rightarrow N_2$) [1, 4]. Transmembrane nitrate reductase (NAR) or periplasmic nitrate reductase (NAP) catalyzes the reduction of nitrate to nitrite, and cytochrome cd1 nitrite reductase (NIR-S) or copper nitrite reductase (NIR-K) catalyzes

*Correspondence:
Sujin Lee
sujinlee.sl@gmail.com
[1]Department of Environment and Energy Engineering, Gwangju Institute of Science and Technology, Gwangju 61005, Republic of Korea

nitrite reduction to nitric oxide (NO). Three nitric oxide reductase families, cNOR, qNOR, and qCuANOR, were shown to catalyze the reduction of nitric oxide to nitrous oxide (N_2O), which is further reduced to dinitrogen gas by nitrous oxide reductase [1, 5].

During the nitrogen cycle, N_2O is naturally produced as an intermediate product of denitrification and a byproduct of nitrification. It is a long-lived, the third most greenhouse gas, contributing to ozone layer destruction and climate change [6]. Approximately 40% of global N_2O emissions are derived from human activities such as agriculture, industry, fossil fuel combustion, and waste treatment [7]. Agricultural practices, such as the use of nitrogen fertilizers and land management activities, cause the most significant anthropogenic N_2O emissions. Atmospheric N_2O concentration is increasing more rapidly than predicted, requiring effective strategies to mitigate N_2O emissions from agricultural lands [7, 8]. One of the proposed sustainable approaches is the microbial reduction of N_2O to N_2 by denitrifying bacteria [9–12].

Denitrifying bacteria are characterized based on the presence of metabolic genes encoding reductases for denitrification. Unlike other reductases involved in denitrification with broad phylogenetic and taxonomic diversity, N_2O reductase is the only enzyme known to catalyze the reduction of N_2O to N_2 [13, 14]. Therefore, the gene encoding N_2O reductase, *nosZ*, is a genetic marker for identifying complete denitrifying bacteria [1, 15, 16]. Although nitrogen-transforming bacteria were specifically classified based on the metabolic process they participate in, further studies have found that they are metabolically and functionally versatile [1, 17]. In particular, soybean-nodulating *Bradyrhizobium* species support soybean growth and productivity via nitrogen fixation. They were also shown to harbor the genes required for denitrification, proposing their additional role in the nitrogen cycle. N_2O reductase activity was demonstrated in specific isolates and *nosZ* mutants of *B. diazoefficiens* and *B. ottawaense* [11, 12, 18, 19]. A comprehensive analysis is needed to evaluate the potential of diverse *Bradyrhizobium* communities to mitigate N_2O emissions. Understanding the distribution of indigenous *Bradyrhizobium* populations with denitrification capability is essential for utilizing them in agricultural and environmental management.

In this study, we analyzed the populations of nitrogen-fixing *Bradyrhizobium* species isolated from the soybean root nodules in South Korea at the strain-level, focusing on their possession of the *nosZ* gene. Developing nitrogen-fixing *Bradyrhizobium* inoculants with complete denitrification ability specifically adapted to the unique environmental and agricultural conditions in South Korea is a sustainable and promising strategy to enhance agricultural productivity and reduce environmental impacts.

Materials and methods

Isolation of soybean-nodulating *Bradyrhizobium* strains

An updated *Bradyrhizobium* strain collection, based on our previous report [20] and including newly identified isolates, was used to examine the presence of the *nosZ* gene encoding N_2O reductase. Briefly, root nodules were collected from 97 soybean plants across 14 regions in 8 provinces of South Korea (excluding Jeju) in September 2019 [20]. Details of the sampling locations, including GPS coordinates, agricultural field types, and host soybean cultivars, are provided in Supplementary File 1. Root nodules were surface-sterilized in 0.1% $HgCl_2$ for 30 s, followed by sequential washes in sterile water, 70% ethanol, and sterile water 7 to 10 times. The sterilized nodules were crushed in 1 mL of sterile water to release the bacterial suspension, which was serially diluted up to 10^{-4} and plated onto arabinose-gluconate (AG) agar medium [21, 22]. Plates were incubated at 28 °C for 7 to 10 days to selectively isolate pure *Bradyrhizobium* strains associated with soybean nodules [20].

