Revista ARACÊ
ISSN: 2358-2472

METAGENOMIC ANALYSIS OF BACTERIA PRODUCING THE ENZYME ADENYLATE CYCLASE (EC 4.6.1.1) UNDER DIFFERENT SOIL AND FOREST MANAGEMENTS

ANÁLISE METAGENOMICA DE BACTÉRIAS PRODUTORAS DA ENZIMA ADENILATO CICLASE (EC 4.6.1.1) SOB DIFERENTES MANEJOS DE SOLO E FLORESTA

ANÁLISIS METAGENÓMICO DE BACTERIAS PRODUCTORAS DE LA ENZIMA ADENILATOCICLASA (EC 4.6.1.1) BAJO DIFERENTES MANEJOS DE SUELO Y BOSQUE

https://doi.org/10.56238/arev7n8-204

Submission date: 07/20/2025 **Publication date:** 08/20/2025

Kauanny Wictoria Plenz[1], Larissa Marques Negrett[2], Ryan Fernandes Vieira de Souza[3], Maricy Raquel Lindenbah Bonfá[4], Rodrigo Matheus Pereira[5]

ABSTRACT

The soil microbial community plays an essential role in ecological processes, such as nutrient cycling and plant health, and is regulated by molecular mechanisms such as signaling via adenylate cyclase (E.C. 4.6.1.1). This enzyme is a key component of the cAMP pathway, with functional relevance in various microbial contexts. The aim of this study was to quantify and taxonomically identify adenylate cyclase-producing bacteria in soils under different agricultural management and in native forest areas in the region of Dourados - MS, Brazil. To this end, five soil samples were analyzed using metagenomic sequencing and bioinformatics tools, including contig assembly, ORF prediction, local database alignment, taxonomic and statistical analyses. 18,139 sequences associated with the target enzyme were identified, with greater abundance in soils of continuous pasture and crop-livestock integration. The phyla Actinobacteria and Proteobacteria were predominant, and the genera Sinorhizobium, Micromonospora and Bifidobacterium stood out, with significant variations between managements. The analysis showed that management practices directly affect the composition and diversity of bacteria producing the enzyme adenylate cyclase.

---

[1] Undergraduate Student in Biotechnology. Universidade Federal da Grande Dourados (UFGD).
E-mail: kakawictoria8@gmail.com Lattes: http://lattes.cnpq.br/6400558585644143
Orcid: https://orcid.org/0009-0005-8399-2803
[2] Undergraduate Student in Biotechnology. Universidade Federal da Grande Dourados (UFGD).
E-mail: Larissa.negrett028@academico.ufgd.edu.br Lattes: http://lattes.cnpq.br/4947328505344222
Orcid: https://orcid.org/0009-0000-9617-4889
[3] Master's Student in Bioinformatics. Universidade Federal de Minas Gerais (UFMG).
E-mail: ryanfvs@ufmg.br Lattes: http://lattes.cnpq.br/5252229894109187
Orcid: https://orcid.org/0009-0006-1393-7224
[4] Dr. in Food Science. Universidade Federal da Grande Dourados (UFGD).
E-mail: maricybonfa@ufgd.edu.br Lattes: http://lattes.cnpq.br/5670504878145026
Orcid: https://orcid.org/0000-0002-6978-4769
[5] Dr. in Agricultural Microbiology. Universidade Federal da Grande Dourados (UFGD).
E-mail: rodrigopereira@ufgd.edu.br Lattes: http://lattes.cnpq.br/8155952045293310
Orcid: https://orcid.org/0000-0001-6025-5118

Keywords: Environmental Genomics. Microbial Ecology. Liases. Microbiology.

RESUMO

A comunidade microbiana do solo desempenha um papel essencial em processos ecológicos, como ciclagem de nutrientes e saúde vegetal, e é regulada por mecanismos moleculares como a sinalização via adenilato ciclase (E.C. 4.6.1.1). Esta enzima é um componente chave da via do AMPc, com relevância funcional em vários contextos microbianos. O objetivo deste estudo foi quantificar e identificar taxonomicamente bactérias produtoras de adenilato ciclase em solos sob diferentes manejos agrícolas e em áreas de floresta nativa na região de Dourados - MS, Brasil. Para tanto, cinco amostras de solo foram analisadas utilizando ferramentas de sequenciamento metagenômico e bioinformática, incluindo montagem de contigs, predição de ORFs, alinhamento de banco de dados local, análises taxonômicas e estatísticas. Foram identificadas 18.139 sequências associadas à enzima alvo, com maior abundância em solos de pastagem contínua e integração lavoura-pecuária. Os filos Actinobacteria e Proteobacteria predominaram, com destaque para os gêneros Sinorhizobium, Micromonospora e Bifidobacterium, com variações significativas entre os manejos. A análise mostrou que as práticas de manejo afetam diretamente a composição e a diversidade de bactérias produtoras da enzima adenilato ciclase.

