Production and one-step purification of serratiopeptidase enzyme from *Serratia marcescens* with potent anti-inflammatory and antioxidant power

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Background and objective
Inflammation is a huge problem facing the world. NSAIDs are the most popular and widespread drugs used for lowering the impaired symptoms accompanied with inflammation. This group has plentiful disorders to the gastrointestinal tract reaching to gastric ulcer. The objective of this study was to produce a natural product having an anti-inflammatory power without the side effects concomitant with the used drugs. The serratiopeptidase enzyme has a potent anti-inflammatory action without any abnormal disorders.
Materials and methods
Different media and cultural conditions were evaluated for the optimal production of the serratiopeptidase enzyme from a local bacterial strain. Partial purification was employed using the ammonium sulfate precipitation technique.
Results and conclusion
Serratiopeptidase enzyme was produced by the bacterial strain *Serratia marcescens* and its anti-inflammatory and antioxidant power were declared. The best medium was chosen for the optimal productivity and the optimum pH was 6 that recorded the highest anti-inflammatory and anti-oxidant activity at 67.5 and 71.8%, respectively, which is powerful than any chemical drugs used. Serratiopeptidase enzyme produced by this strain was partially purified by ammonium sulfate precipitation revealed an increase in specific activity by two times than the crude one with 14.7 recovery. The authors conclude the use of serratiopeptidase enzyme for treating the inflammation instead of the traditional ways.

Keywords:
anti-inflammatory, antioxidant, *Serratia marcescens*, serratiopeptidase

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Introduction

Nonhealed inflammation is one of the most predominant problems facing the medical circles worldwide. Inflammation is a self-defense response of the body's immune system against most infectious and noninfectious disorders such as sinusitis, arthritis, lung diseases, cardiovascular disorders, etc. It is characterized by heat, redness, and pain. There are many groups of chemically synthesized anti-inflammatory agents that were conventionally used in the market. NSAIDs are the most potent and widely prescribed group especially for inflammatory joint problems. Their effects are mediated through inhibition of the biosynthesis of prostaglandins, a hormone responsible for mucosal protection of the stomach, leading to gastric ulcers.

Unlike these drugs, serratiopeptidase is a naturally occurring enzyme with no inhibitory effects on prostaglandins and with no effect on the gastrointestinal tract. Serratiopeptidase is a proteolytic enzyme available for clinical use from many years. Serratiopeptidase binds to α-2-macroglobulin in the blood which obscured its antigenicity but retains its enzymatic activity and is slowly, transferred to the site of inflammation. Serratiopeptidase hydrolyzes bradykinin, histamine, and serotonin responsible for the edematous status. Serratiopeptidase reduces swelling, improves microcirculation and expectoration of sputum, etc. [1]. Also, serratiopeptidase is widely used to reduce pain and inflammation associated with some diseases such as arthritis, chronic bronchitis, and atherosclerosis [2].

Materials and methods

Microorganism

Serratia marcescens bacterial strain was obtained from the cultural collection of El-Diwany Laboratory,

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Natural and Microbial Products Division, National Research Center. The experimental research that is reported in this manuscript has been performed with the approval of an appropriate ethics committee. No experiments had done on humans.

Production media

Nutrient broth was prepared and inoculates with *S. marcescens* mother culture. Culture broth was maintained in 150 rpm for 24 h at 27°C for the preparation of preproduction media. 2% of the preproduction media are taken for the main production media with different compositions as follows (g/l):

(1) Medium 1: KH_2PO_4 7, K_2HPO_4 2, $MgSO_4$ 0.1, yeast extract 1.0, casein 2.
(2) Medium 2: Beef extract 5, peptone 8, NaCl 2, dextrose 5, casein 7.5.
(3) Medium 3: Luria Bertani (LB) medium.
(4) Medium 4: Nutrient broth (NB) composed of glucose 5, beef extract 5, peptone 5, NaCl 5.
(5) Medium 5: It composed of 3% ground sesame in tap water.
(6) Medium 6: It composed of 3% ground peanuts in tap water.
(7) Medium7: It composed of 3% rice bran in tap water.

pH was adjusted to 7.

Enzyme assay

Qualitative estimation of serratiopeptidase activity

This was done using 10% gelatin medium poured in equal volumes in test tubes. One tube was inoculated with 0.5 ml culture filtrate of pregrown *S. marcescens* and another one was inoculated with water and used as control. Both tubes were incubated for 2 h at 37°C, and then kept at 4°C for 30 min. The positive one shows the liquefaction of gelatin.

