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Effect of temperature and mutation on serratiopeptidase secreted from *Serratia marcesence*

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Abstract

A total of 44 blood samples obtained from patients infected with septicemia in Baghdad Hospital and the duration of study the two months (April and May) the 15 isolates of *Serratia marcesence* were identified biochemically test and Epi20 analysis. The ability of isolates to hydrolysis of casein were tested in 28, 32, 37 and 40^{o}C temperature during 24 and 48hrs. All isolated gave hydrolysis of casein except 1 and 2 isolates gave a hazy zone and the optimal temp that gave a maximum hydrolysis of casein was 32^{o}C after exposure this isolates to different time of mutation 20, 40, 60sec. UV light all isolates gave hydrolysis of casein except 1 and 2 isolate gave only hazy zone. The maximum hydrolysis of casein was in (20sec.)of mutation in 32^{o}C. The aim of this study to know the optimal temperature and mutation that gave increasing of serratiopeptidase production because the (SRP) was a proteolytic enzyme with wide application in the medical.

Keywords: *Serratia marcesence*, Isolates, Temprature, Mutation.

Introduction

Serratiopeptidase or serrapeptase or serralysin is a proteolytic enzyme that has been widely used successfully for pain and inflammation (Kakinumu, A. 1982; Selan *et al.,* 1993) due to arthritis (Matsudo *et al.,* 1981) trauma, surgery sinusitis, bronchitis (Nakamura *et al.,* 2003). Carpal tunnel and painful, swelling of the breasts. There is some preliminary indication that it may be useful for arthrosclerosis. This enzyme is absorbed through the intestine an transported directly into the blood stream. SRP is known to be produce extracellular in submerged and solid state fermentation by the bacteria. *Serratia marcesence* strain was grown in production media and incubated in different incubation temperature 28, 32, 37 and 40^{o}C respectively. *Serratia marcesence* strain was UV mutated and maximum SRP producing selected by its caseinolytic of property. After examined of casein hydrolysis, plates were left at room temperature for another 24hrs (Mohankumar *et al.,* 2011; William and Joseoh, 1972). Culture media especially the organic nitrogen source and various studies have reported (Satpal *et al.,* 2011; Debajit *et al.,* 2012) there are no reports in the production of SRP by using mutant strain of *Serratia marcesence* (Jin-li *et al.,* 2005) mutant were selected on the basis of zone of proteolysis on skim milk agar plates (William, 1978; Ka-Man, 2005).

Materials and Methods

Microorganism: 15 strains of *Serratia marcesence* isolated from blood of patient with septicemia in Baghdad Hospital duration from two months (April and May) this isolates were identified biochemically tests and Epi20 analysis.

Incubation temperature: *Serratia marcesence* was grown in production media incubated at different incubation temperature (28, 32, 37 and 40^{o}C) respectively. This study occur on the basis of zone of proteolysis on skim milk agar plates (24and 48hrs). All strains were seeded on the skim milk agar by pointed inoculation (William and Joseoh, 1972).

UV mutation: All strains was used for mutation was first grown on the nutrient agar plate for 24hrs (William and Joseoh, 1972; Jin-li *et al.,* 2005). Cell were scraped and suspended in sterile saline and then diluted to concentration range 10^{5}-10^{7} cell/ml. under sterile condition the dilution cell were exposed to ultra violate rays of 15W ultra violate lamp for 20sec with mild agitation at a distance of 30cm to give a survival rate of about 15%.

Production medium: In batch production, the medium reported by Pansuriya *et al.* (2011) was used which contained maltose (45g/l), soybean meal (65g/l), KH2PO4 (8.0g/l) and Nacl (5.0g/l) PH7. The medium was sterilized in an autoclave for 15min at 121^{o}C.