Palavras-chave: Genômica Ambiental. Ecologia Microbiana. Liases. Microbiologia.

RESUMEN

La comunidad microbiana del suelo desempeña un papel esencial en los procesos ecológicos, como el ciclo de nutrientes y la salud de las plantas, y está regulada por mecanismos moleculares como la señalización a través de la adenilato ciclasa (E.C. 4.6.1.1). Esta enzima es un componente clave de la vía del AMPc, con relevancia funcional en varios contextos microbianos. El objetivo de este estudio fue cuantificar e identificar taxonómicamente las bacterias productoras de adenilato ciclasa en suelos bajo diferentes manejos agrícolas y en áreas de bosque nativo en la región de Dourados - MS, Brasil. Para este fin, se analizaron cinco muestras de suelo mediante secuenciación metagenómica y herramientas bioinformáticas, incluyendo ensamblaje de contig, predicción de ORF, alineamiento de bases de datos locales, análisis taxonómicos y estadísticos. Se identificaron 18,139 secuencias asociadas con la enzima diana, con mayor abundancia en suelos de pastoreo continuo e integración cultivo-ganadería. Los filos Actinobacteria y Proteobacteria fueron predominantes, destacando los géneros Sinorhizobium, Micromonospora y Bifidobacterium, con variaciones significativas entre los manejos. El análisis mostró que las prácticas de manejo afectan directamente la composición y diversidad de las bacterias productoras de la enzima adenilato ciclasa.

Palabras clave: Genómica Ambiental. Ecología Microbiana. Enlaces. Microbiología.

1 INTRODUCTION

There are direct and indirect influences of soil microbial communities on various processes, including nutrient cycling, organic matter dynamics, soil structure, carbon transformations and sequestration (Benerjee, et al. 2023). These communities of microorganisms also contribute to plant health, in which plants recruit microorganisms from the rhizosphere that are essential for their growth and development, and these soil microorganisms can shape the composition and functioning of the microbiota associated with plants (Benerjee, et al. 2023).

The enzymes adenylate cyclases (ACs) generate the adenosine 3',5'-monophosphate (cAMP) signaling pathway, being a highly conserved regulatory mechanism that plays a fundamental role in a wide range of fundamental cellular processes, therefore, this enzyme is considered one of the main components of the cAMP signaling and regulation pathways (Khannpnavar, et al. 2020). As an example of the association of this enzyme in cellular processes, studies show that the gram-negative saprophytic bacterium *Myxococcus xanthus* uses adenylate cyclase as an osmosensor, while the bacterium *Burkholderia pseudomalei* also showed a large increase in adenylate cyclase expression when exposed to salt stress, possibly indicating that it also uses this enzyme as an osmosensor (Duangurai, et al. 2018).

Metagenomics is essential for studying the direct sequencing and DNA analysis of microbial assemblages, which are methods used to characterize the functional potential of microbial communities (Taş, et al. 2021). Information obtained from metagenomics can help identify protein-coding genes (ORFs), thus providing a greater understanding of microbial processes that participate in biogeochemical cycles in ecosystems, such as the adenylate cyclase enzyme in the cAMP pathway.

In other areas, adenylate cyclase is also involved in fungal virulence in *Aspergillus flavus*, Yang (2016) provided evidence that the adenylate cyclase AcyA is responsible for cAMP synthesis in *A. flavus*, demonstrating that AcyA has pleiotropic effects on growth, conidiation, virulence and FA biosynthesis. In addition, severe defects in fungal growth, sporulation and sclerotia formation were observed in the microorganism that suffered the acyA deletion.

The aim of this study was to quantify and identify taxonomically the bacteria that produce the enzyme adenylate cyclase (E.C. 4.6.1.1) in soils under different types of management and forest in the region of Dourados - MS, Brazil.

2 METHODOLOGY

Figure 1

Flowchart of the bioinformatics activities carried out in this study

Source: Research data, 2025.

The soil samples studied came from Embrapa Agropecuária Oeste in Dourados - MS, Brazil. After metagenomic DNA extraction and illumina sequencing, the samples were analyzed using bioinformatics tools. The quality of the sequencing was measured using the FastQC program, filtering out those of low quality or too short using the Prinseq-lite program (Schmieder, et al. 2011). The sequences were assembled using the IDBA-UD program (Peng, et al. 2012). The coding regions were identified using the FragGeneScan program (Rho, et al. 2010), which identified the ORFs (open reading frames). All the ORFs were generated in the work by Rissi, 2015. In the current work, they were prospected for adenylate cyclase (E.C. 4.6.1.1).