Identification of soybean-nodulating *Bradyrhizobium* strains

Purified isolates were taxonomically identified and classified based on 16S rRNA sequencing, as previously described [20, 23]. The 16S rRNA gene was amplified using universal primers 27F (5'-AGAGTTTGATCMTGGCTCA) and 1492R (5'-GYTACCTTGTTACGACTT) with the AccuPower® PCR premix (Bioneer Co., Korea). PCR amplification was performed on a Mastercycler Gradient thermal cycler (Eppendorf Co., Germany) under the following conditions: initial denaturation at 96 °C for 4 min; 30 cycles of denaturation at 94 °C for 30 s, annealing at 57 °C for 30 s, and extension at 72 °C for 1 min; followed by a final extension at 72 °C for 10 min. The 16S rRNA PCR products were purified and sequenced for identification and classification (Macrogen Co., Korea).

Detection of the *nosZ* gene

PCR analysis was conducted to assess the presence of the *nosZ* gene in 233 *Bradyrhizobium* isolates retrieved from the stock collection. To ensure the reliability of the analysis, species represented by fewer than five isolates were excluded. Consequently, 227 out of 233 isolates were included in the present study (Supplementary File 2). Each bacterial strain was cultured in 200 µL of AG medium at 28 °C for 7 days. 2 µL of each liquid culture was directly added to the AccuPower® PCR premix (Bioneer Co., South Korea) with target gene-specific primers. The primer sets used were: nosZ_1804F (5'-CGCRACGGCAASAAGGTSMSSGT)

and nosZ_2090R (5'-CAKRTGCAKSGCRTGGCAGAA) for the *nosZ* gene, and otsAF (5'-GAGGCCTATGGCA ATCTTCA) and otsAR (5'-TACTCCTTTGCGACGAG GTT) for the *otsA* gene. The *otsA* gene, encoding trehalose-6-phosphate synthase required for trehalose biosynthesis, was amplified in all strains examined, serving as a control [24]. PCR amplification was performed under the following thermal cycling conditions: initial denaturation at 96 °C for 2 min; 25 cycles of denaturation at 95 °C for 16 s, annealing at 60 °C for 30 s, and extension at 72 °C for 45 s; final extension at 72 °C for 2 min. 7 µL of PCR product was analyzed on 1.5% (w/v) agarose gel in tris-acetate-EDTA (TAE) buffer with ethidium bromide (0.5 µg/mL) using the Mupid-exU Electrophoresis system (Takara Bio Inc., Japan). Gels were visualized using a UV Transilluminator (Wealtec Co., USA).

Results and discussion

To assess the populations of indigenous *Bradyrhizobium* with the potential for N_2O reduction, we employed strains reported in our previous study [20], along with additional newly identified isolates. A total of 227 *Bradyrhizobium* strains were analyzed, each of which belongs to one of the following genomic groups, representing their relative abundance in agricultural fields: *B. algeriense* (2.2%), *B. diazoefficiens* (59.9%), *B. elkanii* (14.5%), *B. guangxiense* (4.0%), *B. japonicum* (8.4%), *B. ottawaense* (8.4%), and *B. subterraneum* (2.6%) (Supplementary File 3). Three elite species, *B. diazoefficiens*, *B. elkanii*, and *B. japonicum*, account for 82.8% of the total population, reflecting their competitive adaptability and ecological fitness to the agricultural environments in South Korea. *B. diazoefficiens* was widespread across the country, and *B. elkanii* and *B. japonicum* were isolated mainly from the southwestern and central-western regions, respectively, highlighting their ecological dominance and environmental adaptation.

Out of 227 indigenous *Bradyrhizobium* strains, 162 (71.4%) were found to contain the *nosZ* gene in their genomes, indicating their potential N_2O reductase activity (Fig. 1). The presence of the *nosZ* gene varies significantly among strains, even within the same species, underscoring the importance of understanding strain-level genetic and functional diversity for research and application purposes. The majority of *B. diazoefficiens* (90.4%), *B. guangxiense* (100%), and *B. ottawaense* (84.2%) harbor the *nosZ* gene. The populations of *B. algeriense* (60.0%), *B. japonicum* (47.4%), and *B. subterraneum* (33.3%) also possess the *nosZ* gene with relatively lower frequencies. Interestingly, no PCR products were obtained from *B. elkanii* isolates, indicating that this species likely does not carry the *nosZ* gene (Fig. 1). Together, our strain collection showed that 6 of 7 *Bradyrhizobium* genomic groups contain the *nosZ* gene with strain-specific variations. Compared to previous research in Japan and Argentina [25, 26], the relatively high proportion of *nosZ*-containing populations identified in this study may reflect the distinct environmental conditions, crop management practices, and unique evolutionary pressures shaping indigenous *Bradyrhizobium* communities in South Korea.

