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RESEARCH ARTICLE

Exploring the bioactive potential of *Serriatia marcescens* VITAPI (Acc: 1933637) isolated from soil

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BACKGROUND: *Serratia* is one of the most important groups of bacteria which produces proteolytic enzymes effectively and known to possess anti-inflammatory properties. The main focus of the current study was to extract the enzyme serratiopeptidase and pigment prodigiosin from *Serratia mascescens*. Prodigiosin is a red colored pigment produced by the bacterium *Serratia marcescens.* It is emerging as a valuable molecule because of its large applications. It has already been proved that pigmented strain of *Serratia marcescens* is less virulent than non-pigmented strains. Moreover the strain we have obtained is from farm soil which indicates that prodigiosin production can be carried safely using this strain.
METHODS: In the present study, the isolate VITASP strain was confirmed by morphological, biochemical and molecular studies. The enzyme and pigment were analyzed for anti-oxidant, anti-inflammatory and cytotoxic properties.
RESULTS: The isolate was further confirmed and identified as *Serratia marcescens* with 99% similarity. The extracted pigment showed potent radical scavenging effect with 86% and the enzyme was found to inhibit 83%, which was significant in comparison to ascorbic acid standard. The *in vitro* anti-inflammatory effect of pigment in controlled experimental conditions revealed its protection at 88% and the enzyme with 90%. Aspirin was used as the reference drug. The present findings exhibited a concentration dependent inhibition. The cytotoxic bioassay of pigment showed the IC_{50} value as (50) μg/mL with 63% cytotoxicity which was statistically significant compared to positive control.
CONCLUSIONS: Therefore, it appears to be an essential remedial and application research. It may turn out to be highly beneficial to mankind in solving many problems associated with human health.

Keywords *Serratia mascescens*, prodigiosin, serratiopeptidase, bioactivity

Introduction

Inflammation is a normal response to protect the tissues from various noxious stimuli and one of the most normal clinical conditions. Many degenerative diseases such as rheumatoid arthritis, heart disease, asthma, cancer and inflammatory bowel disease are often associated with inflammatory processes. A variety of enzymes and pigments has been used as therapeutic agents in a number of clinical conditions and are co-administered with non-steroidal anti inflammatory agent (Iwalewa et al., 2007; Subathra Devi et al., 2013; Subathra Devi et al., 2014). Secondary metabolites of bacterial origin include various enzymes, pigments, antibiotics, etc. which could be of importance to mankind in many ways. Microbial compounds with antioxidant potential plays an important role in cell damage during inflammatory process and they prevent damage by scavenging reactive oxygen species. It has immense potential in designing therapeutics for infectious diseases and their prevention. Therefore the discovery of these microbial bioactive compounds can revolutionize drug discovery. Hence, microbial pigments and enzyme production is now one of the emerging fields of research to demonstrate its potential for various industrial applications (Roxvall et al., 1990). From an industrial point of view, the necessity to obtain a suitable medium to simultaneously enhance the growth of *Serratia marcescens,* pigment and enzyme production is also important. Due to the fast growing interest in the anti-inflammatory drugs the pharmaceutical companies and scientific researchers have focused on the mass production of anti-inflammatory compounds. There is a need to search for high yielding strains

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for the mass production. The present investigation was carried out to explore the bioactivity of pigment and enzyme produced from *Serratia marcescens* VITAPI (Acc: 1933637) isolated from soil.

Materials and method

Isolation and characterization of isolate *Serratia marcescens*

Serratia sp. was isolated from soil of VIT University campus, Vellore, Tamil Nadu, India. The bacterial isolate was subjected to biochemical test in order to identify the genus level characterization. The molecular characterization was done using 16S rRNA sequencing using universal primers.

