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ISOLATION, IDENTIFICATION AND PRODUCTION OF ENDOGLUCANASE FROM *BACILLUS SUBTILIS* STRAINS USING CARBON AND NITROGEN NATURAL SOURCES

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ABSTRACT : This study were isolated and identified locally in the province of Thi-Qar, Iraq. These strains have shown their ability to produce of Enzyme Endo-β-1, 4-glucanase was determined after growing on carboxymethyl cellulose CMC medium. The ability of isolates was detected in the cellulose analysis after using the stain of Congo red and NaCl solution.The isolates were identified using Morphological, biochemical testsas VITEK2 and Molecular biology 16S rRNA tests.The gene sequence was compared with data available Gen Bank, NCBI data showed that they were new strains of *B. subtilis* strain M3 (MF455196) , *B. subtilis* strain M4 (MF480418), *B. subtilis* strain M5(MF480424) and *B. subtilis* strain M6(MF480420) bacteria. Isolates were recorded in NCBI GenBank and were design for each evolutionary tree isolation. Endo-β-1, 4-glucanase was produced and the culture conditions like temperature, pH, and Incubation time,Incubator Shake and medium components like Carbon sources, nitrogen sources and role of natural substrates were optimized. Enzyme activity was measured using of dinitro salicylic acid DNS detector and spectrophotometer at 540 nm.

Key words **:** *B. subtilis strain*, Produce Endo-b-1, 4-glucanase, carbon source , nitrogen source, DNS reagent .

INTRODUCTION

These microorganisms are consisting of a large variety of and gram-positive (only positive in the early stages of growth), as a well motile rod by peritrichous flagella. They are usually found in the soil and surrounded endospore, oval, elliptical or cylindrical shape, which is resistant to negative environmental conditions and is a survival characteristic for this type which allows it to resist harsh temperatures and dry environments ,Microaerophilic in some time, anaerobically when nitrate, glucose, and oxygen is absent depending on Fermentation (Promon *et al*, 2015).Excellence *B. subtilis* producing a variety of extracellular enzymes that are important chemicals in industrial applications (Thaz *et al*, 2015). These are important enzymes with stable and limited activity cellulase enzyme (Vimal *et al*, 2016).

Cellulose is a component part plant cell walls consisting of linear chains and dense Carbohydrate polymers consists of D-glucose residues connected by β-1, 4glucosidic linkages (Karmakar *et al*, 2011).The cellulase enzyme is responsible for breaking the glycosidic bonds between the cellulose and classified into Endoglucanases, Exoglucanases and cellobiase (Yang *et al*, 2014; Guan *et al*, 2017).The objective of our study was to isolate and diagnose *B. subtilis* producing an Endoglucanases using cheap natural sources as alternatives source to reduce to costly carbon cost of and nitrogen sources and benefiting from biological treatment of agricultural and industrial waste.

MATERIAL AND METHODS

Bacteria isolation

Samples were collected from different soils of Thi-Qarprovince and took at depth of 3-9 cm and were collected 100 g from each different site to 1g of the sample, and made four a serial dilution, then 100 ìL of each dilution was spread on carboxymethyl cellulose agar CMC plates were Incubated for 48 hours at 37°C (Chelab and Faisal, 2016).

Screening of Bacteria

After incubation,growingcolonies were flooded with 1% Congo red for 15 minutes at room temperature, Appeared clear zones of hydrolysis around the colonies was indicated cellulose analysis in the nutrient plates, clear zones varied among strain of isolated bacteria. The required isolates were purified and stored at 4 °C.

Identification of Bacteria

Morphological characteristics were used to identify isolates,optical microscopy, electron microscopy and