Quantitative estimation of serratiopeptidase activity

Serratiopeptidase activity was assayed by the modified method of Tsuchida *et al.* [3]. In brief, 0.5 ml of the enzyme was mixed thoroughly with 2.5 ml of 0.75% of casein solution (using Tris buffer pH 8.5). The mixture was incubated at 37°C for 30 min. Then the reaction was terminated by the addition of 2 ml of 15% trichloroacetic acid. The incubated solution was filtered through Whatman No. 1 filter paper. Then 1 ml from the filtrate was estimated using the Lowry method. The final solution was measured at 750 nm. One unit of serratiopeptidase activity was defined as the amount of enzyme required to liberate 1 μmol (181 μg) of tyrosine in 30 min at 37°C.

Protein assay

Protein concentration was measured by the method of Lowry *et al.* [4] using bovine serum albumin (Sigma Scientific Services Co., St. Louis, Missouri, USA) as the standard. The specific activity was expressed as the enzyme.

In-vitro anti-inflammatory assay

Anti-inflammatory activity was tested using the method of Rahman *et al.* [5]. The samples or standard drug diclofenac sodium were mixed with 0.45 ml bovine albumin serum. Samples were incubated at 37°C for 20 min, heated at 57°C for 3 min, after cooling the samples, 2.5 ml phosphate buffer at 6.3 pH was added to the samples. The absorbance was measured using a ultraviolet-visible spectrophotometer at 255 nm.

In vitro total antioxidant capacity assay

Phosphomolybdic acid-free radial scavenging was assayed according to Prieto *et al.* [6]. A measure of 1 ml of samples and standard ascorbic acid was mixed with 3 ml of reagent solution. Tubes were incubated at 95°C for 90 min. After cooling, the absorbance of each sample was measured at 695 nm.

Partial purification of serratiopeptidase

Sequential ammonium sulfate precipitation

The enzyme was first precipitated from the cell-free supernatant by the addition of ammonium sulfate at different saturation levels ranging from 20 to 80% and kept for 2 h at 4°C and the precipitated protein was collected by centrifugation at 10 000 rpm for 20 min and at 4°C and then resuspended in a minimal volume of 0.1 M phosphate buffer, pH 8.0 to obtain the concentrated enzyme suspension. The enzyme solution was finally dialyzed against the same buffer and this was repeated till the buffer outside the bag was free from sulfate ions; then the enzyme solution was lyophilized and weighed.

Results and discussion

S. marcescens was tested for the serratiopeptidase enzyme production qualitatively as shown in Fig. 1. The left tube showed positive result, where the protease enzyme liquefied the substrate by hydrolyzing the gelatin.

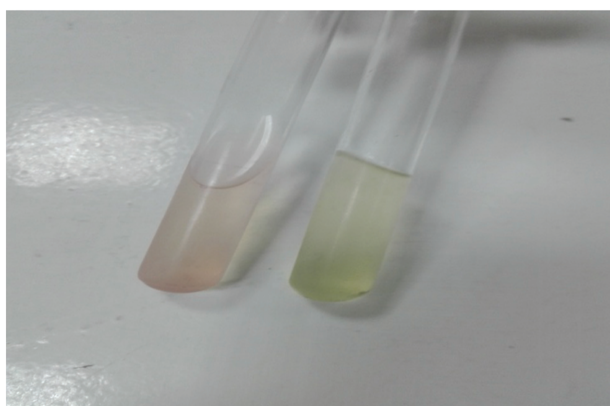
As shown in Fig. 2, *S. marcescens* can successfully grow and produce serratiopeptidase enzyme in a wide range of unselective media with even simple compositions. Medium no. 2 was selected because of its higher enzyme productivity and activity. The highest productivity obtained by medium no. 2 related to

the presence of casein in its composition which acts as a protein source that was hydrolyzed by proteolytic enzymes produced by *S. marcescens*. These results are in agreement with that of Subbaiya *et al.* [7], who produce serratiopeptidase enzyme in a pilot scale using casein as the sole carbon source. Also, medium no.2 showed the highest anti-inflammatory activity by inhibiting the denaturation of heat-induced albumin as shown in Table 1 with maximum inhibition (74.27%) at a concentration of 25 μg/ml compared either with different media or with diclofenac sodium as the most potent drug used in the market.

