Research Article

# Production and Partial Purification of Tannase from Serratia Marcescens Isolated from Different Sources

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## Abstract

Tannase has different benefits in food, chemical and pharmaceutical fields. Seventeen *Serratia marcescens* isolates were collected from septicemia, wound infections and hospital environment(babies incubators).These isolates were identified by biochemical tests and Vitek 2 system that contained Vitek GNI card then conformed by16S rRNA gene products(amplified size 179 bp) for genotypic detection. After that, they screened for higher tannase production and *Serratia marcescens* b9 was a better producer of tannase with a larger diameter of dark green zone. The tannase activity was increased to 63U/ml when this isolate was cultivated under the optimal conditions which consisted of using nutrient broth supplemented with ber leaves at pH value 5.5 and a temperature equals to 37°C for 72 hour. In partial purification of tannase ammonium sulfate was more efficient than organic solvents, since it was found that 70% saturation of ammonium sulfate led to precipitate of tannase with tannase activity of 80U/ml. In contrast, 30% of ethanol, acetone and isopropanol led to precipitate of tannase with different levels of activity ranged between 45-47U/ml. Consequently, ber leaves has a potential as an effective and much cheaper (economical) substrate for tannase production in comparison with traditionally used substrates like tannic acid.

**Keywords:** *Serratia marcescens*, tannase, partial purification, optimum conditions.

## الخلاصة

يمتلك انزيم التانيز عدة تطبيقات غذائية, كيميائية وصيدلانية. تم جمع 17 عزلة تعود لبكتريا *Serratia marcescens*من حالات تسمم الدم واصابات الجروح وبيئة المستشفى(حاضنات الاطفال). شخصت تلك العزلات بالاختبارات الكيموحيوية ونظام الفايتك الثاني واكدت باستخدام ناتج جين 16S rRNA (حجم القطعة المكثرة 179 زوج قاعدة) كتشخيص وراثي. بعد ذلك غربلت العزلات لتحديد العزلات المنتجة لانزيم التانيز وكانت العزلة b9 *Serratia marcescens* هي المنتج الافضل لانزيم التانيز باكبرقطر للمنطقة الخضراء المعتمة. لقد ازدادت فعالية انزيم التانيز الى 63 وحدة/ مل عندما نميت تلك العزلة تحت الظروف المثلى المتضمنة استخدام وسط اوراق السدر الهندي ذو الرقم الهيدروجيني 5.5 وبدرجة حرارة 37 ºم لفترة حضن 72 ساعة. في التنقية الجزئية لانزيم التانيز وجد بان املاح كبريتات الامونيوم كانت اكثركفاءة من المذيبات العضوية, حيث وجد بان نسبة الاشباع 70% للملح ادت الى ترسيب التانيز بفعالية 80 وحدة/ مل. وبالمقابل وجد بان 30% ايثانول, اسيتون وايزوبروبانول ادت الى ترسيب التانيز بنسب مختلفة تراوحت بين 45-47 وحدة/ مل. وبذلك نستنتج بان اوراق نبات السدر الهندي يمكن استخدامها كمادة اساس فعالة ورخيصة (اقتصادية) لانتاج انزيم التانيز بالمقارنة مع المواد الاساس التقليدية المستعملة مثل حامض التانيك.

## Introduction

Serratia marcescens is appeared as Gram-negative rod shaped bacterium, formed a part of *Enterobacteriaceae* family [1]. Although, *S. marcescens* was considered a nonpathogenic saprophytic organism it appeared on decaying organic matter besides to plants and animals. Also it found in foods, water and soil [2]. *S. marcescens* is now become an opportunist pathogen causing nosocomial infections since it associated with a wide range of hospital-acquired infections caused such, septicaemia, keratoconjunctivitis, conjunctivitis wound and eye infections, keratitis, meningitis, pneumonia, endocarditis, osteomyelitis and endophthalmitis besides to respiratory tract infections and urinary tract infections(UTI) [3]. Tannins are polyphenols have the ability to dissolve in water [4] that appeared in plants as secondary metabolites [5]. They have many

biological functions for plants such as the defensive function versus diseases that resulting different microorganisms. Tannin bitterness associated with protection the tissues of plants from aggression of different insects [6].

Tannase (tannin acyl hydrolase, EC 3.1.20) is an enzyme stimulates the hydrolysis of ester and depside linkages in hydrolysable tannins like tannic acid to give gallic acid and glucose [7]. Tannase is inducible intracellular/extracellular enzyme, in nature, for the purpose of commercial production of tannase the microorganisms were used because their tannase have higher stability in comparison with the plants and animals tannase [5].