The DNA sequences from the five soil samples: native forest, continuous pasture, direct planting, crop-livestock integration and conventional planting were compared with a local database created from the adenylate cyclase sequences (E.C. 4.6.1.1) from bacteria in the NCBI (National Center for Biotechnology Information) protein bank. For this comparison, the sequences were aligned using the Blastall program (Boratin, et al. 2013).

The MEGAN6 program (Huson, et al. 2018) was used for the comparison and taxonomic classification of the adenylate cyclase sequences (E.C. 4.6.1.1) present in each soil sample, covering domain, kingdom, phylum, class, order, family, genus and species. The comparison was made by importing the sequences from the results generated by Blastall (Boratin, et al. 2013).

InteractiVenn (Heberle, et al. 2015) produced Venn diagrams, which made it possible to check which microorganisms are common and exclusive in the soil samples.

The Statistical Analysis of Taxonomic Profiles (STAMP) program (Parks, et al. 2014) was used to evaluate statistical differences between the soils, analyzing them in pairwise combinations. It used the G-test (w/Yates') + Fisher's statistical test for $p \leq 0.05$, using the DP:Asymptotic-CC method, being of the Two-sided type (two-tailed test).

3 RESULTS AND DISCUSSIONS

The result of comparing the 5 soil samples with the local database generated 18,139 matching sequences (Table 1). It can be seen that the continuous pasture and integrated crop-livestock soils had the largest microbial community and the enzyme targeted by the study.

Table 1

Number of gene sequences responsible for the adenylate cyclase enzyme and total ORF sequences present in the microbial community of 5 soil samples

Soils	Adenylate cyclase	Total de ORFs
Native forest	1.747	46.430
Continuous pasture	5.637	137.703
Direct planting	3.065	75.866
Crop-Livestock Integration	5.019	128.475
Conventional planting	2.671	71.762

Legend: * ORFs - Open reading frame
Source: Research data, 2025.

The Shannon-Wiener Diversity Index (H') (Spellerberg; Fedor, 2003) is one of the most widely used metrics for quantifying biological diversity in ecological communities, as it considers both species richness and evenness. This index means that H' > 3 indicates high diversity (many well-distributed species, with no clear dominance), and it was possible to see high species diversity in all the samples, except in the soil under conventional planting management, where H' was between 1 and 3, indicating moderate species diversity, that is, some species may be dominant (Table 2).

Table 2

Ecological index in the 5 soil samples.

Soils	H’
Native forest	6.388
Continuous pasture	5.637
Direct planting	3.065
Crop-Livestock Integration	5.019
Conventional Planting	2.671

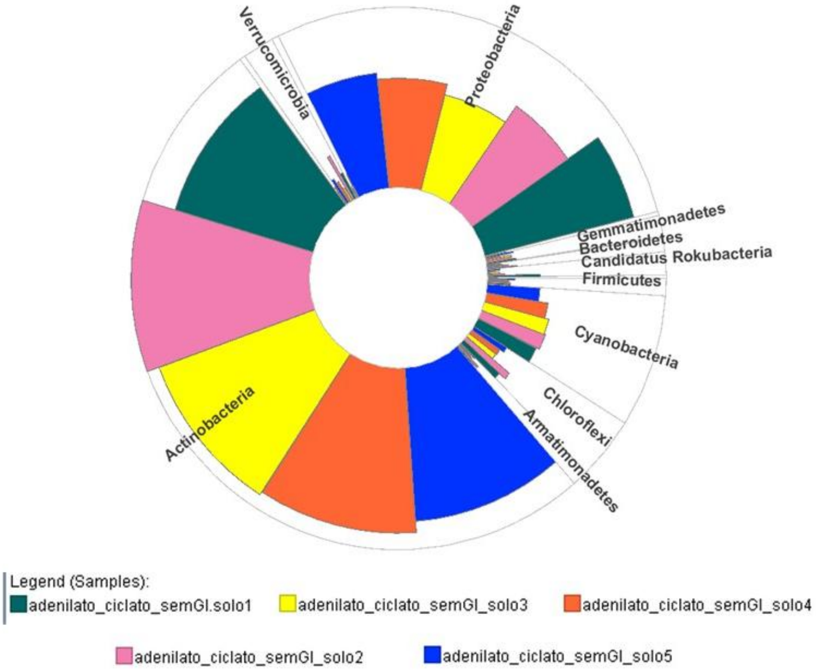
Legend: Shannon-Wiener Diversity Index (H')
Source: Research data, 2025.