On the other hand, the anti-oxidant capacity of serratiopeptidase was evaluated by scavenging of phosphomolybdic acid as shown in Table 1. Most of the media possessed the ability of scavenging of phosphomolybdic acid radical; the inhibition % ranged from 7.83 to 85.03% at a concentration of 50 μg/ml. The highest antioxidant effect was observed in media 4 (85.03%) at a concentration of 50 μg/ml. These results were superior than that of Wang *et al.* [8] who produced the proteolytic enzyme along with the antioxidant with 72% capacity from *Serratia ureilytica.*

By the same, medium no. 2 was the most favored for serratiopeptidase productivity and anti-inflammatory activity when growing *S. marcescens* on the prior media under static conditions but with lower yield than the shaken one. While medium no.6 enhanced the antioxidant capacity than other media that are

Figure 1



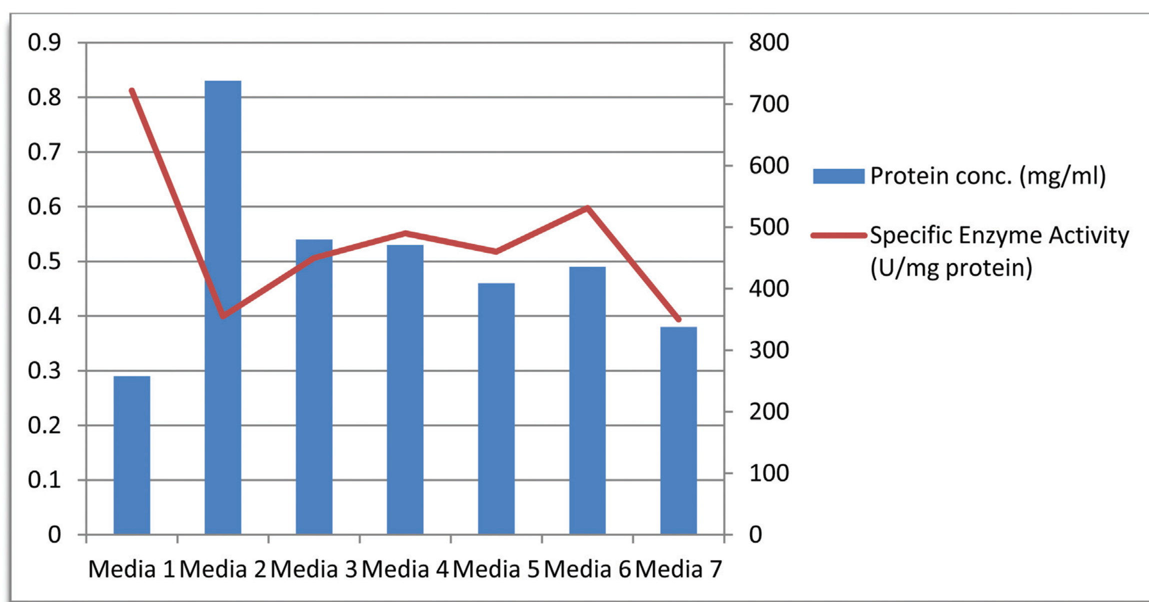
Qualitative estimation of serratiopeptidase production by *Serratia marcescens.*

Table 1 Anti-inflammatory and antioxidant activities of different shaken culture media

Medium no.	Anti-inflammatory 25 μg protein conc.	Antioxidant capacity 50 μg protein conc.
Standard drug	70.62	45.36
Media 1	68.46	7.83
Media 2	74.27	69.38
Media 3	53.87	58.30
Media 4	57.80	85.03
Media 5	45.76	69.89
Media 6	66.82	67.54
Media 7	29.47	71.01
LSD	10.48	8.49

LSD, least significant difference.

Figure 2



Serratiopeptidase activities produced by *Serratia marcescens* at different shaken culture media.

related to the presence of peanuts in the medium enriched with vitamin E (Fig. 3 and Table 2). This is referred to the need of high aeration for growth and metabolism.

Panchanathan *et al.* [9] showed that protease from marine actinobacterium *Streptomyces* spp. MAB18 has antioxidant activity against free radicals of DPPH, O, NO, Fe^{2+} chelating and reducing power.

The effect of different initial pH values on the productivity of *S. marcescens* was studied as shown in Fig. 4 and Table 3. The activity of serratiopeptidase enzyme was nearly the same, while at pH 6 the yield increased obviously. pH 7.5 was the best for anti-inflammatory and antioxidant activities. These results match with that of Mohankumar [1], who declared that pH 6 was the optimal for serratiopeptidase production by *S. marcescens* using phosphate buffer.