Tannase used as a foaming agent for the tea and as a clearing agent and preventing for Phenol-induced braiding in beer, wine and fruits juices production besides to extensively using in chemical, food and pharmaceutical industries [8]. In animals tannins have a toxic nutritional effect, since they can decrease from nutritional digestibility and protein availability, therefore; tannase leads to reduce the nutritional qualities in animal feeding [9]. The liberated gallic acid has an important role in the food industry as antioxidant and in the processing of trimethoxy benzaldehyde in pharmaceutical industry that enters in the production of trimethoprim: an antimalarial as antibiotics [8]. Thus the purpose of this research was to screen the productivity of tannase by *Serratia marcescens* and optimization preventing for Phenol-induced braiding of medium conditions for increasing the yield with lower cost and comparison its partial purification by salts and solvents.

## Materials and Methodology

### *Isolation and identification of S. marcescens*

The bacterial isolates that primarily was identified as *S. marcescens* were isolated from blood and wounds samples from patients submitted to Baghdad hospitals-Iraq and suffering from septicemia and wound infection, besides to hospital environment (babies' incubators) during the period between August-October 2016.

### *Bacteriological analysis of S. marcescens*

Bacteriological analysis was performed depending on Berge's Manual of systemic bacteriology by using morphological and biochemical tests such as the shape of the colonies, oxidase and catalase tests and the motility test [10]. Further, *S. marcescens* isolates were corroborated by using complementary Vitek 2 system that contained Vitek GNI card and used as described by bio Mérieux, France

### *Genotyping detection of S. marcescens*

For genotypic diagnosis 16S rRNA was used. A specific primer revealed in Table 1 was employed to amplify a fragment with size of 179 bp. DNA template was extracted by boiling method that described by [11]. Briefly, few colonies of overnight bacterial isolates were suspended in 1 ml distilled water and boiled for 10 min in water bath. The suspension was centrifuged and the supernatant was used as template DNA. The mixture of PCR contained about 12.5µl of GoTaq Green Master Mix (2x), 5 µl template DNA, 1.5µl primers (for each) final concentration (0.6pmol/µl) and nuclease free water up to 25µl. PCR cycle was started with a step of primary denaturation at 95°C for 5 min after that 35 repeated cycles each cycle started with a step of denaturation for 1 min at 94°C, a step of annealing for 1 min at 60°C and extension step at 72°C for 1 min later a final extension step for 7 min at 72°C. The products of PCR were electrophoresed in 1% agarose gel by using 5µl of the reaction product and 10kb DNA ladder (Kapa, south Afreqa) as a molecular marker and visualized under UV light[12].

### Screening of tannase production from *S. marcescens*

#### *1-Semi-quantitative analysis*

All the bacterial isolates were inoculated to nutrient agar plates containing 2 %( w/v) tannic acid. After incubation period at 37°C for clinical isolates and 30°C for environmental isolates for 24 hour an appearance of a greenish brown zone around the colonies refer to tannase production and then the diameters of

dark green zones that surrounding the colonies was curculated [14].

***2- Quantitative analysis***

All the bacterial isolates were inoculated to nutrient broth supplemented with 2 %( w/v) tannic acid after that incubated at 37°C for clinical isolates and 30°C for environmental isolates for 24 hour.After centrifugation at 8000 rpm for 20 min. The resulting supernatant considered as the crude extract to establish the tannase activity.

Table 1: PCR Oligonucleotide [13].

| No. of cycle | Tm | 5 –3 | Name of primer |
|---|---|---|---|
| 35 | 60° C | F- GGTGAGCTTAATACGTTCATCAATTG<br>R- GCAGTTCCCAGGTTGAGCC | 16S rRNA |

**Tannase assay**

Tannase activity was measured spectrophotometrically by using tannic acid as a substrate and releasing of gallic acid and glucose by modification the method that described by [15]. One ml of crude extract was incubated for 30 min at 37°C with 1ml of 0.1M acetate buffer at pH =5.0 supplemented with 0.5% tannic acid as substrate after that to denaturate of the enzyme activity the mixture was incubated in boiling water bath for 15 min. From this mixture 1 ml was used and mixed with 3,5- Dinitrosalicyclic acid reagent and the final volume was diluted by adding 10 ml of distilled water and the absorbency was measured at 540 nm. Tannase activity was calculated from the following formula:

Enzyme activity (U/ml) = µg of liberated glucose / V x T, Where µg of liberated glucose can be taken from the standard curve, V is the volume of enzyme sample and T is the hydrolysis time.