The use of Megan software in conjunction with STAMP provided results on taxonomic classification and their proportions in the 5 soil managements, generating radial graphs and extended error bars, informing which management practices possibly favored greater abundance or diversity of bacteria producing the enzyme adenylate cyclase.

Fig. 2 shows the Actinobacteria phylum in higher proportions in all management practices, with a greater presence in the continuous pasture soil, as well as the Proteobacteria phylum, but with a greater predominance in the native forest soil.

Figure 2

Radial graph of the proportions of phyla in the soils of native forest (soil 1), continuous pasture (soil 2), direct planting (soil 3), crop-livestock integration (soil 4) and conventional planting (soil 5)



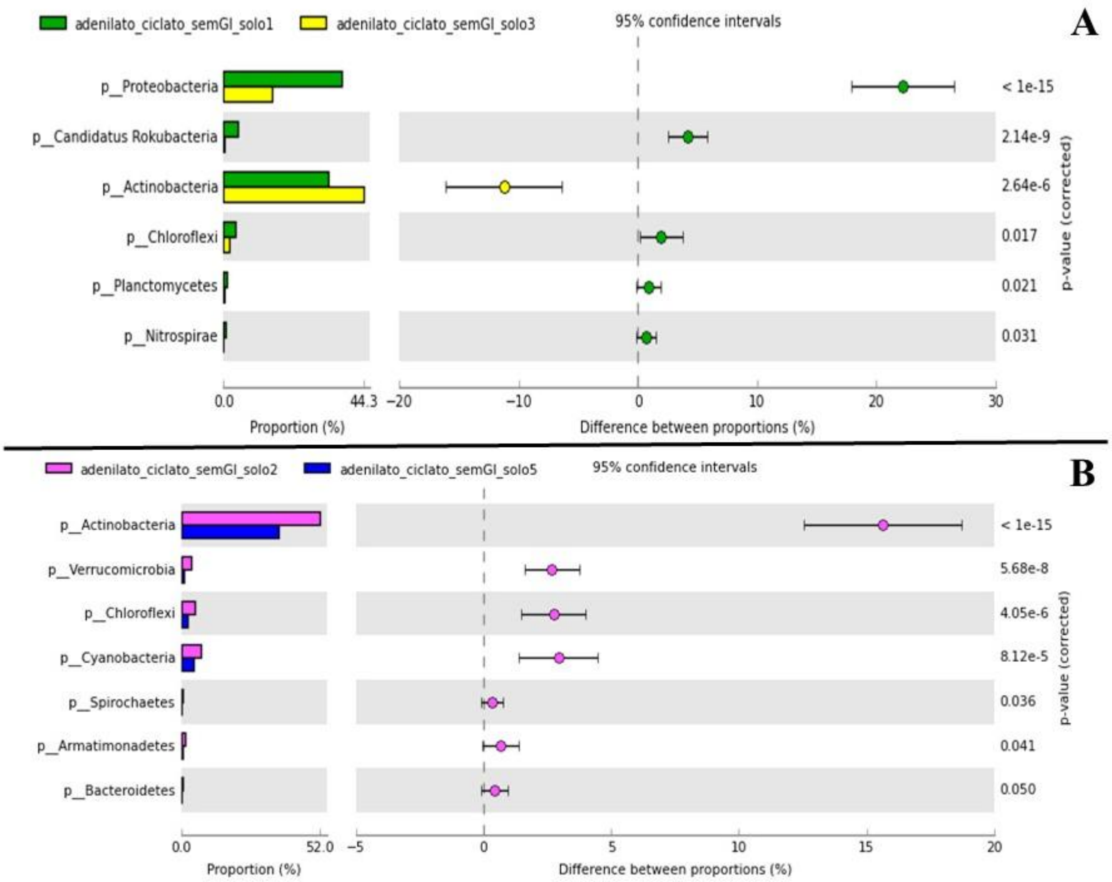
Source: Research data, 2025.

There were statistical differences at 5% significance with 95% reliability in the proportions of the presence of the Actinobacteria phylum between the continuous pasture and conventional tillage soil managements, as well as for the native forest and no-tillage soils (Fig.3a and Fig.3b). The phylum Proteobacteria showed no statistically significant difference between the continuous pasture and conventional tillage soils, and its absence can be seen in Fig.3b.

Evaluating the statistical differences between bacterial phyla in soil samples under different types of management is fundamental to understanding how agricultural and land use practices influence the structure and function of microbial communities. The different types of management strongly influence the taxonomic composition of the soil, its different uses or characteristics can present specific taxonomic groups (Kacergius, et al. 2023).