Although the denitrification pathway has been reported in *Bradyrhizobium*, the complete denitrifying species containing the *nosZ* gene are uncommon [11, 13, 19, 27]. The genome sequencing analysis of *B. diazoefficiens* USDA110 (reclassified from *B. japonicum* USDA110 [28]) revealed that it carries an entire set of denitrification genes, including the *nosRZDFYLX* gene cluster, of which the *nosZ* gene encodes N_2O reductase [29]. The mutant of *B. diazoefficiens* USDA110 lacking the *nosZ*

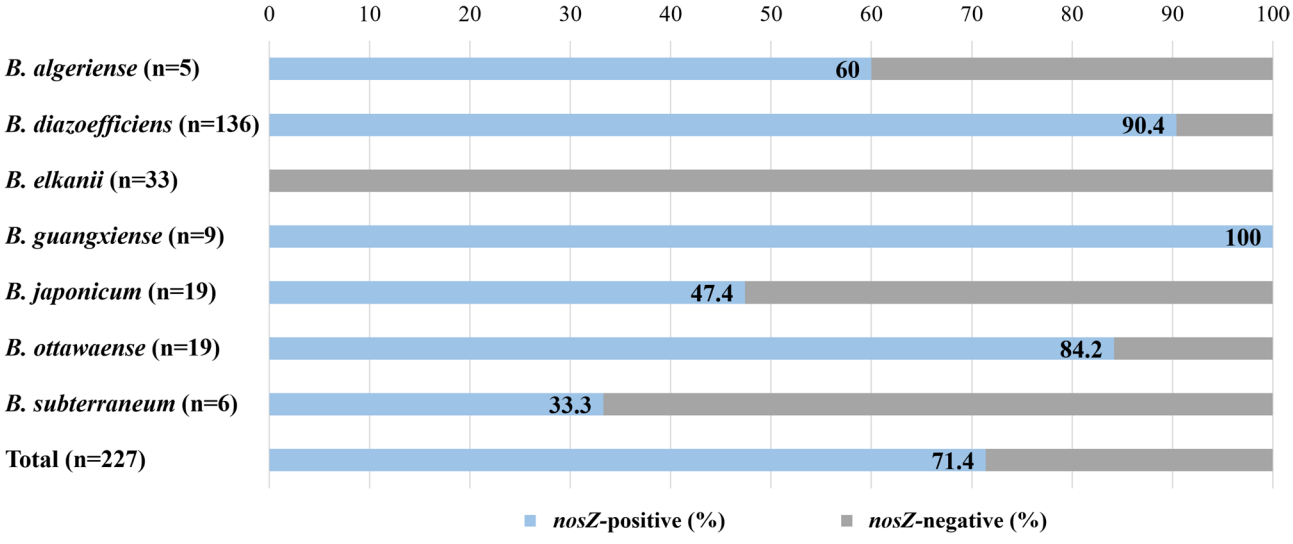

Fig. 1 Relative frequencies of *nosZ*-positive genotype across diverse *Bradyrhizobium* species isolated from soybean nodules in South Korea

gene accumulated N_2O during the microaerobic cultivation with nitrate, confirming its functional role in denitrification [30]. Inoculation of *B. diazoefficiens* strains carrying the *nosZ* wild-type and mutants with enhanced N_2O reductase activity was shown to mitigate N_2O emissions at the field scale [10, 11]. Consistent with previous findings in this species, 90.4% of *B. diazoefficiens* isolates in our study possess the *nosZ* gene (Fig. 1). Given that it is the most prevalent *Bradyrhizobium* species in South Korea, dominating 9 out of 14 sampling sites (Table 1), it is crucial to investigate these isolates' N_2O-reducing activity in local soybean cultivation settings.

Genome sequencing analysis also verified the presence of denitrification genes, including *nosZ*, in *B. ottawaense* MIAE 01942 isolated from soybean nodules [31, 32]. Wasai-Hara et al. [33] showed that *B. ottawaense* strains, isolated from non-leguminous sorghum roots in Japan, exhibit higher *nosZ* gene expression and N_2O-reducing activity under N_2O-respiring conditions than *B. diazoefficiens* USDA110 despite having over 90% identity in the NosZ protein sequence [19]. In our study, *B. ottawaense* strains were predominant in GW_PC (52.2%) and CB_JC (71.4%) (Table 1), two geographically adjacent cities, with a high frequency of *nosZ* gene possession (84.2%), suggesting that they may be well-suited for improving nitrogen management in mountain agriculture [20]. Due to the limited research available, the effects of *B. ottawaense* on nitrogen fixation and denitrification are not fully understood. Given the high potential for N_2O mitigation [19], further research is necessary to understand the physiological and functional characteristics of native *B. ottawaense* isolates.

