

# Prospection of Bioactive Compounds Produced by Bacterial Isolates from Caves: Antioxidant and Antibacterial Activities

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**Keywords:** Antibacterial, Antioxidant, Bacteria, Biotechnology, Caves, Sustainability.

## Introduction

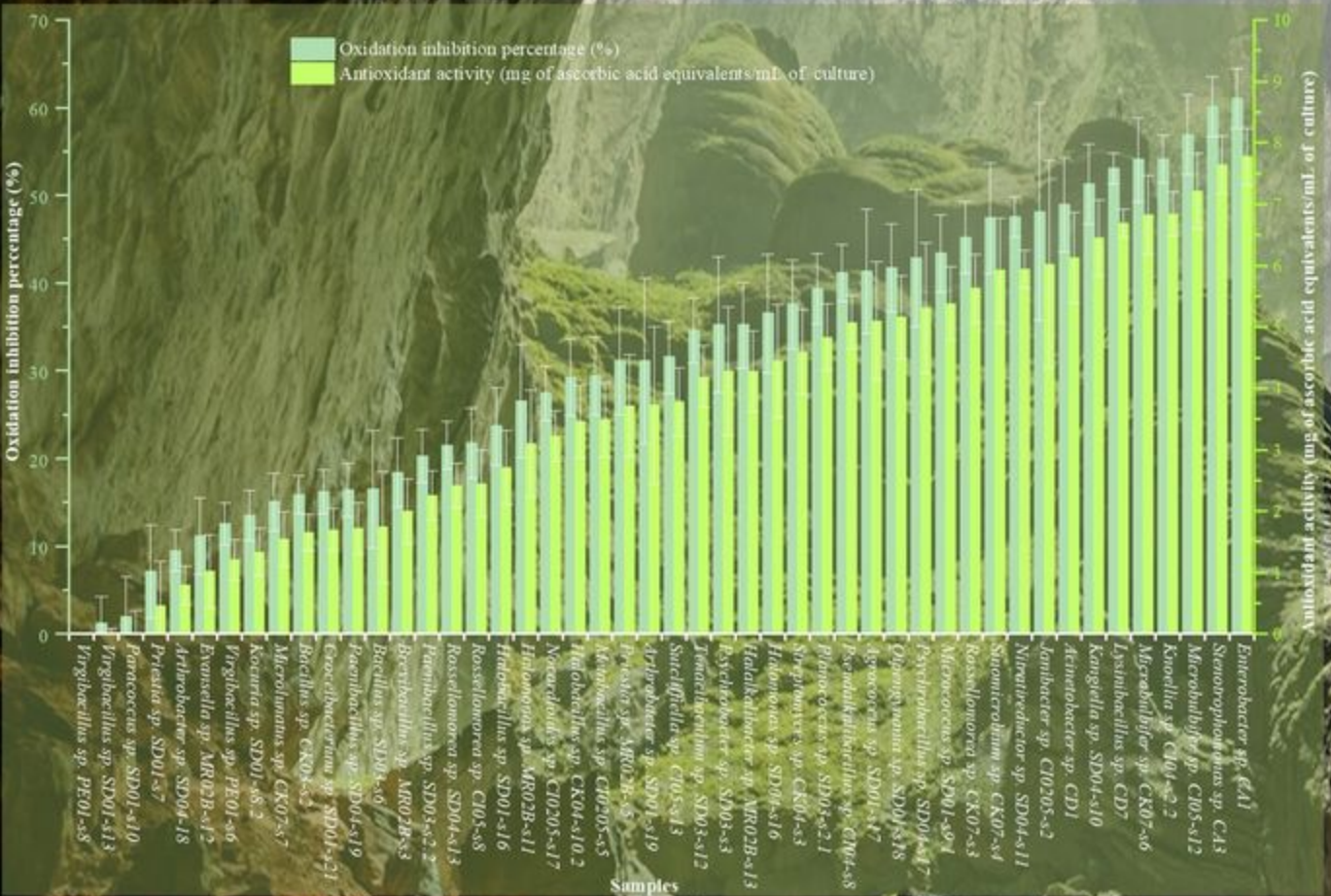
Caves, natural geological formations resulting from rock cavities with adverse abiotic conditions, are considered extreme and inhospitable habitats. This further emphasizes the remarkable adaptability of microorganisms that manage to inhabit them. These unique environmental characteristics enable microorganisms to develop specific metabolisms and produce new bioactive compounds with potential activities, such as antimicrobial and antioxidant properties [1]. This research aims to evaluate the antioxidant and antibacterial activity against Gram-negative and Gram-positive of diluted lyophilised extracts produced by strains isolated from pristine environments such as 3 caves on Selvagem Grande Island (Madeira archipelago, Portugal), 2 caves on Lanzarote Island (Canary archipelago, Spain) and the Paleolithic Escoural Cave (Montemor-o-Novo, Portugal [2]). The results obtained suggest that the selected bacterial isolates produce biologically active compounds that have the potential to serve as viable alternatives to conventional antibiotics or as antioxidants. These findings have wide-ranging implications for health and well-being, covering areas such as nutrition, pharmacology, cosmetics, and even the culinary sector.