Figure 3

Statistical analysis of the difference between the proportions of the Phyla of native forest (soil 1), continuous pasture (soil 2), direct planting (soil 3) and conventional planting (soil 5)

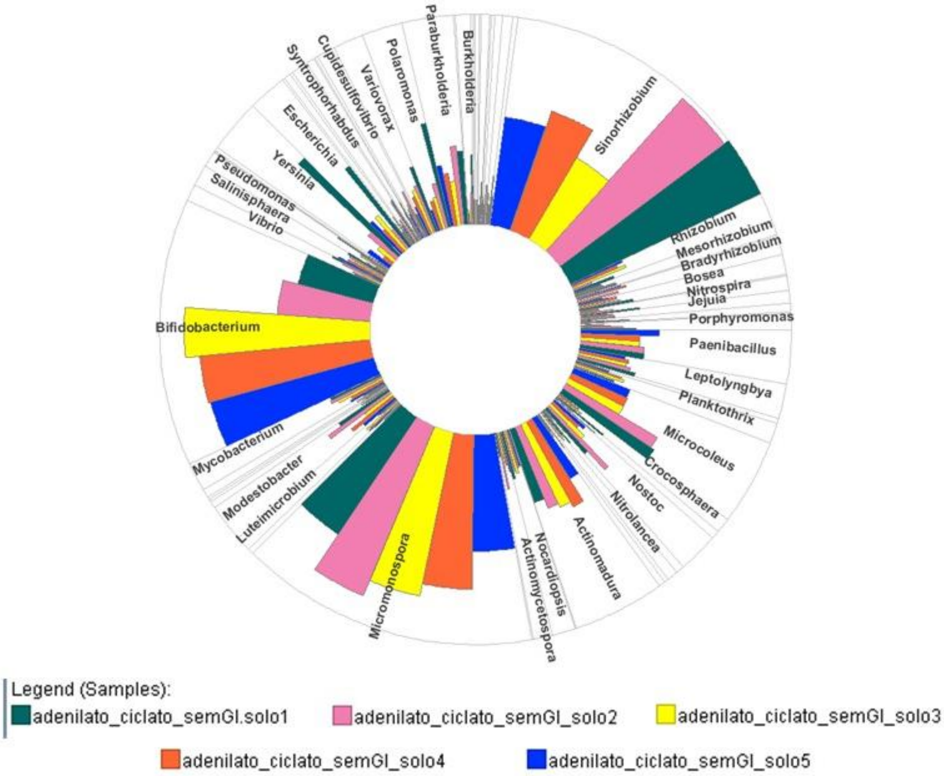


Source: Research data, 2025.

Looking at the genus level, the bacteria producing adenylate cyclase stood out in the highest proportions: *Sinorhizobium*, *Micromonospora* and *Bifidobacterium* (Fig.4). There were statistical differences at 5% significance for the proportions of the genus *Sinorhizobium* between the soils of native forest and no-till, as well as continuous pasture and conventional tillage. The same differences applied to the genus *Bifidobacterium* (Fig.5a, Fig.5b). In turn, *Micromonospora* showed no statistical difference between native forest and direct planting management (Fig.5a).

Figure 4

Radial graph showing gender proportions in native forest soils (soil 1), continuous pasture (soil 2), direct planting (soil 3), crop-livestock integration (soil 4), and conventional planting (soil 5).