We also detected the *nosZ* gene in *B. japonicum*, *B. guangxiense*, and other minor species. No clear evidence in previous studies supports the presence of the *nosZ* gene in *B. japonicum* and *B. guangxiense*, suggesting the need for further genomic and functional analyses to determine their roles in denitrification. Although *B. guangxiense* makes up only 4.0% of the total strains examined (Supplementary File 3), the isolates were distributed across JB, GW, and GB provinces (Table 1), all carrying the *nosZ* gene (Fig. 1). These findings suggest that *B. guangxiense* may function as both a nitrogen-fixer and a denitrifier. Known as a symbiont of peanuts, *B. guangxiense* showed limited compatibility with soybeans [34, 35]. Our findings support its symbiotic relationship with soybeans in agricultural fields, expanding our understanding of the host range and ecological adaptability of *Bradyrhizobium*. A comprehensive analysis is required to clarify the potential roles of diverse *nosZ*-carrying *Bradyrhizobium* in nitrogen fixation, symbiotic relationships, and denitrification within the soybean rhizosphere.

The second-largest group, *B. elkanii*, is prevalent in the warmer southwestern regions of Korea, particularly dominating JB_JU and JN_MA (Table 1), which are characterized by unique temperature patterns and physiochemical soil properties [20]. Consistent with the lack of evidence, we did not detect this gene in any of the 33 isolates examined (Fig. 1). The high prevalence of *B. elkanii* lacking the *nosZ* gene in the JN and JB provinces may be related to a potential risk of elevated N_2O emissions associated with incomplete denitrification. Further comprehensive analysis is needed to evaluate the impact of *Bradyrhizobium* populations on N_2O emissions in these regions, taking into account the environmental factors that influence the denitrification process.

Along with environmental factors, symbiotic compatibility is one of the key determinants influencing the distribution and genetic diversity of *Bradyrhizobium* communities. The JN and JB provinces are major production areas for Taekwang soybeans. According to the Korea Seed and Variety Service, over 90% of Taekwang soybean seeds were distributed to these regions in 2024 [36]. Its exceptional symbiotic compatibility with *B. elkanii* (Supplementary File 1) [20] has likely shaped the distinct localized microbial community structure (Fig. 2). In contrast, the nationwide predominance of *B. diazoefficiens* (Table 1), which is compatible with most soybean cultivars except Taekwang (Supplementary File 1), has led to the high frequency of *nosZ*-positive genotypes in

Table 1 Geographical and strain-level diversity of *nosZ* possession in *Bradyrhizobium* populations isolated from soybean nodules in South Korea

		number of *nosZ*-positive isolates (number of total isolates)														
	Province	**JN**	**JB**				**GW**			**GG**	**GB**		**GN**	**CN**	**CB**	
Species	**City**	**MA**	**JU**	**IS**	**JA**	**GJ**	**PC**	**HC**	**CW**	**PJ**	**AD**	**MG**	**UR**	**TA**	**JC**	**Total**
B. algeriense			0(1)	3(3)		0(1)										3(5)
B. diazoefficiens		1(2)		12(16)	2(5)	29(31)	9(9)	5(6)	30(31)	14(15)	3(3)	6(6)	9(9)	2(2)	1(1)	123(136)
B. elkanii		0(27)	0(5)			0(1)										0(33)
B. guangxiense						6(6)	1(1)	1(1)			1(1)					9(9)
B. japonicum		1(1)			1(2)	1(6)	1(1)				1(1)			4(8)		9(19)
B. ottawaense							10(12)							2(2)	4(5)	16(19)
B. subterraneum					0(1)	0(2)			1(1)					0(1)	1(1)	2(6)
Total		2(30)	0(6)	15(19)	3(8)	36(47)	21(23)	6(7)	31(32)	14(15)	5(5)	6(6)	9(9)	8(13)	6(7)	162(227)