## Methodology

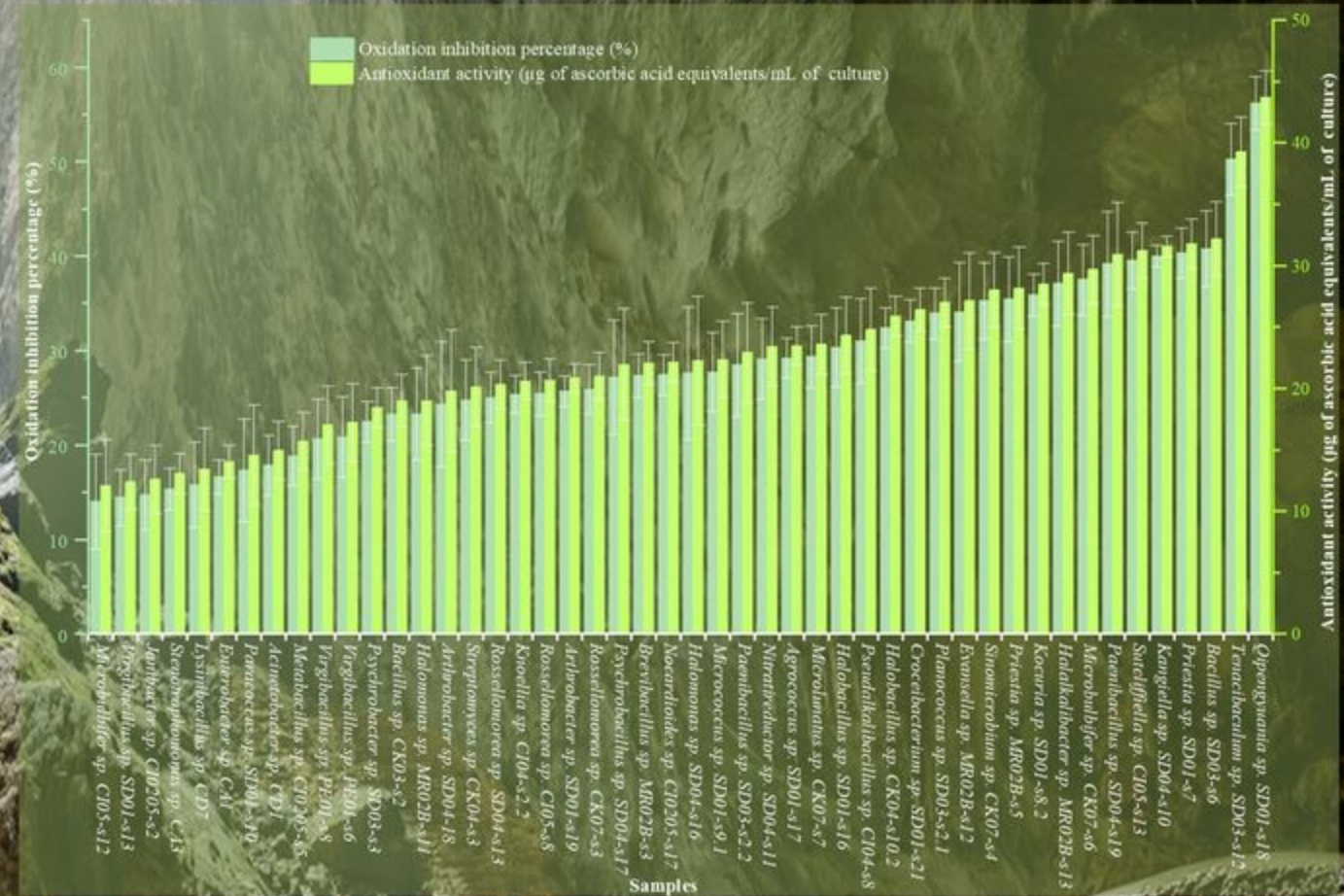


## Results

### Lipidic peroxidation inhibition



### Free radical scavenging potential



### Antibacterial activity in liquid media



### Antibacterial activity by paper disc diffusion method

| Samples                           | Pathogenic bacteria and Bacterial inhibition zones (mm) |                       |                |                      |
|-----------------------------------|---|-----------------------|----------------|----------------------|
|                                   | <i>S. aureus</i>  | <i>S. epidermidis</i> | <i>E. coli</i> | <i>P. aeruginosa</i> |
| <i>Brevibacillus</i> sp. MR02B-s3 | 6.50±0.50   | n.d.                  | 7.60±1.53      | 8.35±0.22            |
| <i>Evansella</i> sp. MR02B-s12    | n.d.  | n.d.                  | 7.10±1.05      | n.d.                 |
| <i>Bacillus</i> sp. CK03-s2       | n.d.  | 12.17±2.72            | n.d.           | n.d.                 |
| <i>Streptomyces</i> sp. CK04-s3   | n.d.  | 8.03±1.95             | n.d.           | n.d.                 |
| <i>Janibacter</i> sp. CI0205-s2   | n.d.  | 7.60±2.08             | n.d.           | n.d.                 |
| <i>Agrococcus</i> sp. SD01-s17    | 11.00±1.32  | n.d.                  | n.d.           | 10.83±0.29           |

Mean values from 3 tests. SD are demonstrated, n.d. – not determined.



