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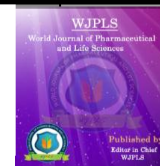

MARINE *SERRATIA MARCESCENS* CHITINASE PRODUCTION: MEDIUM CONSTITUTION AND OPTIMAL CONDITIONS

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ABSTRACT

Serratia an ubiquitous member of Enterobacteriaceae produce many extracellular enzymes. Serratia marcescens used in this study was marine strain previously identified and selected as hyper chitinase producer. In this research the optimum conditions for chitinase production were determined using one factor at time technique (OFAT) in shake flasks (submerged fermentation). The highest chitinase activity was obtained in 75% natural sea water, 0.5% crab shell colloidal chitin as essential carbon source, 0.2 % lactose as secondary carbon source, 0.5% peptone as nitrogen source, 8% NaCl and 2.5% as best inoculum size. The production also was optimum at initial pH: 6.5, temperature 30 ^{0}C, and 48 h incubation period at 150 rpm shaking. These results form the basis for large scale production as well as further improvement for the enzyme properties suitable for various applications in the future.

KEYWORDS: *Serratia marcescens,* chitinase, optimization, submerged fermentation, production.

INTRODUCTION

Chitin is a homopolymer consists of β- (1-4) N-acetyl - D- glucosamine (GLc NAc), and the most abundant natural amino polysaccharide.[1]

Chitinases (E.C.3.2.1.14) are members of glycoside hydrolases, which catalyze the hydrolysis of Chitin into chitooligosaccharides or N-acetyl-β-D-glucosamine (NAG). According to their cleavage pattern, they are classified into exochitinases, endochitinases and chitobiosidases.[2] Due to their versatile applications in many fields ranging from environment, industry, agriculture to medicine.[3,6] chitinases attracted the attention of researchers from multidisciplines, and were extracted from prokaryotic and eukaryotic sources including plants and animals,[7–8] however these later don't meet industrial demands, for these reasons, a strong emphasizes was focused on microbial chitinases and wealthy papers have been reported on bacterial chitinases from different genera such as *Streptomyces , Bacillus, Clostridium, Vibrio, Aeromonas, Xanthomonas, Pseudomonas, Enterobacter and Serratia*.[9]

Serratia marcescens is widely dispersed in nature and produce many extracellular enzymes (amylase, chitinase, protease, gelatinase, and lipase).[10] however, very scare reports were recorded about marine Serratia marcescens chitinase, in this regard, we are interested in investigating and testing factors influencing chitinase production from marine Serratia marcescens.

In our previous papers, many enzymes such chitinase and α-amylase were optimized.[11,12,13,14,15] furthermore, Serratia marcescens was isolated, identified and its α-amylase was optimized, and selected as potent bacterial strain for chitinase production,[16] this study was planned to investigate and optimize the cultivation conditions, which are expected to improve the enzyme production using one factor at time technique (OFAT) in shake flasks submerged fermentations.

MATERIAL AND METHODS

Substrates and chemicals

Chitin was extracted from crustaceans by the method of (Synowiecki *et al*. 1982).[17] Crab shell chitin flakes (Win-lab, UK). Swollen chitin was prepared according Monreal and Reese, (1969).[18] Galactose, glucose, agarose, lactose, N-acetyl glucosamine, bovine serum albumin (BSA), Sodium alginate (Sigma -USA), agar, Peptone tryptone, and yeast extract (Oxoid Hampshire, England).

apple pectin (Fison- Germany), 2 Hydroxy 3,5 dinitrosalselic acid (DNSA) (Merck, Darmstadt-Germany), All other chemicals used were of the highest grade available.

Microorganism and cultivation

Serratia marcescens was isolated from Red sea water, Hurghada-Egypt, identified, and selected as hyper-producer for amylase and chitinase.[16] the strain was cultured in sea water supplemented with 0.5 % yeast extract and incubated at 30°C; solid media were prepared by adding 20 g/l Bacto agar.

Chitinase production and monitoring

The initial medium for chitinase production comprised (g/l): 1% colloidal chitin, 0.2 % yeast extract dissolved in sea water pH: 7.5, this medium was inoculated with 3% of an overnight seed culture of Serratia marcescens, and then incubated at 37°C in an orbital shaker set at 180 rpm for 24h. Furthermore, the changes in the enzyme activity and protein content were monitored each 24h during 4 days to investigate the effect of incubation period on chitinase production.

Chitinase production Optimization

The factors influencing chitinase production were determined using one factor at time technique (OFAT) in shake flasks (submerged fermentation). The tested factors and their levels were detailed as follow:

1. Effect of substrate concentration, carbon and nitrogen sources

Colloidal chitin initial concentration (0.1–1% w/v), carbon sources (glycerol, glucose, galactose, lactose, sucrose, starch, dextran, cellulose, chitosan, pectin, alginate, agar and tween 80), and different inorganic (ammonium chloride, ammonium sulphate, potassium nitrate) and organic (urea, peptone, tryptone and yeast extract) nitrogen sources were tested at a concentration of 0.5% in sea water medium.

