Biotechnological production of new Green Biocides for application in Cultural Heritage

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Background

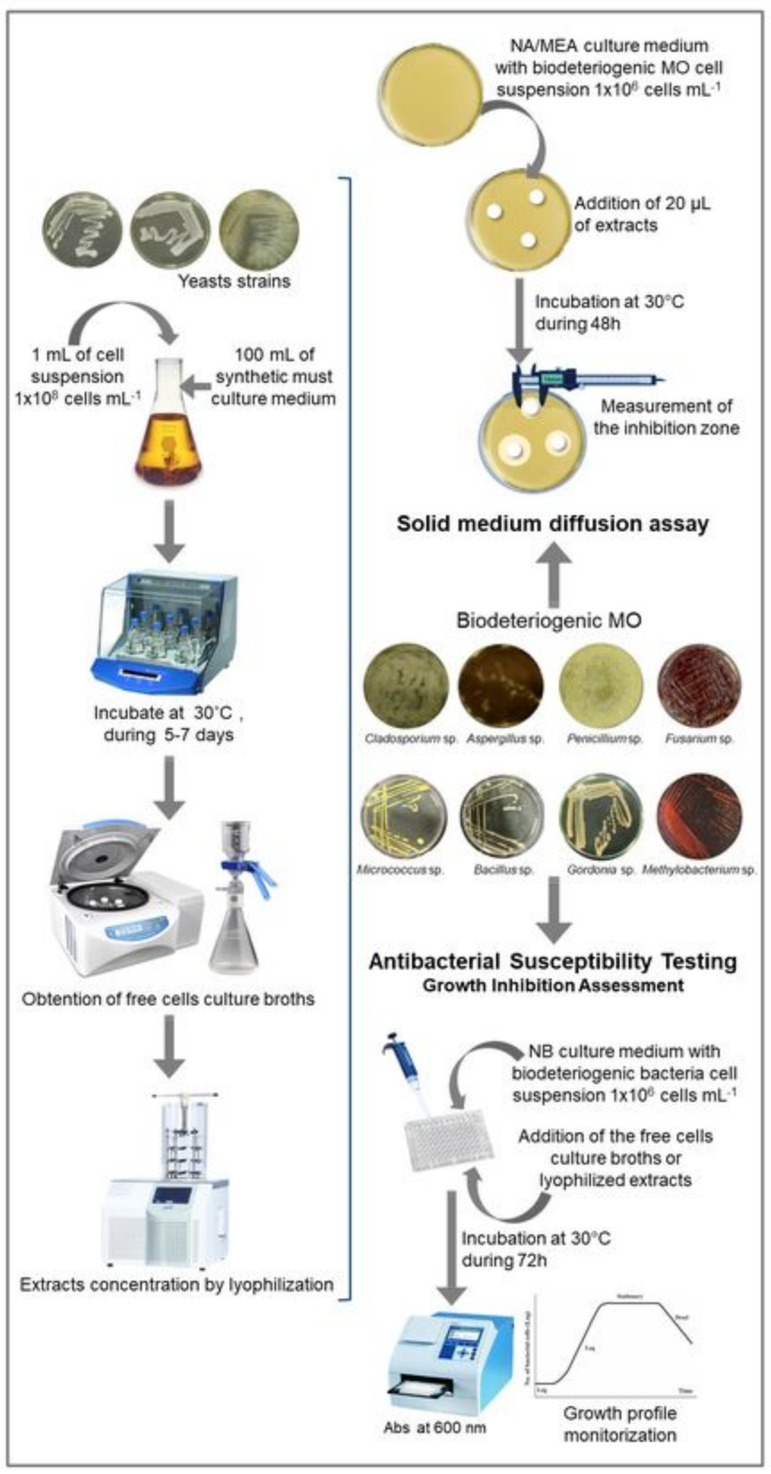
Biodeterioration phenomenon has been considered a high priority issue in the context of Cultural Heritage safeguarding, highlighting the need for innovation in this area. In many cases, synthetic polymers are used in an attempt to control the biodeterioration of heritage, but they exhibit drawbacks such as high toxicity, their effect is not long-lasting, and many microorganisms quickly develop resistance to these products [1]. Instead of using conventional products, biotechnological methods have emerged as an alternative to produce environmentally friendly biocides. These bioactive molecules are synthesized by microorganisms as a defense mechanism, often referred to as killer toxins. Once these biocompounds are produced and purified, they can be used to control microbiological proliferation in heritage assets. These biocides should offer more effective and sustainable alternatives while being safe for human health and the environment without negative impact on assets [2].