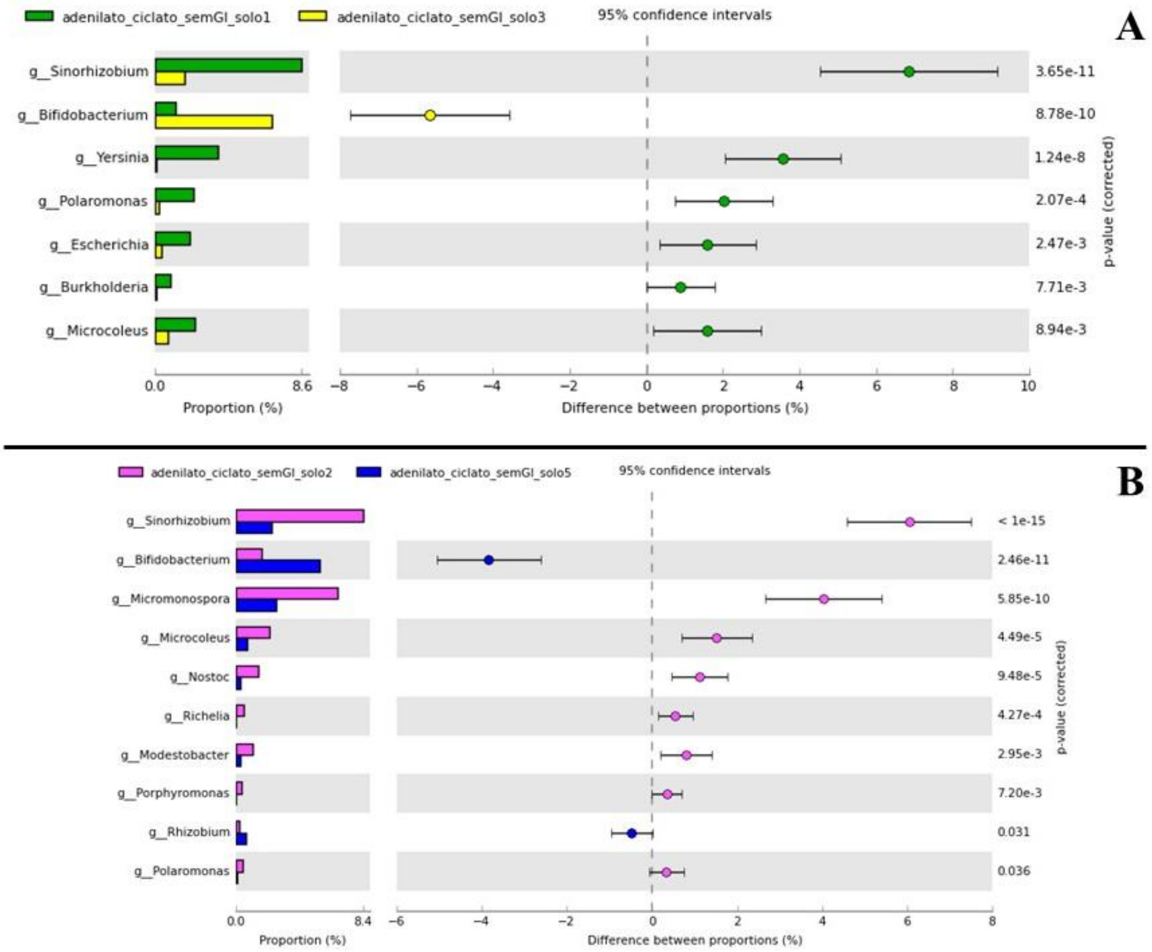
Source: Research data, 2025.

The genus *Sinorhizobium* is fundamental to sustainable agriculture due to its ability to establish symbiosis with legumes, forming root nodules where biological nitrogen fixation (BNF) occurs (shahrajabian, et al. 2021). In addition, it is understood that environmental factors such as different types of management can influence the efficiency of symbiosis and nitrogen fixation.

Thus, this possibly explains the greater presence of this genus in the soil under native forest (Fig.4), since signaling via cAMP, mediated by adenylate cyclases, allows *Sinorhizobium* to actively respond to plant signals, controlling infection and optimizing symbiosis (Tian, et al. 2012), and managements that promote a greater abundance of adenylate cyclase-producing bacteria tend to favor more stable and adaptable microbial communities (Souza, et al. 2015). In other words, the analysis of this enzyme serves as a possible functional indicator of soil health and resilience, allowing us to interpret how agricultural practices impact microbial structure and functioning.

Figure 5

Statistical analysis of the difference between the proportions of the genera of native forest (soil 1), continuous pasture (soil 2), direct planting (soil 3) and conventional planting (soil 5)

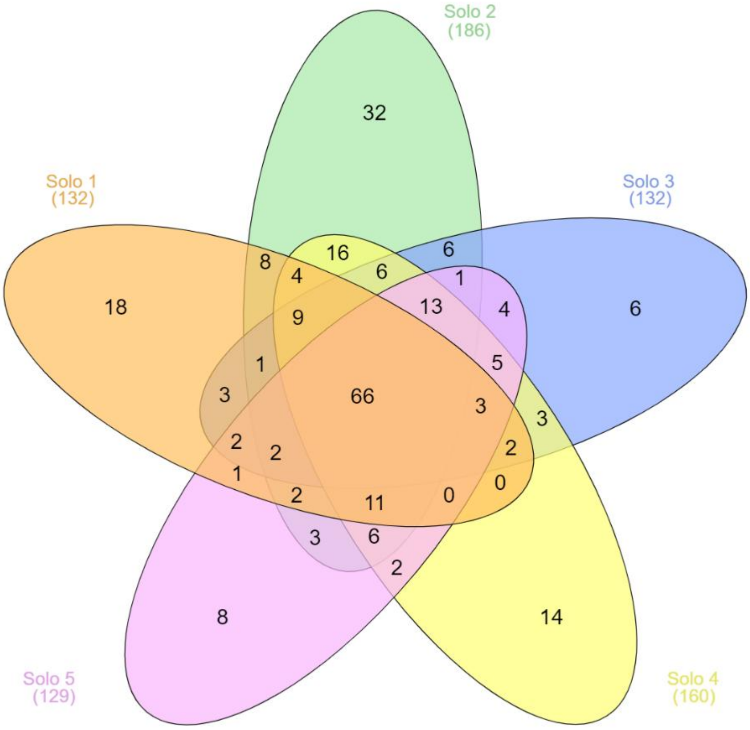


Source: Research data, 2025.