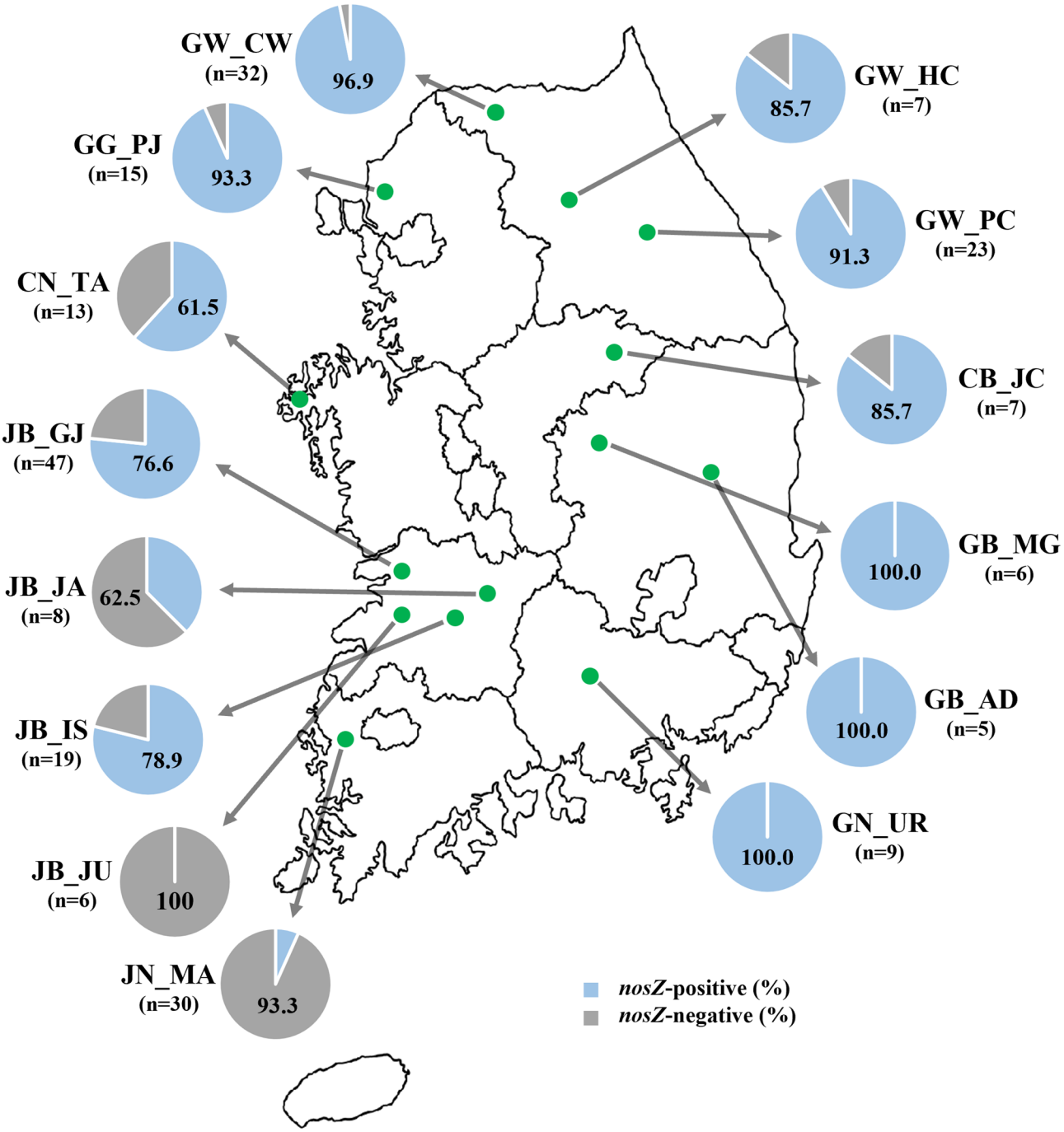

Fig. 2 Distribution of *nosZ*-carrying *Bradyrhizobium* species in the sampling locations in South Korea (*nosZ*-positive in blue and *nosZ*-negative in gray) CB_JC; Chungcheongbukdo_Jecheon, CN_TA; Chungcheongnamdo_Taean, GB_AD; Gyeongsangbukdo_Andong, GB_MG; Gyeongsangbukdo_Mungyeong, GG_PJ; Gyeonggido_Paju, GN_UR; Gyeongsangnamdo_Uiryeong, GW_CW; Gangwondo_Cheorwon, GW_HC; Gangwondo_Hongcheon, GW_PC; Gangwondo_Pyeongchang, JB_GJ; Jeollabukdo_Gimje, JB_IS; Jeollabukdo_Imsil, JB_JA; Jeollabukdo_Jinan, JB_JU; Jeollabukdo_Jeongeup, JN_MA; Jeollanamdo_Muan

the GG, GW, GB, GN, and parts of JB provinces (Fig. 2) [20]. These findings underscore that host-symbiont compatibility and preference need to be carefully considered when introducing microbial inoculants.