This work was carried out within the scope of the ART3*mis* Project (2022.07303.PTDC) with the aim of producing killer toxins from yeast strains and evaluating their antimicrobial activity against different species of biodeteriogenic microorganisms isolated from Cultural Heritage.

The range of antimicrobial effects exhibited by the metabolites generated from specific killer yeast strains was assessed in both solid and liquid growth media, targeting bacterial strains and also tested in solid medium against biodeteriogenic fungi.

Methodology

Biocides production and Antimicrobial tests

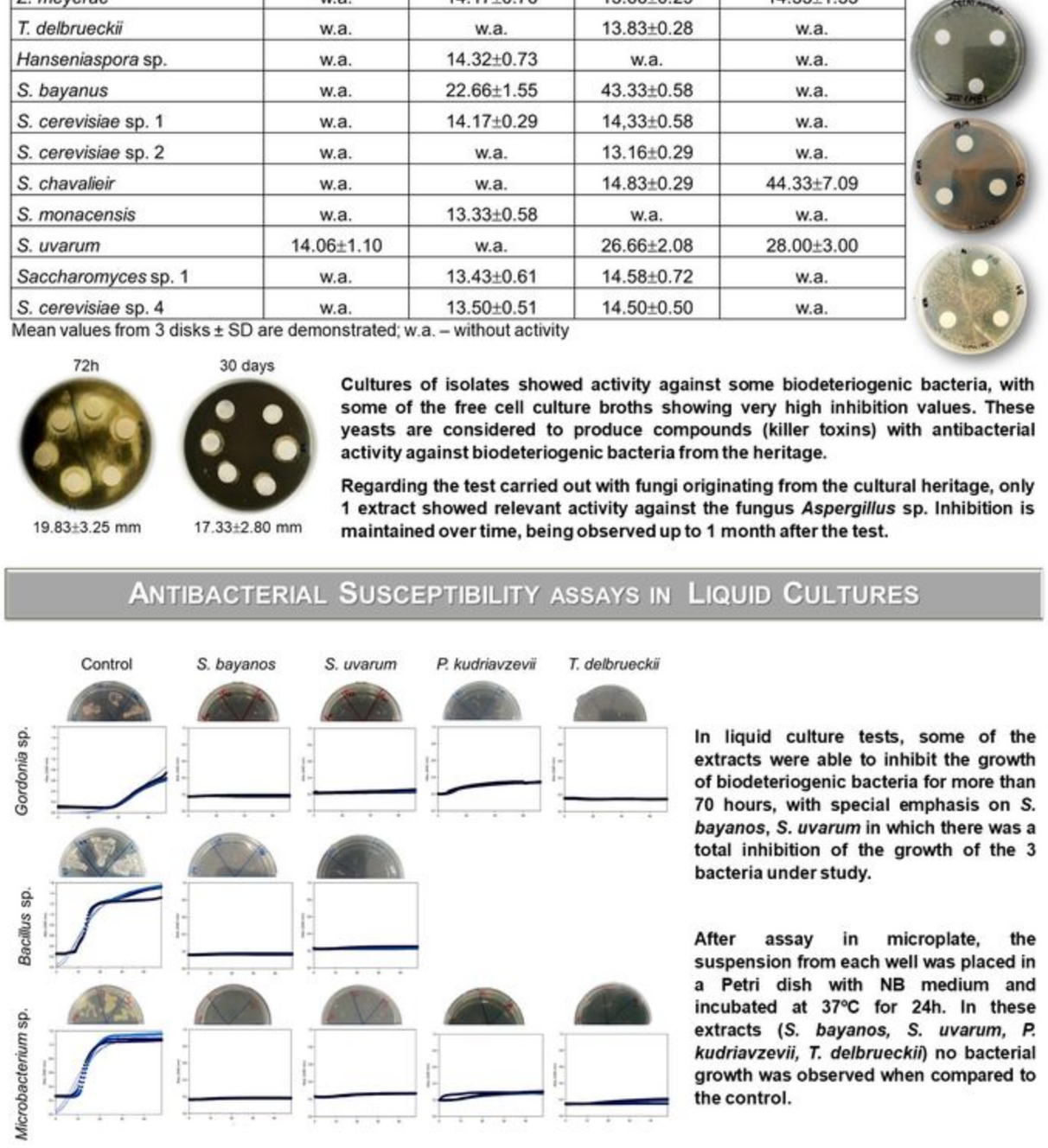


Results

Screening of Antimicrobial Activity

Samples	Biodeteriogenic bacteria and Bacterial inhibition zones (mm)			
	Bacillus sp.	*Microbacterium sp.*	*Gordonia sp.*	*Methylobacterium sp.*
P. kudriavzevii	w.a.	13.83±0.58	14.23±0.40	w.a.
Z. meyerae	w.a.	14.17±0.76	13.66±0.29	14.33±1.53
T. delbrueckii	w.a.	w.a.	13.83±0.28	w.a.
Hanseniaspora sp.	w.a.	14.32±0.73	w.a.	w.a.
S. bayanus	w.a.	22.66±1.55	43.33±0.58	w.a.
S. cerevisiae sp. 1	w.a.	14.17±0.29	14,33±0.58	w.a.
S. cerevisiae sp. 2	w.a.	w.a.	13.16±0.29	w.a.
S. chavalieir	w.a.	w.a.	14.83±0.29	44.33±7.09
S. monacensis	w.a.	13.33±0.58	w.a.	w.a.
S. uvarum	14.06±1.10	w.a.	26.66±2.08	28.00±3.00
Saccharomyces sp. 1	w.a.	13.43±0.61	14.58±0.72	w.a.
S. cerevisiae sp. 4	w.a.	13.50±0.51	14.50±0.50	w.a.

Mean values from 3 disks ± SD are demonstrated; w.a. – without activity