Entering 739 species (Fig.6), distributed among the five soil samples in the InteracVenn program, resulted in a graphic representation of 66 species in common, among them *Rhizobium tibeticum*, *Sinorhizobium americanum*, *Paraburkholderia caribensis*, *Micromonospora noduli*, *Yersinia enterocolitica*, *Micromonospora saelicesensis*, *Actinomadura parvosata*, *Paenibacillus xylanexedens* and *Planktothrix agardhii*, as well as being the most abundant among the samples. It can be seen from that the continuous pasture soil has the highest number of exclusive species, while the direct planting soil has the lowest.

Figure 6

Venn diagram showing the species found in soil samples from native forest (soil 1), continuous pasture (soil 2), direct planting (soil 3), crop-livestock integration (soil 4), and conventional planting (soil 5)



Source: Research data, 2025.

5 CONCLUSION

A total of 18,139 sequences of bacteria containing the enzyme adelinate cyclase were obtained from soils under different types of management and forest in the Dourados - MS region. Among these bacteria, there was a greater presence of the phyla Actinobacteria and Proteobacteria, with statistical differences between the soils under continuous pasture and conventional planting, as well as for the native forest and direct planting soils. There was no statistically significant difference in the Proteobacteria phylum between continuous pasture and conventional tillage. At the genus level, there was a higher proportion of *Sinorhizobium*, *Micromonospora* and *Bifidobacterium*, with statistical differences at 5% significance between the native forest and direct planting soils, as well as continuous pasture and conventional tillage. The genus *Micromonospora* showed no statistically significant difference between the native forest and direct planting management.

It was found that the soil under continuous pasture had a higher diversity of species producing the enzyme adenylate cyclase, while the soil under conventional tillage management had a lower diversity. This study provides valuable input for future studies, expanding knowledge about bacteria that produce the enzyme adenylate cyclase under different types of soil management in Brazil.

ACKNOWLEDGEMENTS

The authors would like to thank the Federal University of Grande Dourados (UFGD) for its institutional support, for the high quality of the academic training it offers and for encouraging scientific research and development in biotechnology. We would also like to thank FUNDECT (Fundação de Apoio ao Desenvolvimento do Ensino, Ciência e Tecnologia do Estado de Mato Grosso do Sul) for its financial support, which is crucial for carrying out this work. We acknowledge the contribution of the UFGD Bioinformatics Laboratory, for its technical infrastructure, collaborative environment and the availability of computational tools that made the analysis possible. The laboratory's technical and scientific support was essential for the development of this study.

REFERENCES

Banerjee, S., & van der Heijden, M. G. A. (2023). Soil microbiomes and one health. Nature Reviews Microbiology, 21(1), 6–20. https://doi.org/10.1038/s41579-022-00779-w

Boratin, G. M., Camacho, C., Cooper, P. S., Coulouris, G., Fong, A., Ma, N., Madden, T. L., Matten, W. T., McGinnis, S. D., Merezhuk, Y., Raytselis, Y., Sayers, E. W., Tao, T., Ye, J., & Zaretskaya, I. (2013). BLAST: A more efficient report with usability improvements. Nucleic Acids Research, 41(W1), W29–W33. https://doi.org/10.1093/nar/gkt282

Duangurai, T., Indrawattana, N., & Pumirat, P. (2018). Burkholderia pseudomallei adaptation for survival in stressful conditions. BioMed Research International, 2018, Article 9123926. https://doi.org/10.1155/2018/9123926

Gorgojo, J. P., Fernández, J., Estein, S. M., & Baldi, P. C. (2023). Adenylate cyclase toxin of Bordetella parapertussis disrupts the epithelial barrier granting the bacterial access to the intracellular space of epithelial cells. PLOS ONE, 18(11), e0291331. https://doi.org/10.1371/journal.pone.0291331

Heberle, H., Meirelles, G. V., da Silva, F. R., Telles, G. P., & Minghim, R. (2015). InteractiVenn: A web-based tool for the analysis of sets through Venn diagrams. BMC Bioinformatics, 16, 169. https://doi.org/10.1186/s12859-015-0611-3

Huson, D. H., Auch, A. F., Qi, J., & Schuster, S. C. (2018). MEGAN-LR: New algorithms allow accurate binning and easy interactive exploration of metagenomic long reads and contigs. Biology Direct, 13, 6. https://doi.org/10.1186/s13062-018-0208-7