Standard curve was performed by preparing series dilutions ranged between 100-1000 µg/ml of glucose solution. One unit of tannase activity is defined as the amount of enzyme releasing 1µmol $min^{-1}$ of glucose under assay conditions.

## Determination of Protein concentration

The protein concentration of tannase was measured using Bradford dye method with bovine serum albumin as a standard [16].

## Optimization of growth conditions for tannase production from *S. marcescens*

***1- Effect of culture medium on tannase production from S. marcescens***

A- In tannic acid broth (TAA), that composed of (g/l) peptone 5.0, sodium chloride salt 5.0, yeast extract 1.5, beef extract 1.5 and tannic acid 5.0 [17], pH 5, the tannic acid was replaced with various tannin-rich agro-residue wastes such as tamarind seed, mango leaves, guava leaves, eucalyptus bark, amla bark, leaves and fruit, jamun leaves, pomegranate rind, mulberry leaves, keekar leaves, amaltash leaves and ber leaves as carbon sources for tannase production. The chosen isolate was inoculated to each of these media and incubated at 37°C for 24 hour. The resulting culture was centrifuged at 8000 rpm in cooling centrifuge for 15 min and the resulting supernatant used as a crude extract then the tannase activity measured.

B- Nutrient broth supplemented with 2 %( w/v) tannic acid and the best tannin-rich agricultural waste, separately, was prepared and inoculated with the chosen isolate then incubated at 37°C for 24 h. After centrifugation the tannase activity was measured for each crude extract.

***2- Effect of pH on tannase production from S. marcescens***

Nutrient broth supplemented with 2 %( w/v) the best tannin-rich agricultural waste was prepared with different pH values ranged from 2 to 8. The resulted media were inoculated with the selected isolate and incubated at 37ºC for 24hours after that the tannase activity was measured.

***3-Effect of incubation temperature on tannase production from S. marcescens***

Nutrient broth supplemented with 2%(w/v) the best tannin-rich agricultural waste at pH 5.5 was inoculated and incubated with the chosen isolate at several temperatures (15,20,25,30, 35,37,40 and 50ºC) for 24 h later tannase activity was measured.

***4-Effect of different incubation periods on tannase production from S. marcescens***

Nutrient broth supplemented with 2%(w/v) the best tannin-rich agricultural waste was inoculated with chosen isolate and incubated at 37°C for several incubation periods ranged to 24, 48, 72 and 96 hour then tannase activity was measured.

## Extraction and partial purification of tannase from *S. marcescens*

The selected isolate was grown in the nutrient broth supplemented with 2 %( w/v) the best tannin-rich agricultural waste at 37°C for 72hour. the culture after incubation time was centrifuged in cooling conditions at 8000 rpm for 20 min and crude supernatant was subjected to partially purification by two methods: the first included the precipitation with ammonium sulphate at concentration (20-80%) saturations and in the second method ethanol, acetone and isopropanol at a concentration of 95% were added to the crude extract to obtain 20, 30, 40,50,60,70 and 80 % concentrations in ice bath. The resulting samples were stored at 4°C overnight, the samples were centrifuged at 1000 rpm for 15 min and the precipitates were dissolved in 0.1M acetate buffer (pH 5.0).The tannase activity, protein concentration and the specific activity were measured. The samples that obtained from precipitation step with ammonium sulfate were dialyzed overnight against the same buffer in cold conditions.

## Results and Discussions

In this study, 17 *S. marcescens* isolates were collected including 1(5.8%) isolate from wound infection, 2 (11.7%) isolates from hospital environment(babies incubators) an 14(82.3%) isolates from septicemia as shown in Figure 1. These isolates were diagnosed by using many morphological and biochemical tests and conformed by Figure 2 that showed positive agarose gel electrophoresis results for 16S rRNA gene products (amplified size 179 bp) and used for genotypic detection of *S. marcescens* isolates.

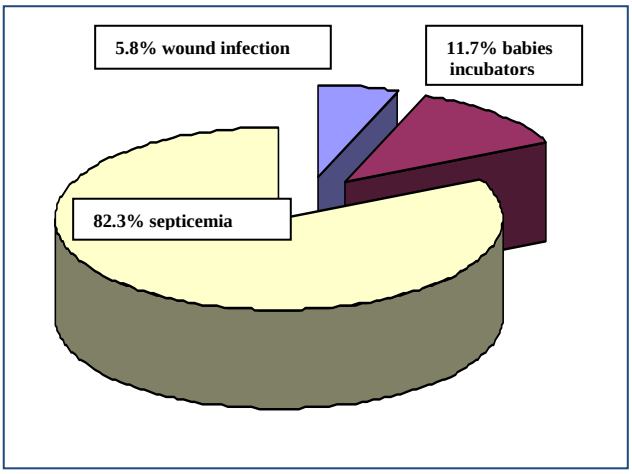



Figure 1: Percentage of *S. marcescens* isolation from different sources.