From the results summarized in Tables 4 and 5, an extracellular serratiopeptidase was precipitated from the culture filtrate of *S. marcescens* using different saturation levels of ammonium sulfate ranging from 25 to 100%. The results showed that the 40% ammonium sulfate saturation fraction possessed the highest enzyme activity (3905 Ul/ml) with an increase of purification fold to two times and recovered activity 14.7%. These results are in agreement with that of El-Khonezy *et al.* [10], who illustrated that 40–-60% saturation of ammonium sulfate was the best for protease enzyme recovery (4.28) with 3.5 purification fold. Also, Nageswara *et al.* [11]

Table 2 Anti-inflammatory and antioxidant activities of different static culture media

Medium no.	Anti-inflammatory 25 µg protein conc.	Antioxidant capacity 50 µg protein conc.
Standard drug	70.62	45.36
Media 1	54.28	65.1
Media 2	69.05	69.28
Media 3	61.95	80.03
Media 4	59.15	52.55
Media 5	45.75	69.89
Media 6	20.31	90.27
Media 7	20.31	56.36
LSD	10.48	8.49

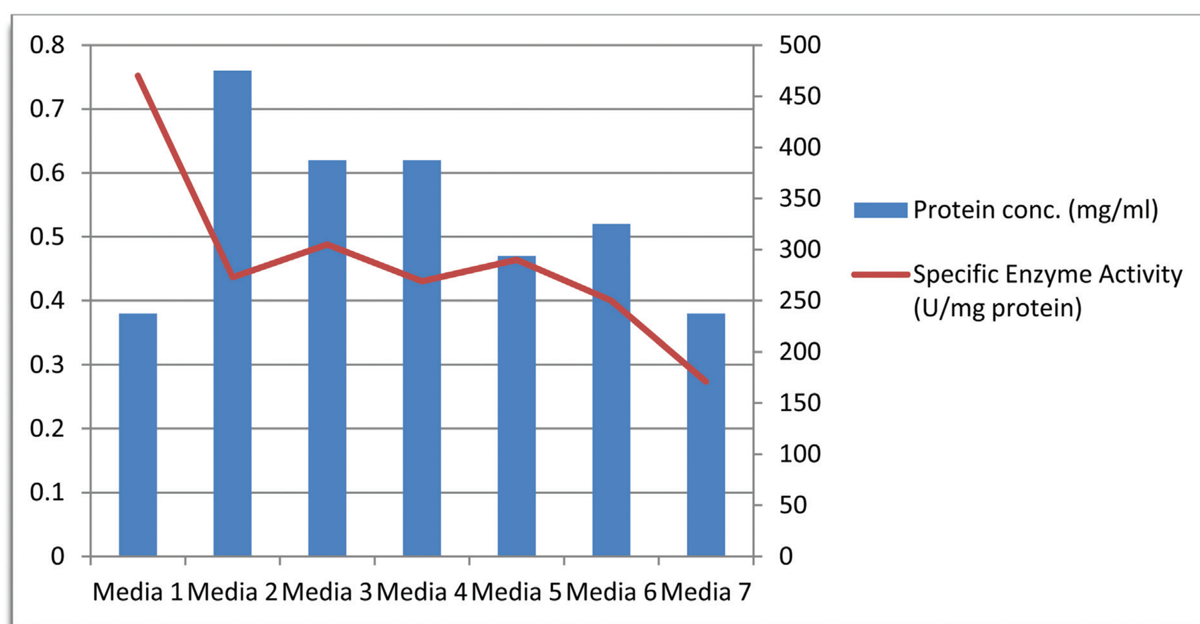
LSD, least significant difference.

Table 3 Anti-inflammatory and antioxidant activities at different pHs in shaken culture

pH	Anti-inflammatory %	Antioxidant capacity
Standard drug	70.62	45.36
5	63.88	77.29
5.5	64.47	75.29
6.5	67.56	71.81
7	62.45	79.71
7.5	70.32	80.06
LSD	10	0.14