2. Effects of Fermentation conditions

The tested parameters were pH (6 to 9), Temperature (20, 30, 37 0C), salinity (2, 6, 8, and12%Nacl), agitation rate (0 to 200 rpm/min), inoculum size (1, 2.5, 5, and 7.5).

Protein and Chitinase assay: after production the culture was centrifuged at 7,000 rpm for 5 min and the cell free supernatant was used for soluble proteins determination as described by Bradford (1976)[19] whereas chitinase activity was analyzed by estimating the released reducing ends of sugar according to the method of Miller[20] using N-acetyl - D-glucosamine (NAG) as standard .One unit of chitinase activity was defined as the amount of enzyme required to release 1 µ mol of NAG per minute during these conditions.

RESULTS AND DISCUSSION

Chitinase Production Optimization

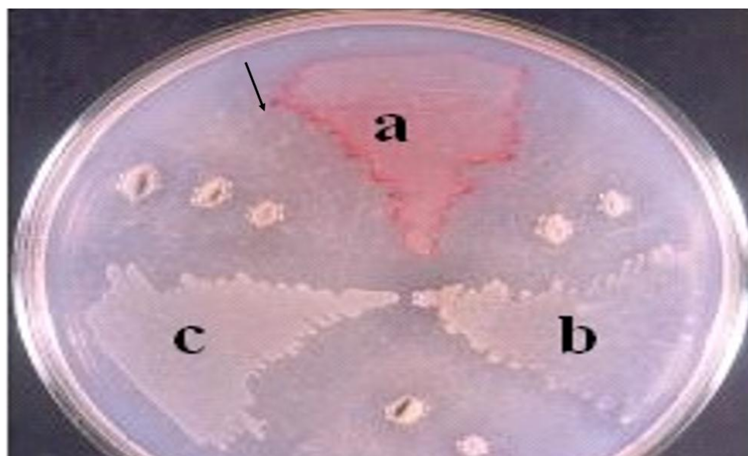
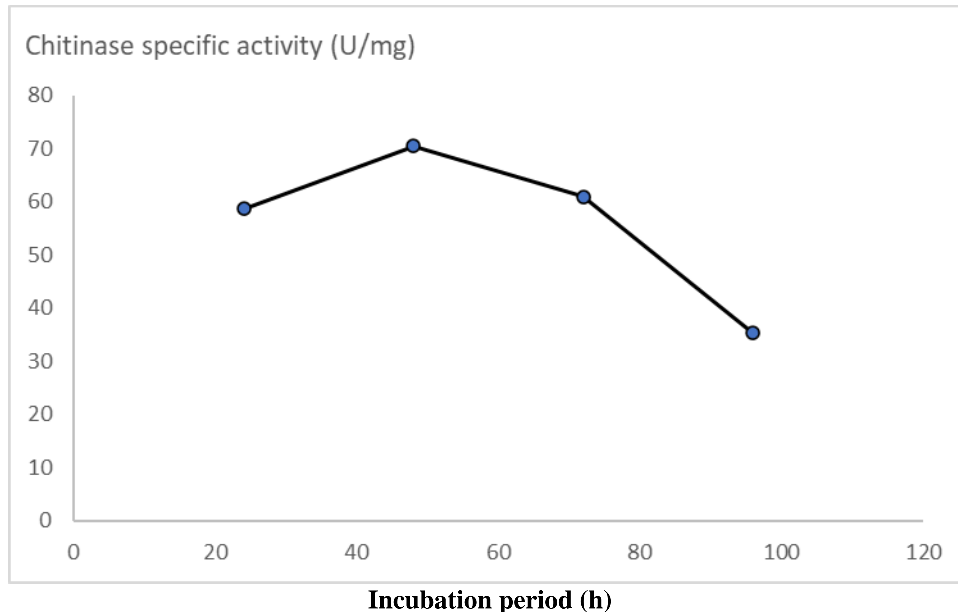


Fig. (1): a: Serratia marcescens chitinolytic activity on marine colloidal chitin agar. b and c other chitinolytic isolates.

Effect of incubation period

Time course of Serratia marcescens chitinase production illustrated in Fig. (2) showed that the maximum chitinase specific activity was obtained at 48h and dropped gradually thereafter. similar results were obtained by Bautista, and Dalmacio, 2003 for Serratia marcescens LMP42. Chitinase production.[21] Whereas Xia et al. 2011.[22] found 32 h was ideal for harvesting chitinase of Serratia marcescens LMP42.This result was in conformity with many bacterial chitinases that produced highly within just one or two days.