Production and extraction of enzyme

Growing cultures of *Serratia mascescens* was transferred to 100 mL trypticase soy broth. 10 mL of the inoculum was transferred to 90 mL sterile production broth and incubated at 27°C for 24 h in a shaker at 120 r/min. At the end of fermentation, the culture medium was centrifuged at 10000 r/min to obtain the enzyme which was further subjected to partial purification. Ammonium sulfate was added with constant stirring to the culture supernatant in small quantities in order to achieve saturation. The concentration of the supernatant was made 60%–70% by precipitation with ammonium sulfate. The precipitates were dissolved in 50mM phosphate buffer (pH 7). Dialysis was conducted over night against the same buffer. The resultant dialysates were regarded as partially purified serratiopeptidase and used for further studies.

Production and extraction of pigment prodigiosin

Serratia mascescens was grown in powdered trypticase soy broth for 2 days at 37°C and further centrifuged at 10000 r/min for 15 min. The pigment from the supernatant was extracted with ethyl acetate at 10000 r/min for 15 min and dried. The extracts were evaporated to dryness and the powdered sample was used for further analysis. The pigment was dissolved in 10% dimethyl sulfoxide (DMSO) to give a final concentration of DMSO not more than 0.5% and it should not affect the cell survival.

Anti-inflammatory assay

Anti- inflammatory activity of pigment and enzyme extracted from *Serratia marcescens* was assessed by in vitro HRBC membrane stabilizing method. The test was performed at (1 mg/mL). Aspirin was used as positive control. The hemoglobin content in the supernatant was measured using spectrophotometer at 560 nm.

Antioxidant assay

Stock solution (0.004% w/v) of DPPH (1,1-diphenyl-2-picryl-hydrazyl) was prepared. The pigment (1 mg/mL) and enzyme was added. The reaction mixture was wrapped in aluminum foil and kept at 30°C for 30 min in dark. Ascorbic acid was used as control. Spectrophotometric measurements were done at 517 nm. Scavenging of DPPH radicals by the extract was calculated using this formula:

$$\frac{(\text{Absorbance of control} - \text{Absorbance of test}) \times 100}{\text{Absorbance of control}}$$

Cytotoxic assay

Based on the previous reports, the cytotoxic assay was done only for pigment. The HepG2 cells were obtained from NCSS Pune and cultured in RPMI-1640 medium on 10 cm tissue culture dishes (Greiner Bio-one™, Germany) supplemented with 10% heat inactivated fetal bovine serum. Cells were incubated in humidified incubator with 5% CO_2 at 37°C and sub-cultured when confluence was reached up to 80%. The 3-(4, 5-dimethylthiazol-2-yl)-2-5-diphenyl tetrazolium bromide) MTT assay was performed to assess the viability of the cells. After starvation, the cells were treated with different concentrations of pigment (1, 10, 20, 50µg/mL) for 24 h. Spectrophotometric absorbance of the purple blue formazan dye was measured in a micro plate reader at 570 nm (Biorad 680). Cytotoxicity was determined using Graph pad prism5 software. Doxorubicin (5µg/mL) was used as positive control.

Results

The isolate VITAPI (Fig. 1) was identified by detailed conventional biochemical methods, and partial 16S rRNA analysis. Based on gene sequencing, strain VITAPI was identified as *Serratia* sp. The phylogenetic analysis showed 99% similarity to *Serratia marcescens* (Fig. 2). The gene sequence of VITAPI was submitted to GenBank database and an accession number was assigned. The strain name was designated as *Serriatia marcescens* VITAPI (Acc: 1933637)

Serratiopeptidase enzyme and prodigiosin pigment were produced from the strain *Serratia marcescens.* As part of the investigation on the mechanism of anti-inflammatory activity, the pigment and the enzyme showed percentage inhibition of hemolysis which was found with 10% and 12%. The protection was found to be 88% and 90% at 1mg/mL concentration. Aspirin was used as a positive control (Fig. 3 A, B) These provide evidence for membrane stabilization as an additional mechanism of their anti-inflammatory effect.

