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ISOLATION AND IDENTIFICATION OF CHITINASE PRODUCING *SERRATIA MARCESCENS* FROM SOIL

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ABSTRACT

In the present study an attempt was made to isolate *Serratia marcescens* that would synthesize chitinase which can be used for the effective biodegradation of a vast majority of crustacean waste. Out of the 36 isolates screened the isolate no. (22) showed maximum chitinase activity and was selected for further research. The isolates were identified as *Serratia marcescens* using the standard identification parameters like gram staining followed by the biochemical assays. Further characterization and purification of chitinase needs to be carried out.

Keywords: Chitin, Chitinase, *Serratia marcescens*, Isolation, Identification

INTRODUCTION

Chitin is one of the most abundant renewable biopolymer on earth that can be obtained as a cheap renewable biopolymer from marine sources **[1]**. It is biocompatible, biodegradable and bio-absorbable, with antibacterial and wound-healing abilities and low immunogenicity; therefore there have been many reports on its biomedical applications **[1, 2]**. Chitosan, a derivative of chitin, was produced in 1859, and since then, research has been conducted to learn about the properties of chitin and chitosan and develop commercial application for their large scale uses. Chitosan has some advantages over chitin because it is more water-soluble **[3, 4]**. Chitin is a polysaccharide composed of β-(1, 4)-N-acetyl-D-glucosamine units. Chitinases belong to glycosyl hydrolase families 18 and

19 according to the classification made by Henrissat and Bairoch **[5]**. Chitinases are enzymes that catalyse the hydrolysis of β-1, 4-*N*acetylglucosamine linkages present in chitin. As chitin is a major component of fungal cell walls, and is absent in plants, chitinases play a role in plant defence against pathogens. Supportive evidence for the defensive role of chitinases includes chitinase inhibition of fungal growth *in vitro* **[4]** enhanced resistance to pathogens in plants that constitutively express high levels of chitinase **[6]**, and visualization of *in vitro* chitin breakdown **[7]**.

Serratia marcescens is a gram-negative, rod-shaped, motile bacterium belonging to the family Enterobacteriaceae. A human pathogen, *S. marcescens* is involved in nocosomial infections, particularly catheter-associated bacteremia, urinary tract infections and wound infections, **[6, 8]**. It is commonly found in the respiratory and urinary tracts of hospitalized adults and in the gastrointestinal system of children. It can grow in temperatures ranging from 5–40°C and in pH levels ranging from 5 to 9 **[9]**. The present study was carried out to isolation and identification of *Serratia marcescens* from soil.

MATERIALS AND METHODS

Sample Collection

A total of 150 soil samples were collected from various regions in Babylon Governorate through April / 2012 to July / 2012, The debris from the top of the soil was swept off and the soil was collected from a depth of two inches using a spatula and stored in a clean bags and transported to the lab. according to what stated in thesis of **[10]**.

Isolation of *Serratia marcescens*

One gram of each sample was added to 10 ml of sterilized normal saline solution in test tubes and mixed thoroughly **[11]**. Serial dilutions for each sample were achieved, and then 100 µl aliquots from the appropriate dilution were spread on nutrient agar plates. After incubation at 28ºC for 18 hrs, red pigmented colonies were selected for further identifications.

Identification of *Serratia marcescens*

Morphological and Cultural Characteristics

Colony size, shape, color, and odor for bacterial isolates were studied on nutrient agar, brain heart infusion agar and tryptic soya agar plates. Lactose fermentation ability was investigated on MacConkey agar plates after incubation at 37ºC for 24 hr **[12]**.

Microscopically Examination

A single colony of each isolate was fixed on a clean slide to study Gram stain reaction and spore forming under light microscope **[13]**.

Biochemical Tests

For identification of *Serratia marcescens*, several biochemical tests were done and these include the following tests: inability to ferment lactose, a negative oxidase, positive results to catalase, gelatin liquefaction test, citrate utilization test, and growth at 40°c and motility tests **[13]**. Besides to using MicrogenTM GnA+B-ID System (Microgen Bioproducts Ltd/ UK) to differentiate *Serratia marcescens* from the other types.

Detection of Chitinolytic Activity on Plates

Local isolates were streaked on tryptic Soya agar plates and incubated at 30ºC for 24 h. A single colony was then taken and placed on the center of chitinase agar medium that contained 2.0 g colloidal chitin, 0.7 g K_2HPO_4, 0.3g KH_2PO_4, 0.5 g M gSO4.7H2O (pH=7.0) and 2 % w\w agar in 100 ml of distilled water. Plates were then incubated at 30 ºC for 7 days. Ability of *Serratia marcescens* in chitinase production was measured according to the ratio of hydrolysis based on the presence of clear halo around each colony, as follows:

Ratio of hydrolysis = Halo Diameter /Colony Diameter **[14, 15]**.