Kačergius, A., Kačergius, T., Stankevičius, R., & Kačergius, A. (2023). Comparison of the structure of soil microbial communities of different ecosystems using the microbiome sequencing approach. Soil Systems, 7(3), 70. https://doi.org/10.3390/soilsystems7030070

Kapetanaki, S. M., Burton, M. J., Steinhardt, R. C., & Korkhov, V. M. (2024). Crystal structure of a bacterial photoactivated adenylate cyclase determined by serial femtosecond and serial synchrotron crystallography. IUCrJ, 11(Pt 6), 991–1006. https://doi.org/10.1107/S2052252524010170

Khannpnavar, B., Mehta, V., Qi, C., & Korkhov, V. (2020). Structure and function of adenylyl cyclases, key enzymes in cellular signaling. Current Opinion in Structural Biology, 63, 34–41. https://doi.org/10.1016/j.sbi.2020.03.005

Lindner, F., & Diepold, A. (2022). Optogenetics in bacteria—applications and opportunities. FEMS Microbiology Reviews, 46(2), fuab055. https://doi.org/10.1093/femsre/fuab055

Parks, D. H., Imelfort, M., Skennerton, C. T., Hugenholtz, P., & Tyson, G. W. (2014). STAMP: Statistical analysis of taxonomic and functional profiles. Bioinformatics, 30(21), 3123–3124. https://doi.org/10.1093/bioinformatics/btu494

Peng, Y., Leung, H. C. M., Yiu, S. M., & Chin, F. Y. L. (2012). IDBA-UD: A de novo assembler for single-cell and metagenomic sequencing data with highly uneven depth. Bioinformatics, 28(11), 1420–1428. https://doi.org/10.1093/bioinformatics/bts174

Rho, M., Tang, H., & Ye, Y. (2010). FragGeneScan: Predicting genes in short and error-prone reads. Nucleic Acids Research, 38(20), e191. https://doi.org/10.1093/nar/gkq747

Schmieder, R., & Edwards, R. (2011). Quality control and preprocessing of metagenomic datasets. Bioinformatics, 27(6), 863–864. https://doi.org/10.1093/bioinformatics/btr026

Shahrajabian, M. H., Sun, W., & Cheng, Q. (2021). The importance of Rhizobium, Agrobacterium, Bradyrhizobium, Herbaspirillum, Sinorhizobium in sustainable agricultural production. Notulae Botanicae Horti Agrobotanici Cluj-Napoca, 49(3), 12183. https://doi.org/10.15835/nbha49312183

Souza, R. C., Hungria, M., Cantão, M. E., Vasconcelos, A. T. R., Nogueira, M. A., & Vicente, V. A. (2015). Metagenomic analysis reveals microbial functional redundancies and specificities in a soil under different tillage and crop-management regimes. Applied Soil Ecology, 86, 106–112. https://doi.org/10.1016/j.apsoil.2014.10.010

Spellerberg, I. F., & Fedor, P. J. (2003). A tribute to Claude Shannon (1916–2001) and a plea for more rigorous use of species richness, species diversity and the 'Shannon–Wiener' index. Global Ecology and Biogeography, 12(3), 177–179. https://doi.org/10.1046/j.1466-822X.2003.00015.x

Taş, N., de Jong, A. E., Li, Y., Trubl, G., Xue, Y., & Dove, N. C. (2021). Metagenomic tools in microbial ecology research. Current Opinion in Biotechnology, 67, 184–191. https://doi.org/10.1016/j.copbio.2021.01.006

Tian, C. F., Zhou, Y. J., Zhang, Y. M., Li, Q. Q., Zhang, Y. Z., Li, D. F., Wang, S., Wang, E. T., Du, B. H., & Sui, X. H. (2012). Plant-activated bacterial receptor adenylate cyclases modulate epidermal infection in the Sinorhizobium meliloti–Medicago symbiosis. Proceedings of the National Academy of Sciences, 109(17), 6751–6756. https://doi.org/10.1073/pnas.1120260109

Yang, K., Liang, L., Ran, F., Liu, Y., Li, Z., Lan, H., Gao, P., Zhuang, W., & Zhang, D. (2016). Adenylate cyclase AcyA regulates development, aflatoxin biosynthesis and fungal virulence in Aspergillus flavus. Frontiers in Cellular and Infection Microbiology, 6, 190. https://doi.org/10.3389/fcimb.2016.00190

Yawo, H., Kandori, H., Koizumi, A., & Kageyama, R. (Eds.). (2021). Optogenetics: Light-sensing proteins and their applications in neuroscience and beyond. Springer. https://doi.org/10.1007/978-981-15-8763-4