

ANTIMICROBIAL POTENTIAL OF MICROORGANISMS AFTER LYOPHILIZED STORAGE

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Species belonging to the fungal genera *Trichoderma* and *Penicillium*, as well as bacteria of the genus *Bacillus*, are recognized producers of bioactive metabolites with antifungal and antibacterial properties that have extensive applications in sustainable agriculture and biological plant protection.

Aim: To evaluate the antimicrobial activity of selected microbial strains after six months of storage in a lyophilized state using different protective media.

Materials and Methods. Four fungal strains (*Penicillium* sp. 1, *Penicillium* sp. 6, *Trichoderma* sp. 3, and *Trichoderma* sp. 14) and one bacterial strain (*Bacillus* sp. 13), previously identified as exhibiting high antimicrobial activity against a broad spectrum of phytopathogens, were used in this study. Antimicrobial activity was assessed prior to lyophilization and re-evaluated after six months of storage in a lyophilized state. Various protective media based on skim milk supplemented with glucose or sucrose at different concentrations and 0.01% ascorbic acid were used during the lyophilization process.

Antimicrobial activity was determined using the agar diffusion method, and inhibition zones were measured against selected phytopathogenic microorganisms. Test cultures included the fungal phytopathogens *Alternaria alternata*, *Botrytis cinerea*, *Fusarium solani*, and *Fusarium oxysporum*, as well as the phytopathogenic bacteria *Agrobacterium tumefaciens*, *Corynebacterium michiganensis*, *Erwinia carotovora*, and *Xanthomonas campestris*. Nutrient agar was used for bacterial cultures, while malt agar was used for fungal cultures.

Results. Screening performed prior to lyophilization identified *Penicillium* sp. 1, *Penicillium* sp. 6, *Trichoderma* sp. 3, and *Trichoderma* sp. 14 as the most active strains, producing inhibition zones of 20–34 mm against phytopathogenic fungi and 15–20 mm against phytopathogenic bacteria.

After six months of lyophilized storage, *Penicillium* strains retained substantial antimicrobial activity, exhibiting inhibition zones of 20–27 mm against phytopathogenic fungi and 12–16 mm against phytopathogenic bacteria, depending on the protective medium used. Similarly, *Trichoderma* strains maintained inhibition zones of 20–32 mm against phytopathogenic fungi and 12–15 mm against phytopathogenic bacteria.

The *Bacillus* sp. 13 strain also preserved its antagonistic properties after six months of storage in a lyophilized state. The strain exhibited activity against three phytopathogenic fungi (*A. alternata*, *F. solani*, and *F. oxysporum*), with inhibition zones of 18–32 mm depending on the protective medium used. Furthermore, *Bacillus* sp. 13 demonstrated inhibition zones of 15–20 mm against the phytopathogenic bacteria *A. tumefaciens* and *C. michiganensis*.

Conclusions. The results demonstrate that lyophilization is an effective and reliable method for preserving microorganisms of biotechnological importance, ensuring the maintenance of their antimicrobial activity during long-term storage. The protective media tested provided adequate preservation of the antagonistic properties of the investigated strains.