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ORIGINAL ARTICLE

Chitinolytic enzyme production and genetic improvement of a new isolate belonging to *Streptomyces anulatus*

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Abstract Thirty bacterial isolates were obtained from different sources and sites at Jeddah, Saudi Arabia, on chitin agar medium; 9 of the 30 isolates were cultured in liquid medium containing chitin as sole carbon and nitrogen sources. Isolate SM21, which was isolated from shrimp shells, showed the best growth and chitinase production in liquid medium. According to its morphological, physiological and biochemical characteristics, SM21 belongs to the genus *Streptomyces* and was identified as *Streptomyces anulatus* SM21. Identification was confirmed using 16S rDNA analysis. The chitinase enzyme was precipitated with 80% NH_4SO_4 and purified using DEAE-cellulose ion exchange chromatography followed by Sephadex G-100 gel filtration. The molecular weight determined using sodium dodecyl sulfate polyacrylamide gel electrophoresis was 28 kDa. Genetic improvement using the protoplast fusion technique was carried out between the identified *Streptomyces* isolate and *Streptomyces coelicolor* SM1. These two species, which have different resistance profiles to streptomycin and tetracycline (400 μg/ml and 10 μg/ml, respectively), were used in an intraspecific protoplast fusion using PEG 6000. The percentage of real protoplasts that could regenerate successfully was 71% for *S. coelicolor* SM1 and 80% for *S. anulatus* SM21. Out of three recombinant fusants obtained, one (named Fu3) showed higher chitinase production compared to both parents (5 fold increase).

Keywords Chitinase · *Streptomyces* · 16S rDNA · Protoplast fusion · Chitin · Molecular weight

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Introduction

Chitin is a major structural component of fungi and the exoskeleton of insects, crustaceans and other arthropods. It occurs as an insoluble unbranched linear chain of β-1,4-linked N-acetyl D-glucosamine residues. It is the second most abundant renewable carbohydrate polymer in nature after cellulose (Gooday 1990; Madigan and Martinko 2006) and possibly the most abundant in marine environments. In oceans, insoluble chitin is an important nutrient source for maintaining the ecosystem in the marine environment. A wide range of microorganisms have the ability to degrade chitin by producing chitinases for nutrition, antagonism and combating parasites (Faramarzi et al. 2009). Marine bacteria, *Vibrio* (Keyhani et al. 2000), *Bacillus*, *Clostridium* (Konagaya et al. 2006) and *Micromonospora* (Nawani et al. 2002) can degrade chitin to chito-oligosaccharides, which can be metabolized and further used by others microorganisms as a sole source of nitrogen and carbon. Actinomycetes are rich sources of chitinase, which can be induced by the presence of chitin in the cultivation medium (Narayana and Vijayalakshmi 2009). It has been suggested that chitin hydrolysis by such microorganisms plays a pivotal role in the bioconversion of chitin and marine

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