

# 桿菌屬臨床分離菌株抗真菌作用之分析

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## ABSTRACT

The object of this study is to examine 34 clinical isolates of *Bacillus* spp. from blood of pseudo bacteremia patients in Linkou Chang Gung Memorial Hospital, focusing on species classification, antifungal activity, and identification of antifungal gene and active components. Bacterial classification was based on phenotyping (catalase test, reduction of nitrate, hemolysis test, indole production test, citrate utilization test, urease test, Voges-Proskauer test) and genotyping (polymerase chain reaction). All 34 clinical isolates of *Bacillus* spp. belonged to 8 species: *B. cereus*, *B. thuringiensis*, *B. coagulans*, *B. licheniformis*, *B. pumilus*, *B. megaterium*, *B. circulans* and *B. firmus*. From the results of antifungal analysis, *B. thuringiensis* CG 2, *B. thuringiensis* CG 4, *B. pumilus* CG 12, *B. cereus* CG 15, *B. cereus* CG 20 and *B. cereus* CG 26 exhibited significant antifungal activities. These six isolates were further analysed for their antifungal mechanism on *Paecilomyces variotii* T??37. It ' s well known that *Bacillus subtilis* F29-3 produces one antibiotic--fengycin, which effectively inhibits the growth of filamentous fungi. According to the sequence of *fenB*, 3 PCR primer sets were designed to analyze whether any of these 34 clinical isolates of *Bacillus* spp. contained *fenB* gene of fengycin. With one primer set, a 1.0 Kb fragment can be amplified in all 6 isolates with antifungal activity. The sequence of the PCR product showed high homology to *ilvD*. Whether *ilvD* is related to the antifungal activity remained to be elucidated. Furthermore, to identify the active components contributing to the antifungal activity, the fermentation supernatant were obtained from 6 isolates with antifungal activity and *B. subtilis* F29-3. After filtration, the supernatants showed no antifungal activity to *Paecilomyces variotii* T??37. It appeared that only viable bacteria expressed antifungal activity.

Keywords : *Bacillus* spp., fengycin, antifungal activity

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