An analysis of the five most commonly used biofertilizers of *B. japonicum*, *B. elkanii*, and *B. diazoefficiens* in South America revealed that four of them, except *B. diazoefficiens*, are incomplete denitrifiers [37]. These strains produced up to 26 times more N_2O than *B.*

diazoefficiens, highlighting the importance of selecting appropriate microbial inoculants. Our study serves as a foundational screening effort to identify indigenous *Bradyrhizobium* strains having the potential for N_2O reduction. It provides valuable information for developing regionally adapted and host-specified *Bradyrhizobium* inoculants optimized for enhancing nitrogen fixation while mitigating N_2O emissions. Hara et al. [38] recently argued that the impact of *Bradyrhizobium* inoculation on the soil microbial community may be less significant compared to the effects of agricultural land-use types and soil chemical properties. Developing effective and sustainable agricultural strategies incorporating symbiotic nitrogen-fixing bacterial inoculation requires a comprehensive assessment of environmental conditions, crop adaptation, symbiotic compatibility, and agricultural management practices.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s13765-025-00999-7.

Supplementary Material 1

Supplementary Material 2

Supplementary Material 3

Acknowledgements
Not applicable.

Author contributions
J.R. designed and performed the experiments and curated the data. H.-G.H. conceptualized the study and reviewed the manuscript. S.L. designed the experiments, analyzed the data, and wrote the manuscript. All authors have read and approved the final manuscript.

Funding
This work was supported by the National Research Foundation of Korea (NRF), funded by the Korean government (Ministry of Science and ICT [MSIT]) (grant no. RS-2021-NR060081). S. L. was supported by the National Research Foundation of Korea (NRF) (grant no. RS-2023-00281371).

Data availability
All data supporting the findings of this study are available in this article.

Declarations

Competing interests
The authors declare no competing interests.