The pigment could be useful for treating radical related pathological damage, especially at higher concentration of pigment at 1 mg/mL with 86% inhibition. The antioxidant

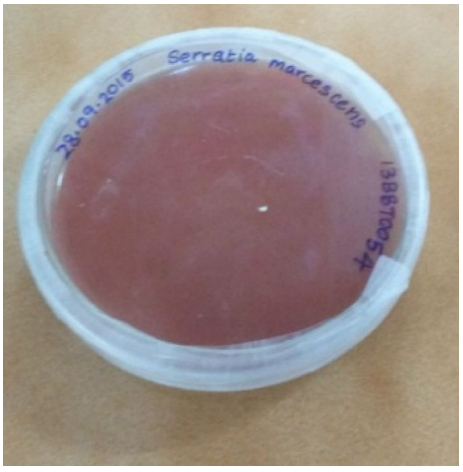

Figure 1 Pure culture of *Serratia marcescens* VITASP isolate from soil.

property were also noticed for the enzyme which indicated its scavenging potential with 83% inhibition. Ascorbic acid was used as a positive control (Fig. 4). Hence the pigment is capable of being used to develop as drugs with antioxidant properties. The pigment results were found comparatively higher when compared to the enzyme.

Cytotoxic assay was performed only for pigment. The experimental data demonstrated that pigment was a valuable source with high cytotoxic activity against Hep G2 cells. Cell death stages were assayed by morphological observations like cell shrinkage and membrane blabbing. Decline in cell proliferation was observed in pigment treated cells when compared to control. The results revealed the IC_{50} value as (50) µg/mL with 63% cytotoxicity which was statistically significant when compared to positive control, doxorubicin. A drug concentration-dependent cell death was observed. The analysis of the relationship between the activity and pigment directed to a conclusion; it has very good anti-proliferative activity (Fig. 5 A, B). This dose dependent cytotoxicity showed that prodigiosin could be used in the development of anti-cancer drugs.

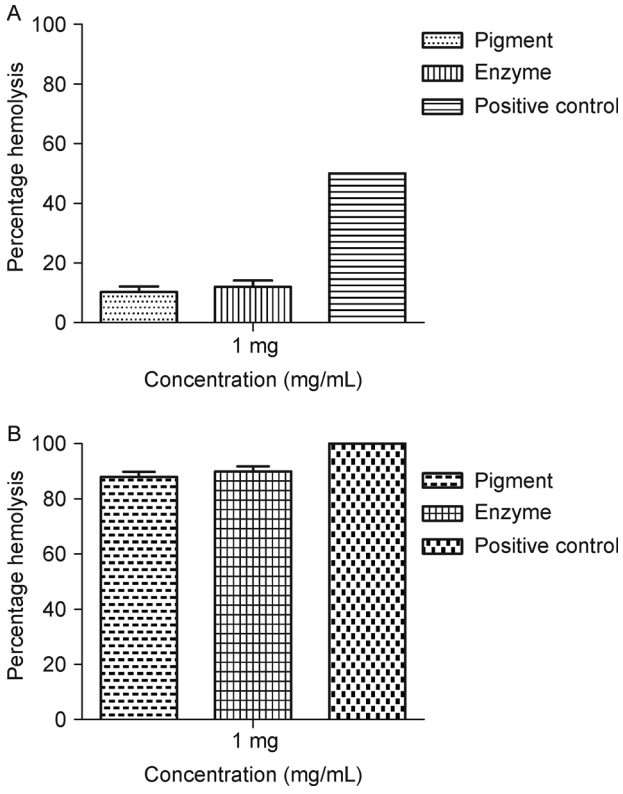

Figure 3 Anti-inflammatory activity of prodigiosin pigment and serratiopeptidase enzyme. (A) percentage of hemolysis, (B) percentage of protection.

