
Isolation and identification of 4 *Bacillus* spp. isolated from the local biofertilizer and its antimicrobial activity test.

Key words: Biofertilizer, 16s rRNA, sequencing, antibiotics.

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Level of study : Master of Science
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Academic Year : 2010

Abstract

Biofertilizer is an alternative to solve the risk of chemical fertilizer. Biofertilizer usually not only provide the plants with nutrients but also numbers of beneficial microorganisms. *Bacillus* species can also be found in biofertilizer. Inoculation of *B. cereus* can promote the plant roots growth by its antibiotics ability and phosphate solubilization. This experiment, *Bacillus* spp. were isolated from locally produced biofertilizer and the concentrate. *B. subtilis* and *B. megaterium* candidates were identified followed the guideline from Bergey's manual. 55 colonies were isolated from biofertilizer, the concentrate and the soil, 24 strains were *Bacillus* spp. candidates. Four of these candidates were selected for sequencing and identification using 16s rDNA, there were No.12, No.14, No.17 and No.25. According to the 16s rDNA sequence of the four selected *Bacillus* spp. (No. 12, 14, 17 and 25), their 16s rDNA showed high similarity to *Bacillus cereus* and *B. megaterium*. All four candidates were subjected to antibiotics production test by growing in nutrient broth, 10% of nutrient broth, SS media, minimal media and improved media for production of antibiotics (supplemented with methionine). The antibiotic sensitivity tests were conducted against seven bacteria, a yeast and two filamentous fungi, which include *Escherichia coli*, *Salmonella* sp., *Staphylococcus aureus*, *Saccharomyces cerevisiae*, *B. cereus*, *B. subtilis*, *Pseudomonas syringae* pv. *sesame*, *Trichoderma reesei* and *Colletotrichum gloeosporioides*. The improved media supplemented with methionine was the only media that showed antimicrobial activity. Depend on the antibiotics concentration, broth from all four candidates at 24h, 48h and 72h showed inhibition effect on the growth of *E. coli*, *Salmonella* sp., and *S. aureus*. The 48h and 72h extract broth could inhibit *P. syringae* pv. *sesame* growth. The antibiotics supernatant of No.17 and No.25 extracted at 48h showed limited inhibition on *C. gloeosporioides* growth rate. All four candidates' extract broths could not inhibit growth of *B. subtilis*, *B. cereus*, *S. cerevisiae* and *T. reesei*. The antimicrobial activity against *E. coli*, *Salmonella* sp. and *S. aureus* increased within 3 days and the 72th hours yielded the best result.