Received: 12 March 2025 / Accepted: 2 May 2025
Published online: 20 June 2025

References

1. Kuypers MMM, Marchant HK, Kartal B (2018) The microbial nitrogen-cycling network. Nat Rev Microbiol 16(5):263–276. https://doi.org/10.1038/nrmicro.2018.9
2. Zehr JP, Jenkins BD, Short SM, Steward GF (2003) Nitrogenase gene diversity and microbial community structure: a cross-system comparison. Environ Microbiol 5(7):539–554. https://doi.org/10.1046/j.1462-2920.2003.00451.x
3. Daims H, Lücker S, Wagner M (2016) A new perspective on microbes formerly known as nitrite-oxidizing bacteria. Trends Microbiol 24(9):699–712. https://doi.org/10.1016/j.tim.2016.05.004
4. Zumft WG (1997) Cell biology and molecular basis of denitrification. Microbiol Mol Biol Rev 61(4):533–616. https://doi.org/10.1128/mmbr.61.4.533-616.1997
5. Hochstein LI, Tomlinson GA (1988) The enzymes associated with denitrification. Annu Rev Microbiol 42:231–261. https://doi.org/10.1146/annurev.mi.42.100188.001311
6. Tian H, Xu R, Canadell JG, Thompson RL, Winiwarter W et al (2020) A comprehensive quantification of global nitrous oxide sources and sinks. Nature 586(7828):248–256. https://doi.org/10.1038/s41586-020-2780-0
7. Intergovernmental Panel on Climate Change (2023) Climate change 2021 – The physical science basis: working group I contribution to the sixth assessment report of the intergovernmental panel on climate change. Cambridge University Press. https://doi.org/10.1017/9781009157896
8. United Nations Environment Programme and Food and Agriculture Organization (2024) Global nitrous oxide assessment. Nairobi. https://doi.org/10.59117/20.500.11822/46562
9. Bueno E, Mania D, Frostegard Å, Bedmar EJ, Bakken LR et al (2015) Anoxic growth of *Ensifer meliloti* 1021 by N_2O-reduction, a potential mitigation strategy. Front Microbiol 6. https://doi.org/10.3389/fmicb.2015.00537
10. Itakura M, Uchida Y, Akiyama H, Hoshino YT, Shimomura Y et al (2013) Mitigation of nitrous oxide emissions from soils by *Bradyrhizobium japonicum* inoculation. Nat Clim Change 3(3):208–212. https://doi.org/10.1038/nclimate1734
11. Akiyama H, Hoshino YT, Itakura M, Shimomura Y, Wang Y et al (2016) Mitigation of soil N_2O emission by inoculation with a mixed culture of Indigenous *Bradyrhizobium diazoefficiens*. Sci Rep 6(1):32869. https://doi.org/10.1038/srep32869
12. Hénault C, Barbier E, Hartmann A, Revellin C (2022) New insights into the use of rhizobia to mitigate soil N_2O emissions. Agriculture 12(2):271. https://doi.org/10.3390/agriculture12020271
13. Zumft WG, Kroneck PMH (2006) Respiratory transformation of nitrous oxide (N_2O) to dinitrogen by Bacteria and Archaea. Adv Microb Physiol 52:107–227. https://doi.org/10.1016/S0065-2911(06)52003-X
14. Murali R, Pace LA, Sanford RA, Ward LM, Lynes MM et al (2024) Diversity and evolution of nitric oxide reduction in bacteria and archaea. Proc Natl Acad Sci 121(26): e2316422121. https://doi.org/10.1073/pnas.2316422121
15. Scala DJ, Kerkhof LJ (1998) Nitrous oxide reductase (*nosZ*) gene-specific PCR primers for detection of denitrifiers and three *nosZ* genes from marine sediments. FEMS Microbiol Lett 162(1):61–68. https://doi.org/10.1111/j.1574-6968.1998.tb12979.x
16. Orellana LH, Rodriguez RLM, Higgins S, Chee-Sanford JC, Sanford RA et al (2014) Detecting nitrous oxide reductase (*nosZ*) genes in soil metagenomes: method development and implications for the nitrogen cycle. mBio 5(3). https://doi.org/10.1128/mbio.01193-14
17. Zhao Y, Zhu-Barker X, Cai K, Wang S, Wright AL et al (2024) Quest for the Nitrogen-Metabolic versatility of microorganisms in soil and marine ecosystems. Microorganisms 12(7):1283. https://doi.org/10.3390/microorganisms12071283
18. Itakura M, Tabata K, Eda S, Mitsui H, Murakami K et al (2008) Generation of *Bradyrhizobium japonicum* mutants with increased N_2O reductase activity by selection after introduction of a mutated *DnaQ* gene. Appl Environ Microbiol 74(23):7258–7264. https://doi.org/10.1128/AEM.01850-08
19. Wasai-Hara S, Itakura M, Fernandes Siqueira A, Takemoto D, Sugawara M et al (2023) *Bradyrhizobium Ottawaense* efficiently reduces nitrous oxide through high *nosZ* gene expression. Sci Rep 13(1):18862. https://doi.org/10.1038/s41598-023-46019-w
20. Kim Y-e, Shin H, Yang Y, Hur H-G (2022) Geographical distribution and genetic diversity of *Bradyrhizobium* spp. Isolated from Korean soybean root nodules. Appl Biol Chem 65(1):39. https://doi.org/10.1186/s13765-022-00708-8
21. Sadowsky MJ, Tully RE, Cregan PB, Keyser HH (1987) Genetic diversity in Bradyrhizobium japonicum serogroup 123 and its relation to Genotype-Specific nodulation of soybean. Appl Environ Microbiol 53(11):2624–2630. https://doi.org/10.1128/aem.53.11.2624-2630.1987
22. Tong Z, Sadowsky MJ (1994) A selective medium for the isolation and quantification of *Bradyrhizobium japonicum* and *Bradyrhizobium elkanii* strains from soils and inoculants. Appl Environ Microbiol 60(2):581–586. https://doi.org/10.1128/aem.60.2.581-586.1994