Discussion

Screenings for bioactive compounds from natural sources are progressively important for the pharmaceutical industry (Chou, 1997). The result of DPPH scavenging assay carried out in the present study indicated pigment was potentially active. This suggests that pigment do contain compounds that are capable of donating hydrogen to a free radical in order to remove odd electron, which are responsible for radical's reactivity. Pigment showed a potential decrease in the oxidative stress. The pigment, Prodigiosin, from microbial

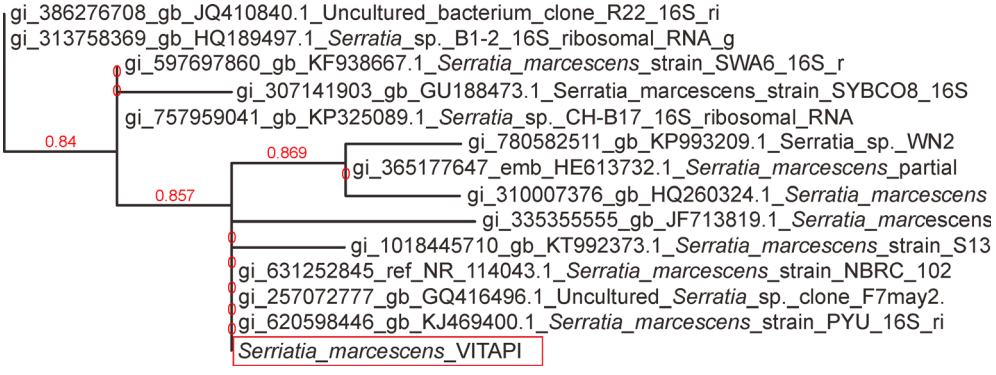

Figure 2 Phylogenetic tree of *Serratia marcescens* VITASP.

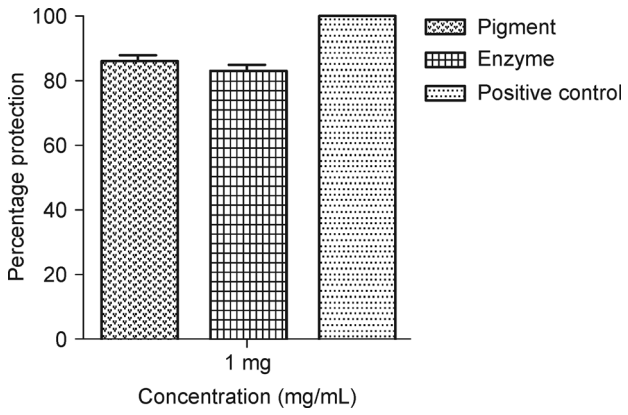

Figure 4 Radical scavenging activity of Prodigiosin Pigment and serratiopeptidase enzyme *extracted* from *Serratia marcescens* VITASP.

sources possess biological properties and belongs to the group Tripyrrole (Miyata et al., 1970; Williams et al., 1971; Nakahama et al., 1986; Carbonell et al., 2000; Miguel and Carrascosa José, 2013). Prodigiosin has anti-cancer, anti-microbial and anti-parasitic activity (Arivizhivendhan et al., 2015). In the present study, the prodigiosin extracted from *Serratia marcesens* VITAPI showed remarkable cytotoxic activity against Hep G2 cells. Prodigiosin showed effective cytotoxic activity against human cervix carcinoma cells. (Kavitha et al., 2010). Inspite of extensive therapeutic claims, the prodigioisin production is not yet extended the economically feasible process. We are in need to screen and develop efficient strains to combat cancer. Serratiopeptidase have been used in successful treatment of many diseases. The strain isolated from soil, *Serratia marcesens* VITAPI is a multi-faceted organism. Because, the serratiopeptidase produced from the strain showed a very good anti-inflammatory activity. Previous studies on *Serratia marcescens* VITSD2 (GenBank accession number: KC961637) showed a maximum serratiopeptidase production. Further *in vivo* studies are essential to drive to the subsequent level of research. Serratiopeptidase is administered for inflammatory diseases or to prevent plaque buildup on the arteries, it is well-tolerated. Due to its lack of side effects and anti-inflammatory capabilities, serratiopeptidase is a logical choice to replace harmful NSAIDs. Hence researchers have taken a large step toward finding relief for inflammatory disease sufferers. In this regard the society can be immensely promoted by the therapeutic value of serratiopeptidase which is in great demand. The enzyme judges a future therapy for many of deadly and emerging clinical conditions to which very little or no alternatives are available. With this futuristic approach serratiopeptidase characterization and intensive study on it can pioneer future manipulation of the enzyme for the advancement of its performance.