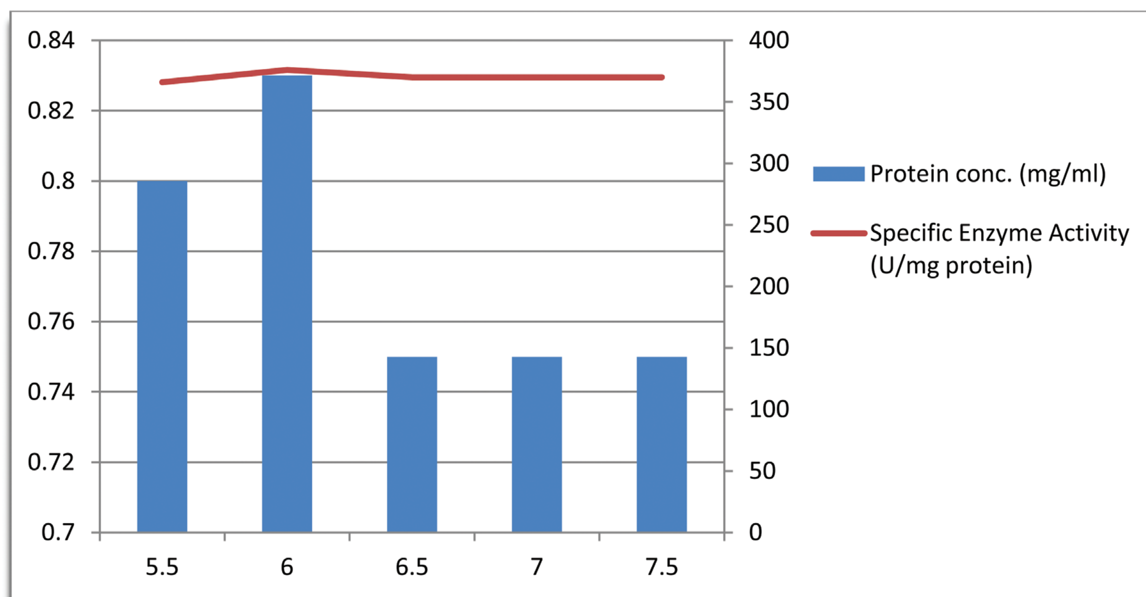
LSD, least significant difference.

Figure 3



Serratiopeptidase activities produced by *Serratia marcescens* at different static culture media.

Figure 4



Effect of initial pH on serratiopeptidase activity.

Table 4 Summary of the results of the partial purification steps of serratiapeptidase enzyme produced by *Serratia marcescens*

Amm. sulfate saturation (%)	Protein content (mg/ml)	Total protein content (mg)	Activity (UI/ml)	Total activity	Specific activity (UI/mg)	Recovered activity (%)	Purification fold
None (CF)	9	1710	3902	741 380	433.5	100	1
25	–	–	–		–	–	–
40	4.46	124.75	3905	109 340	875.6	14.7	2.02
65	5.63	219.5	1482	96 330	263.2	13	0.6
80	3.52	137.2	74	2886	21	0.4	0.05
100	1.17	43.4	55	2035	47	0.27	0.1

CF, culture filtrate.

Table 5 Anti-inflammatory and antioxidant activities of different partial purification steps of culture filtrate produced by *Serratia marcescens*

Ammonium sulfate saturation (%)	Anti-inflammatory (%)	Antioxidant capacity (%)
Standard drug	70.62	45.36
40	70.32	62.12
65	70.33	62.12
80	70.18	62.52
100	48.96	31.43
LSD	10	0.14

LSD, least significant difference.

illustrated the partial purification of serratiopeptidase produced by S. hydrogenans var. MGS13 at 50% saturation of ammonium sulfate yielded a maximum recovery of 52%.

There were no effects of partial purification on anti-inflammatory and antioxidant activities. Serratiopeptidase enzyme purification by 40, 65, and 80 of ammonium sulfate saturation declared the highest anti-inflammatory activity at 25 μg/ml (70.33, 70.32, and 70.18%, respectively). While a low percentage of inhibition was demonstrated at 100% ammonium sulfate saturation compared with the drug. Also, the highest scavenging ability was possessed by serratiopeptidase purification at ammonium sulfate saturation of 65% (70.33%), followed by 40% (70.32%) and 80% (70.18%). The highest anti-inflammatory property of serratiopeptidase from *S. marcescens* was reported by Umesh *et al.* [12], who declared that serine protease inhibits cyclooxygenase (COX-I and COX-II). As this cyclooxygenase produce different inflammatory mediators including interleukins (IL), prostaglandins (PGs), and thromboxane (TXs). Rajinikanth *et al.* [13] found that the anti-inflammatory activity of serratiopeptidase is exhibited by reducing reactive oxygen species, lipid peroxidation, neutrophil accumulation and nitric oxide in acetic acid induced ulcer colitis in mice.

Conclusion

Serratiopeptidase enzyme is a promising substitute for the ordinary anti-inflammatory drugs present in the market without the dangerous and harmful side effects accompanied with their use for long term with an additional antioxidant benefit. Shaken culture having a casein in its constituents at 27°C incubation temperature is the optimal for the production of serratiopeptidase enzyme by *S. marcescens*.

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Nil.

Conflicts of interest

There are no conflicts of interest.

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