Incubation period (h)

Fig. 2: Time course of Serratia marcescens chitinase production.

1.Effect of colloidal chitin concentration

The results illustrated in Fig (3) indicated that 0.5% colloidal chitin was the best concentration for chitinase production. Similar results obtained by Kole and Altosaar, 1985.[23] for Serratia marcescens, strain EB415, furthermore 0.5% swollen chitin was sufficient for Serratia marcescens GG5 chitinase production as reported by Singh, et al. (2005),[24] Whereas Xia et al. 2011,[22] Bautista, and Dalmacio, 2003,[21] found that 0.75% and 0.6% colloidal chitin were the best concentrations that yields the highest chitinase activity from *Serratia marcescens XJ-01* and Serratia marcescens LMP42, respectively. Generally, many investigators reported that the optimal concentration ranged between 0.5 and 1 %.

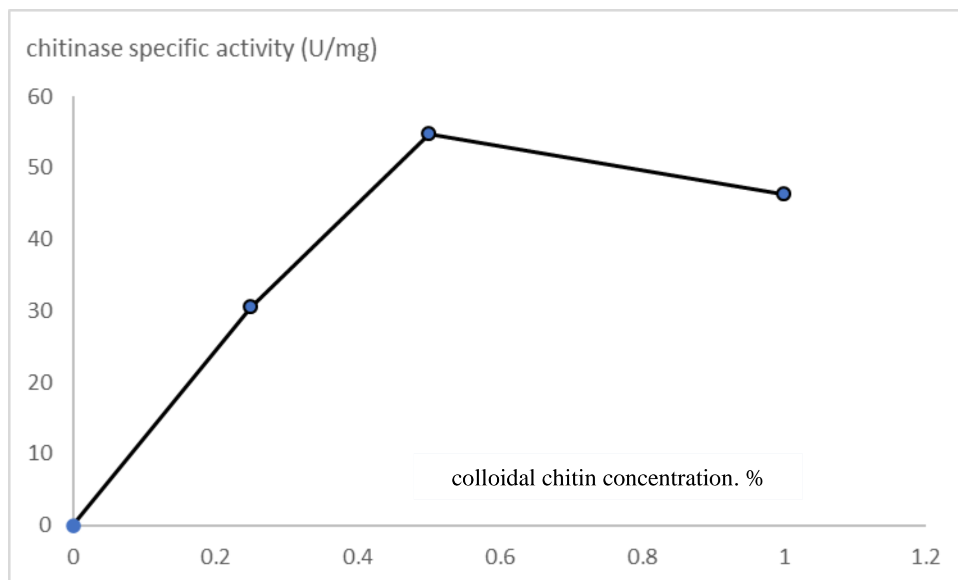


Fig. 3: Effect of colloidal chitin concentration on chitinase production.

2. Effect of carbon sources

Various carbon source supplementations were tested to investigate their effect on chitinase production. It was noticed from Fig. (4) that the lactose was the most efficient secondary carbon source for chitinase production. On the other hand, Singh, et al. (2008) found 0.5% soluble starch enhanced Serratia marcescens GG5 chitinase production,[25] while others reported that, the addition of maltose,[26] pectin[27] also enhanced significantly the chitinase production.

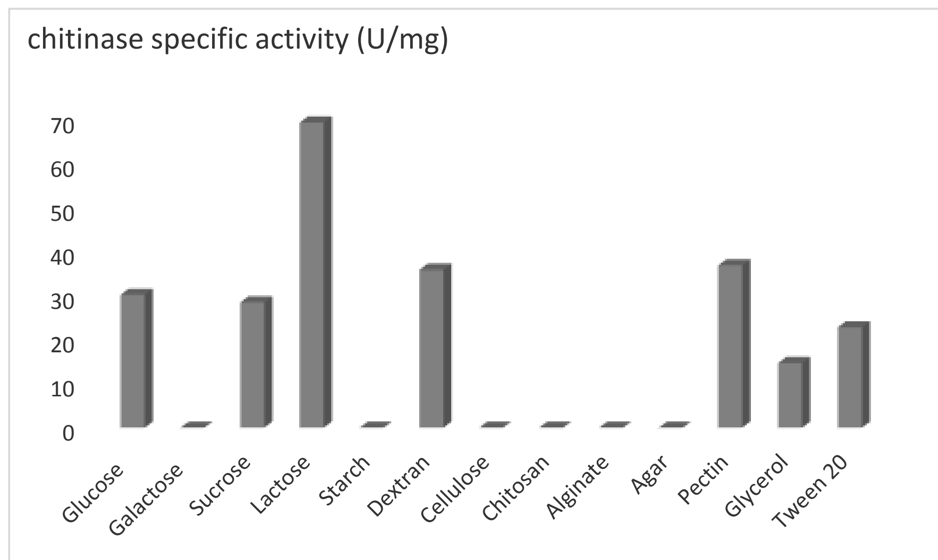


Fig. 4: Effect of carbon sources on chitinase production.