Cultures of isolates showed activity against some biodeteriogenic bacteria, with some of the free cell culture broths showing very high inhibition values. These yeasts are considered to produce compounds (killer toxins) with antibacterial activity against biodeteriogenic bacteria from the heritage.

Regarding the test carried out with fungi originating from the cultural heritage, only 1 extract showed relevant activity against the fungus *Aspergillus* sp. Inhibition is maintained over time, being observed up to 1 month after the test.

Antibacterial Susceptibility assays in Liquid Cultures

In liquid culture tests, some of the extracts were able to inhibit the growth of biodeteriogenic bacteria for more than 70 hours, with special emphasis on *S. bayanos*, *S. uvarum* in which there was a total inhibition of the growth of the 3 bacteria under study.

After assay in microplate, the suspension from each well was placed in a Petri dish with NB medium and incubated at 37°C for 24h. In these extracts (*S. bayanos, S. uvarum, P. kudriavzevii, T. delbrueckii*) no bacterial growth was observed when compared to the control.

Remarks

- **The results achieved are encouraging regarding the development of new ecological biocides that can effectively suppress the biodeteriogenic action of a wide range of microorganisms commonly found in different Cultural Heritage materials.**
- **These outcomes pave the way for the implementation of novel, green, safe, and sustainable solutions derived from fast and cost-effective biotechnological processes.**

Keywords

Patrimony Biodeterioration
Killer Toxins
Green Biocides
Antimicrobial Potential
Sustainable Biotechnology

References

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