## Remarks

- The extracts of the isolates *Enterobacter* sp. 1, *Stenotrophomonas* sp. CA3, *Microbulbifer* sp. CI05-s12, *Knoellia* sp. CI04-s2.2, *Microbulbifer* sp. CK07-s6, *Lysinibacillus* sp. CD7, *Kangiella* sp. SD04-s10, *Qipengyuania* sp. SD01-s18 and *Tenacibaculum* sp. SD03-s12 showed an antioxidant activity greater than 50% inhibition.
- In the future, it would be interesting to carry out a dose-response tests for antioxidant activity.
- Some extracts showed a high inhibition of the growth of pathogenic bacteria, with prominence in *Brevibacillus* sp. MR02B-s3, which showed activity against *Staphylococcus epidermidis*, *Staphylococcus aureus* and *Escherichia coli* and *Agrococcus* sp. SD01-s17 extract which inhibited the growth of *Staphylococcus epidermidis* and *Staphylococcus aureus* during the 24h assay.

**References:**  
 [1] P. Gatinho, C. Salvador, A.M. Silva, A.T. Caldeira, Prokaryotic Communities from Pristine Cave Environments: Biotechnological Potential with Sustainable Production, (2023) 1–22. <https://doi.org/10.3390/su15097471>.  
 [2] A.T. Caldeira, N. Schiavon, G. Mauran, C. Salvador, T. Rosado, J. Mirão, A. Candeias, On the biodiversity and bioterogenic activity of microbial communities present in the hypogenic environment of the Escoural Cave, Alentejo, Portugal, *Coatings*, 11 (2021) 1–17. <https://doi.org/10.3390/coatings11020209>.

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