23. Lane DJ, Pace B, Olsen GJ, Stahl DA, Sogin ML et al (1985) Rapid determination of 16S ribosomal RNA sequences for phylogenetic analyses. Proc Natl Acad Sci 82(20):6955–6959. https://doi.org/10.1073/pnas.82.20.6955
24. Sugawara M, Cytryn EJ, Sadowsky MJ (2010) Functional role of *Bradyrhizobium japonicum* Trehalose biosynthesis and metabolism genes during physiological stress and nodulation. Appl Environ Microbiol 76(4):1071–1081. https://doi.org/10.1128/AEM.02483-09
25. Shiina Y, Itakura M, Choi H, Saeki Y, Hayatsu M et al (2014) Relationship between soil type and N_2O reductase genotype (*nosZ*) of Indigenous soybean bradyrhizobia: *nosZ*-minus populations are dominant in andosols. Microbes Environ 29(4):420–426. https://doi.org/10.1264/jsme2.ME14130
26. Fernández LA, Perotti EB, Sagardoy MA, Gómez MA (2008) Denitrification activity of *Bradyrhizobium* Sp. isolated from Argentine soybean cultivated soils. World J Microbiol Biotechnol 24(11):2577–2585. https://doi.org/10.1007/s11274-008-9828-x
27. Sánchez C, Minamisawa K (2019) Nitrogen cycling in soybean rhizosphere: sources and sinks of nitrous oxide (N_2O). Front Microbiol 10. https://doi.org/10.3389/fmicb.2019.01943
28. Delamuta JRM, Ribeiro RA, Ormeño-Orrillo E, Melo IS, Martínez-Romero E et al (2013) Polyphasic evidence supporting the reclassification of *Bradyrhizobium japonicum* group Ia strains as *Bradyrhizobium diazoefficiens* Sp. nov. Int J Syst Evol MicroBiol 63(Pt9):3342–3351. https://doi.org/10.1099/ijs.0.049130-0
29. Kaneko T, Nakamura Y, Sato S, Minamisawa K, Uchiumi T et al (2002) Complete genomic sequence of Nitrogen-fixing symbiotic bacterium *Bradyrhizobium japonicum* USDA110. DNA Res 9(6):189–197. https://doi.org/10.1093/dnares/9.6.189
30. Velasco L, Mesa S, Xu C-a, Delgado MJ, Bedmar EJ (2004) Molecular characterization of *nosRZDFYLX* genes coding for denitrifying nitrous oxide reductase of *Bradyrhizobium japonicum*. Antonie Van Leeuwenhoek 85(3):229–235. https://doi.org/10.1023/B:ANTO.0000020156.42470.db
31. Revellin C, Hartmann A, Solanas S, Topp E (2018) Long-Term exposure of agricultural soil to veterinary antibiotics changes the population structure of symbiotic Nitrogen-Fixing rhizobacteria occupying nodules of soybeans (*Glycine max*). Appl Environ Microbiol 84(9):e00109–00118. https://doi.org/10.1128/AEM.00109-18
32. Scott A, Topp E, Revellin C, Hartmann A, Fruci M (2024) Complete genome sequence of *Bradyrhizobium Ottawaense* strain MIAE 01942 isolated from soybean nodules grown in antibiotic-amended soil. Microbiol Resource Announcements 13(4):e00004–00024. https://doi.org/10.1128/mra.00004-24
33. Wasai-Hara S, Hara S, Morikawa T, Sugawara M, Takami H et al (2020) Diversity of *Bradyrhizobium* in Non-Leguminous Sorghum plants: *B. ottawaense* isolates unique in genes for N_2O reductase and lack of the type VI secretion system. Microbes Environ 35(1). https://doi.org/10.1264/jsme2.ME19102
34. Li YH, Wang R, Zhang XX, Young JPW, Wang ET et al (2015) *Bradyrhizobium Guangdongense* Sp. Nov. And *Bradyrhizobium guangxiense* Sp. Nov., isolated from effective nodules of peanut. Int J Syst Evol MicroBiol 65(Pt12):4655–4661. https://doi.org/10.1099/ijsem.0.000629
35. Shang JY, Zhang P, Jia YW, Lu YN, Wu Y et al (2022) Scrutiny of NolA and NodD1 regulatory roles in symbiotic compatibility unveils new insights into *Bradyrhizobium guangxiense* CCBAU53363 interacting with peanut (*Arachis hypogaea*) and mung bean (*Vigna radiata*). Microbiol Spectr 11(1):e02096–e02022. https://doi.org/10.1128/spectrum.02096-22
36. Korea Seed & Variety Service, Crop seed production and distribution. Retrieved April 23, 2025. https://www.seed.go.kr/seed/263/subview.do
37. Obando M, Correa-Galeote D, Castellano-Hinojosa A, Gualpa J, Hidalgo A et al (2019) Analysis of the denitrification pathway and greenhouse gases emissions in *Bradyrhizobium* Sp. strains used as biofertilizers in South America. J Appl Microbiol 127(3):739–749. https://doi.org/10.1111/jam.14233
38. Hara S, Kakizaki K, Bamba M, Itakura M, Sugawara M et al (2024) Does rhizobial inoculation change the microbial community in field soils? A comparison with agricultural Land-use changes. Microbes Environ 39(3). https://doi.org/10.1264/jsme2.ME24006

Publisher's note