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Study of the anti-sapstain fungus activity of *Bacillus amyloliquefaciens* CGMCC 5569 associated with *Ginkgo biloba* and identification of its active components

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ABSTRACT

An endophytic bacterium, designated strain *Bacillus amyloliquefaciens* CGMCC 5569 was isolated from Chinese medicinal *Ginkgo biloba* collected from Xuzhou, China. Both the filtrate and the ethyl acetate extract of strain CGMCC 5569 showed growth inhibition activity against the sapstain fungi *Lasiodiplodia rubropurpurea*, *L. crassispora*, and *L. theobromae* obviously (>65%) based on the comparison of the length of zones on the petri dish. From the ethyl acetate extract of the filtrate, the antifungal compounds were obtained as a series of lipopeptides, which including series of fengycin, surfactin and bacillomycin. It showed strong growth inhibition activity *in vitro* against the *L. rubropurpurea*, *L. crassispora* and *L. theobromae* by about 70.22%, 69.53% and 78.76%, respectively. The strong anti-sapstain fungus activity indicated that the endophytic *B. amyloliquefaciens* CGMCC 5569 and its bioactive components might provide an alternative bio-resource for the bio-control of sapstain.

1. Introduction

The concept of biological control as a method for protecting crops and other perishable commodities has received increased attention from the scientific research community in recent years. This is a consequence, in part, of the increasing awareness of both industry and the general public of the environmental impact of chemicals used for crop protection and preservation purposes. Wood discoloration is a complex biological process that can involve a wide variety of microorganisms, often interacting with one another, and influenced by the changing environmental conditions under which the wood is placed. Sapstain is a major problem for timber producers as well as pulp and paper manufacturers since fungi colonization and disfigurement of freshly fell materials prior to drying can result in significant economic losses (Bruce et al., 2003). Although the sapstain fungi cause little or no significant damage to the structure elements of the timber, they have a detrimental effect on the aesthetic value of the wood due to the colonization by their pigmented mycelia. It causes significant economic losses to the China lumber industry recently. Control of sapstain is normally achieved through rapid air or kiln drying of the lumber or through the use of diffusible chemical preservatives. At present, chemical control remains the main measure to reduce the wood discoloration (Yang, 2005); however, it may pose significant risks to environment and public health.

Significant advances in control the disease and stain fungi have been achieved both in research and application by the use of bio-control microorganisms, like bacteria and actinomycetes. Bacteria have been commercialized and used in controlling crop diseases, such as *Bacillus subtilis*, *B. polymyxa*, *B. pumilus*, *B. amyloliquefaciens*, *B. cereus*, and *B. licheniformis*. Recently, *Bacillus* species have been used widely as bio-control agents (Chen et al., 2009; Zhao et al., 2010). *Bacillus* spp. can produce structurally diverse secondary metabolites with a wide spectrum of antifungal activity. Several strains of *B. subtilis* and *B. amyloliquefaciens* have been found to produce lipopeptides, and these bioactive lipopeptides showed a great potential for biotechnological, biopharmaceutical and agricultural applications (Schallmey et al., 2004).

Many of these antifungal substances have been identified, including mycobacillin, iturin, bacillomycin, surfactin, mycosubtillin, fungi-stain, subsporins and rhizocticins (Fiddaman and Rossall, 1993; Kunst et al., 1997; Touré et al., 2004; Stein, 2005; Liu et al., 2010). These compounds, made of amino acids and a fatty acid, are easily biodegradable in the soils (Cho et al., 2003). Considerable interest lies in using *Bacillus* producing lipopetide antibiotics, such as iturin A and surfactin as biocontrol agents due to their antagonistic and repressive activities against plant pathogens. These amphiphilic cyclic biosurfactants have many advantages over other fungicides: low toxicity, high biodegradability and environmentally friendly characteristics (Kim et al., 2004). The endophytic

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