Results and Discussion

The isolates gave higher proteolytic activity after growth in (PM) that contain different carbon sources and maltose was found to be best carbon source in 24 and 48hrs by measuring the diameter of hydrolysis zone (Pansuriya *et al.,* 2011). All isolates gave hydrolysis of casein in 24-48hrs were growth in production media (PM) at optimal temperature for production (SRP) from *Serratia marcesence* (Table 1). This results is agreed with Mohankumar *et al.* (2011) and William and Joseoh (1972), after exposure the isolates to mutation found the best time was 20sec to increase the enzyme production at 32°C. While the 40 and 60sec does not show any effect this indicate that (SRP) production decrease when bacteria mutant in a long time. The hydrolysis can detected by measure the diameter of hydrolysis on casein agar (Figures 1, 2, 3 and 4). All isolates gave hydrolysis of casein in 24 and 48hrs at 32°C isolates were not incubated at 35 to 37°C for the entire 48hrs because of the rapid spreading and over growth of a number of the isolates (William and Joseoh, 1972).

Table (1) : Casein hydrolysis among 15 isolates of *Serratia marcescens* 24-48hrs

Serratia marcescens isolates	Average diameter of clear zone (millimeter)	
	24hrs at 32°C	48hrs at 32°C
1	Hazy zone only	Hazy zone only
2	Hazy zone only	Hazy zone only
3	1.6	2
4	1	1.5
5	1.5	2
6	1.9	2.2
7	2	2
8	1.8	2.2
9	1.5	1.9
10	1.3	2.1
11	2	2.5
12	1.4	2.8
13	2.3	2.8
14	1.2	1.7
15	1.9	2.3

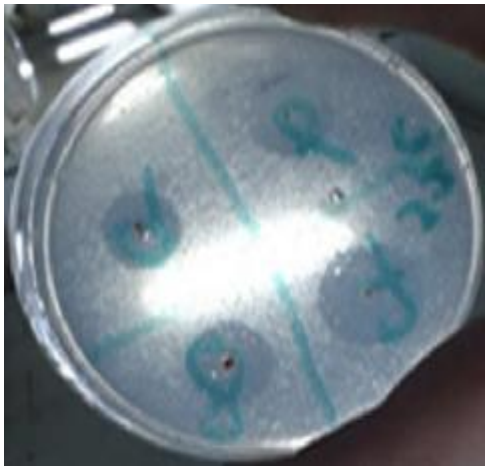

Figure (1): *Serratia marcescens* at 24hrs

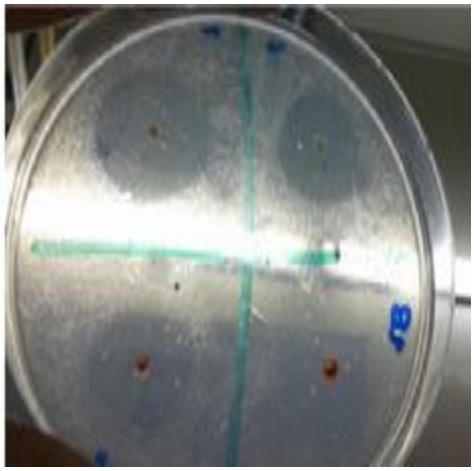

Figure (2): *Serratia marcescens* at 48hrs

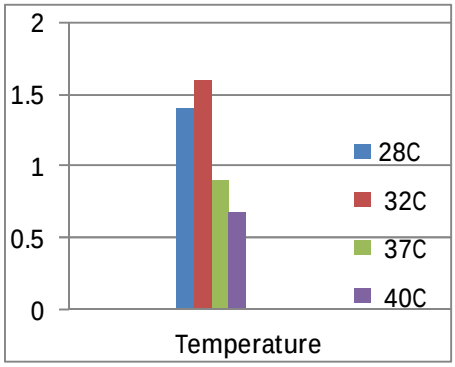

Figure (3): Effect of temperature on hydrolysis of casein in 24hrs

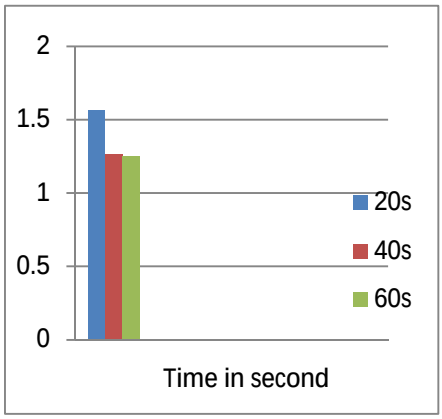

Figure (4): Effect of mutation on hydrolysis of casein in different time.

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