Conclusion

Our key findings demonstrate the hope for the use of this pigment as a therapeutic agent which is antioxidant in nature and does not induce anti-inflammatory activities. Further

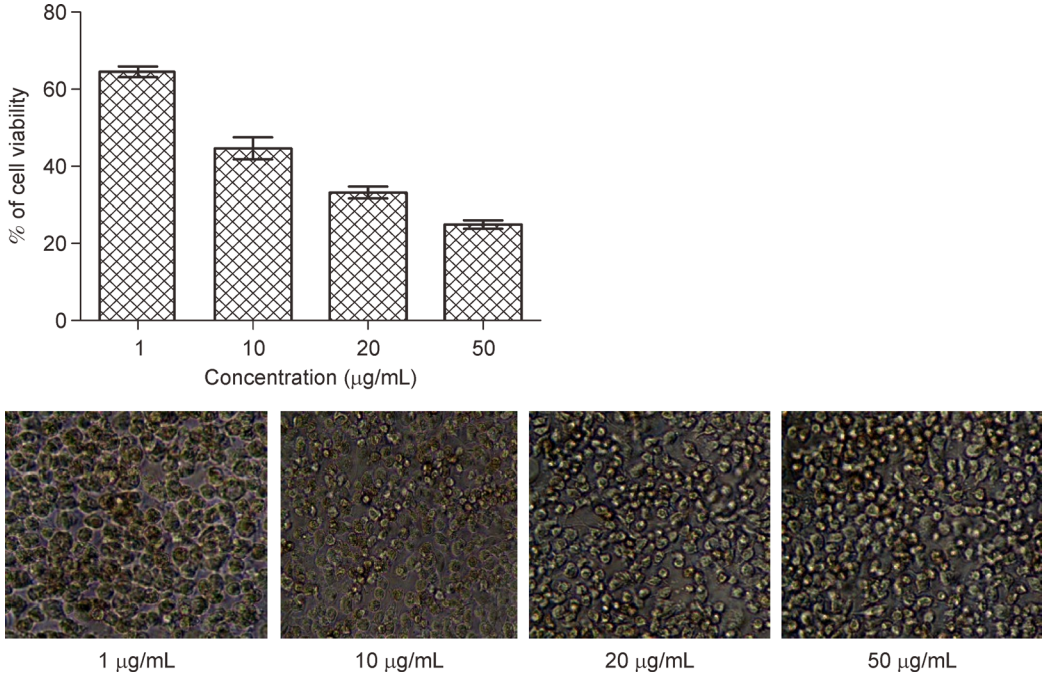

Figure 5 (A) Cytotoxic assay: Cell viability percentage demonstrates the active pigment concentration lethal to Hep G2 cell lines. (B) Morphological changes in HepG2 cell lines after treated with prodigiosin pigment (at different concentrations) extracted from *Serratia marcescens* VITASP.

studies on understanding the chemical nature of the bioactive compound and molecular mechanism of interaction against human cancer cell lines will pave a way for novel drug molecules that combat the dreadful diseases. These findings can help in developing an efficient strain for the production of bioactive molecules.

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Compliance with ethics guidelines

Aruna Muthukumar, Pallavi Pradeep, Isha Thigale, Mohanasrinivasan V., Jemimah Naine S., C. Subathra Devi declare that they have no conflict of interest. This article does not contain any studies with human or animal subjects performed by any of the authors.

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