RESULTS AND DISCUSSION

Isolation of *Serratia marcescens*

A total of 150 soil samples were collected from different locations in Babylon Governorate, 40 of them showed positive result. Results also showed that among the total 40 isolates, only 36 were able to produce prodigiosin red pigment, which gives an indicator that these isolates belong to *Serratia spp.*, while the other 4 isolates may belong to other pathogenic or nonpathogenic bacteria from different genera. These 36 isolates were further characterized and identified according to their cultural, morphological characteristics and biochemical tests.

Identification of Bacterial Isolates

Morphological Characteristics

Bacterial isolates suspected to belong to the genus *Serratia* were cultured on tryptic soya agar and incubated at 30ºC for 18 hours. Results showed that they are circular, convex, smooth, mucoid and red in color (prodigiosin producers) **(Figure 1)** and have musty odor, while they gave diffuse, hazy growth on semi - solid nutrient agar medium **[9]**.

Microscopically Examination

Bacterial isolates were also identified according to their gram staining and other microscopically characteristics. Results showed that bacterial isolates suspected to be *Serratia* were gram negative, rods, non-spore forming and occur singly under light microscope, as mentioned by **[9]**.

Biochemical Tests

According to the results of morphological and microscopically characteristics, bacterial isolates suspected to belong to *Serratia marcescens* were subjected to number of biochemical tests. Results in **(Table 1)** showed that isolates were *Serratia marcescens* because they gave positive results for catalase, DNase, and gelatin liquefaction, while they gave negative results for oxidase and methyl red tests.

Furthermore, these isolates are able to grow at 40ºC but not at 4ºC. Identifying these isolates as *Serratia marcescens* was confirmed by using MicrogenTM GnA+B-ID identification system. Results illustrated in **(Table 2)** confirmed that these isolates were *Serratia marcescens.*

Chitinolytic Activity on Plates

Serratia marcescens isolates were tested for chitinase production by measuring the diameter of clear zone of lysis in synthetic medium agar (SM) **(Figure 2)**. In this experiment, out of the 36 isolates, isolate (no.22) showed maximum zone of chitinolysis (2.9 cm), hence this isolate was selected for further study.

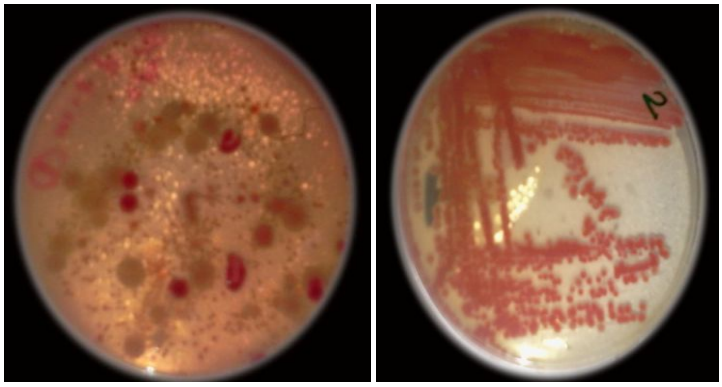

Figure 1: *Serratia marcescens* Colonies

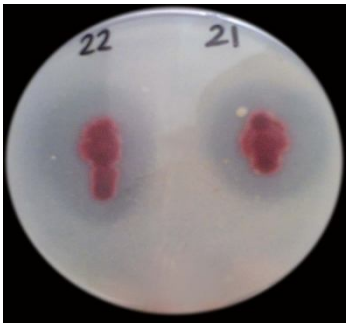

Figure 2: Zone of Chitinolysis

Table 1: Biochemical Tests for the Bacterial Isolates

No.	Biochemical Tests	Results
1.	Gram stain	Gram-negative rod
2.	Catalase Test	+
3.	Motility test	+
4.	DNase	+
5.	Oxidase test	-
6.	Methyl Red Test	-
7.	Gelatin hydrolysis	+
8.	Growth at 40 ºC	+

Table 2: MicrogenTM GnA+B-ID identification system results.

No.	Reaction	result
1.	lysine	+
2.	Ornithine	+
3.	H2S	-
4.	Glucose	+
5.	Mannitol	+
6.	Xylose	-
7.	ONPG	+
8.	Indole	-
9.	Urease	-
10.	V.P.	+
11.	Citrate	+
12.	TDA*	-

NOTE: * TDA: Tryptophan Deaminase

CONCLUSION

In summary, *Serratia marcescens* is capable of producing enzyme chitinase and degrade chitin at substrate level but still a lot more is needed to be studied to harness it at commercial scale and utilize it for the mass scale degradation of chitin wastes generated in the sea-food industries. The pilot scale production of chitinase, fermentation methodology to be adopted for maximum production and assay of enzyme kinetics at various steps is still to be studied. Despite the multiple potential applications of chitin, we believe that the most promising future applications are in the field of nanobiotechnology, which involves drug, gene delivery and scaffold for tissue engineering, and as chito-oligosaccharides in medicine and agriculture.

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