3. Effect of nitrogen sources

Among the organic and inorganic nitrogen sources tested for enhancing chitinase production (Fig. 5), the results indicated that peptone was ideal for chitinase production followed by ammonium chloride. On the other hand, the lower activity was obtained in the presence of KNO3 as a nitrogen source. in contrast 0.5% (NH4)2SO4 was the most preferable for chitinase production by Serratia marcescens XJ-01 as reported by Xia et al. 2011,[22] Generally, it seemed that the preference for nitrogen source varies with microorganisms.

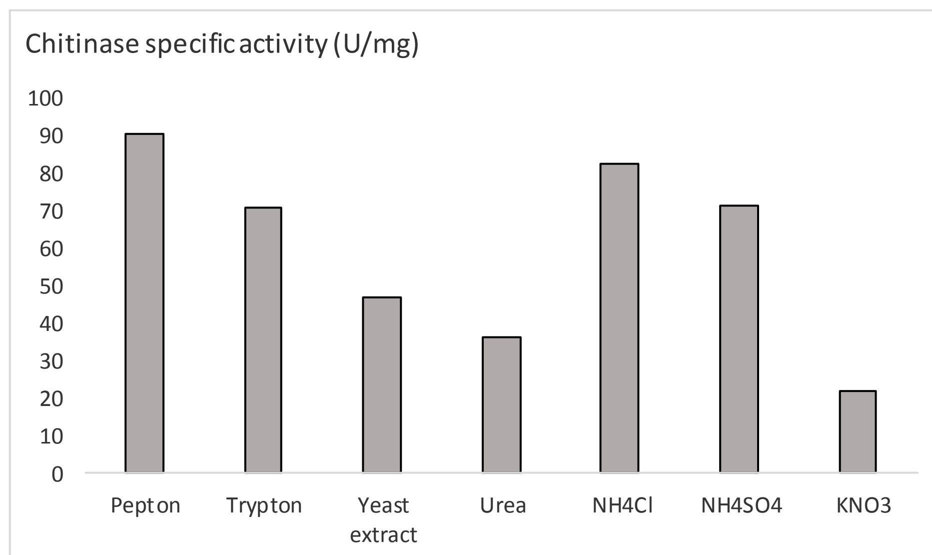


Fig. 5: Effect of nitrogen sources on chitinase production.

4. Effects of fermentation conditions

The optimal conditions for chitinase production by Serratia marcescens in shake flasks using one factor at time technique (OFAT) were summarized in (Tab.1). 8% NaCl, 6.5 initial pH, 30 ^{0}C temperature, 2.5% inoculum size, and 150 rpm shaking, gave the highest chitinase activity. From the available scientific literatures, it seemed that chitinase fermentation conditions were microorganisms to microorganism's variable, for example Aunpad and Panbangrad 2003,[28] Found that NaCl (3%) was optimal for chitinase production by Salinivibrio costicola. whereas, Bautista, and Dalmacio, 2003,[22] found that pH 7, 5% inoculum, and 158 rpm, were optimal condition for chitinase production from Serratia marcescens LMP42. Furthermore Xia et al. 2011,[22] reported that the temperature of 32 ^{0}C, and pH 8 yielded the maximum chitinase activity from Serratia marcescens XJ-01. On the other hand, Gutiérrez-Román et al. 2012,[29] obtained the highest chitinase production (166 units/ml) with an inoculum density of 1. 10^{12} CFU/ml and an initial pH of 6.5. While Ramones et al. 1997,[30] found that 7.7 and 28 ℃ were the suitable pH and temperature for chitinase production from Serratia marcescens QM B1466. respectively,

Table (1): Effect of Fermentation conditions on chitinase production.

Parameter	Value	Chitinase specific activity(U/mg)
Salinity (NaCl %)	2	37,89
	6	44.53
Inoculum size (%)	8	48.92
	12	34,25
	1	56.82
	2.5	85.57
	5	53.30
	7.5	31,63
pH	6	45.12
	6.5	59.80
	7	52.54
	7.5	48.2
	8	43.66
	9	35.24
Temperature (C°)	20	35.81
	30	76.29
	37	55.70
Agitation rate (rpm)	0	34.26
	100	43.88
	150	64.57
	200	55,61

CONCLUSION

Microbial chitinases are inducible and greatly influenced by nutritional and environmental factors, for this raison, the medium constitution optimization and searching for chitinases production key factors, still an urgent need. one factor at time (OFAT) optimization of marine Serratia marcescens chitinase in submerged fermentations was efficient in determining the preferable medium and selecting the crucial effecting factors to maximize the production for future industrial scaling-up with low cost, high yield and potential biotechnological uses.

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