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Effect of nitrogen sources and fermentation conditions on bacillus sp. R2 chitinase production

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Abstract

The nutritional and environmental conditions have a great influence on chitinase production, due to the receptor-inducer system that control enzymes production.in these sense, the aim of this study was to screen several nitrogen sources and optimize the most important fermentation conditions affecting Bacillus sp. R2 chitinase production. The results of one variable at time technique (OVAT) in shake flasks, revealed that among various nitrogen sources tested 0.5% yeast extract led to maximum production. Furthermore, the highest chitinase activity was detected after 24 h incubation period at temperature of 30C, initial pH: 7.5, 2.5 to 3% NaCl concentration and under 180 rpm shaking using 2.5% (8.9 × 108 CFU/ml) as best inoculum size. These optimizations can reduce the cost of chitinase production for the large scale specially

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1. Introduction

Chitin is the second most abundant biodegradable polymer, which exists naturally in the biosphere as a structural polysaccharide of β-1,4-N-acetyl-D-glucosamine. Chitin can be found as part of fungi, plants, crustaceans, insects, arthropods, and algae components [1]. Chitinases (E.C.3.2.1.14), is an important class of glycosyl hydrolases that play a key role in chitin decomposition and utilization as a renewable resource. these enzymes produced by a wide range of organisms including bacteria, actinomycetes, yeasts, fungi, plants, animals and human beings [2-3]. Bacteria produce chitinolytic enzymes for the assimilation of chitinous materials as carbon and nitrogen sources.

In microorganisms, chitinase production is controlled by a receptor-inducer system; therefore, the composition of the culture medium and fermentation conditions can affect significantly chitinase production [4].

During the last decade, Chitinases have drawn much attention in recent years due to their potential biotechnological applications . Beside the enormous application of chitinases in various fields, their commercial production and scale up is of critical importance, and are noticeably influenced by medium components and environmental factors [5,6,7], for this raison, the medium optimization studies and searching for chitinases production key factors, still an urgent need to maximize the production and meet the industrial demands.

In the previous studies, Bacillus sp. R2 was screened as hyper chitinase producer and the effect of Carbone sources on chitinase production was reported [8]. In the present work, the effect of nitrogen sources and fermentation conditions of chitinase production will be investigated using one variable at time technique (OVAT) in shake flasks.

2. Material and Methods

2.1. *Chemicals*

Chitin was extracted from crustaceans and squid by the method of (Synowiecki *et al*. 1982) [9], Crab shell chitin flakes (Win-lab, UK). Swollen chitin was prepared according to Monreal and Reese, (1969) [10]. Peptone tryptone, and yeast extract were obtained from (Oxöid Hampshire, England). N-acetyl glucosamine, and bovine serum albumin (BSA) were from (Sigma -USA), 2 Hydroxy 3,5 dinitrosalselic acid (DNSA) obtained from (Merck, Darmstadt- Germany). All other chemicals and reagents that were used were of highest grade commercially available.

2.2. bacterial strain cultivation and maintenance

Bacillus sp. R2 marine bacterial strain isolated from red sea Egypt and identified biochemically and molecularly by cheba et al 2006 (strain accession number in NCBI GenBank was: DQ923161). LB broth and peptone yeast agar medium(PYA) were used for cultivation. To maintain the isolated bacterial cells, the long-term maintenance was performed by adding 0.5 ml of the early stationary phase cultures grown in marine LB to 50% (v/v) sterile glycerol and the cultures were kept at-20°C.

2.3. Effect of nitrogen source on chitinase production

Sea water (SW) + 0.5 % colloidal chitin + 0.5 % glucose medium was supplemented separately with 0.5 % of one of the following inorganic and organic nitrogen sources: NH4Cl, NH4SO4, urea, tryptone, peptone, yeast extract and 0.25 % peptone + 0.25 % yeast extract. after 24 h incubation at temperature of 30C, chitinase activity was determined.