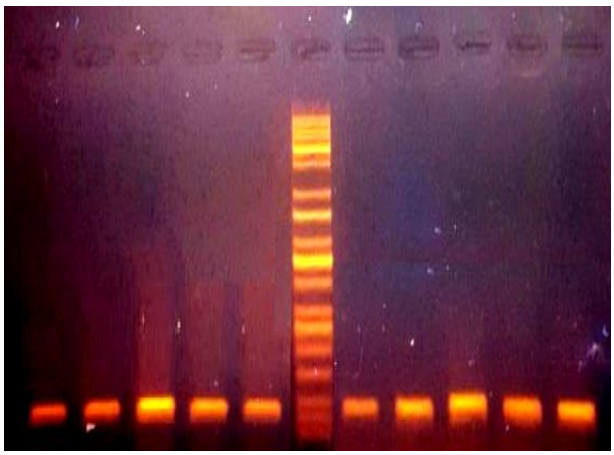


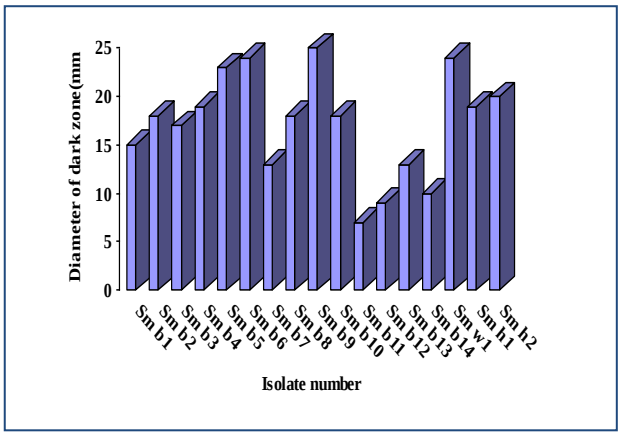
Figure 2: Gel electrophoresis (1% agarose, 7 V/cm for 90 min) for 16S rRNA gene in *Serratia marcescens* isolates. Line M 10Kbp DNA ladder, and all others lines positive results with 179 bp amplicon.

## Screening of tannase production from *S. marcescens*

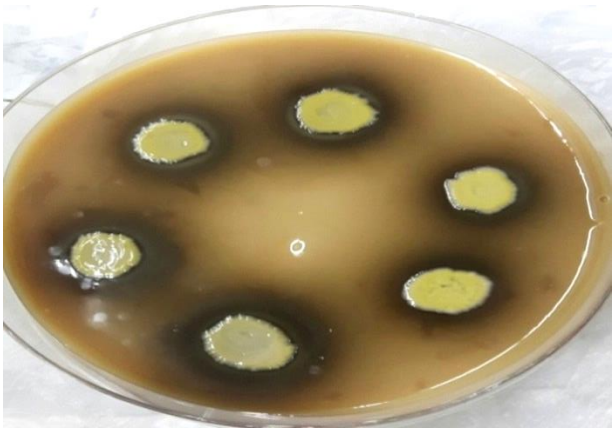
***1-Semi-quantitative analysis***

Tannase enzyme is an extracellular inducible enzyme that stimulates the hydrolysis of ester and break bonds in the tannins releasing of gallic acid and glucose. All 17 *S. marcescens* isolates showed a visible dark green zone of gallic acid around the growing

colonies in nutrient agar plate supplemented with 2% tannic acid (Figure 3). The diameters of these halos were measured as revealed in Figure 4 since they ranged between 7-25 mm and *S. marcescens* b9 was a better producer of gallic acid with a diameter of dark green zone equals to 25 mm so that this isolate considered as a better producer of tannase.




**A**



**B**

Figure (3-A and B):Diameter of dark green halos surrounding the colonies of *Serratia marcescens* isolates.

### *2- Quantitative analysis*

The quantitative screening included using nutrient broth supplemented with 2%(w/v) tannic acid and detection the amount of reduced glucose released by using 3,5-dinitrosalicylic acid reagent. According to the Figure 4 all seventeen *S. marcescens* isolates revealed different levels in tannase production ranged from 103 to 168 U/mg with maximum tannase activity equals to 42 U/ml and 168 U/mg specific activity by *S. marcescens* b9 .

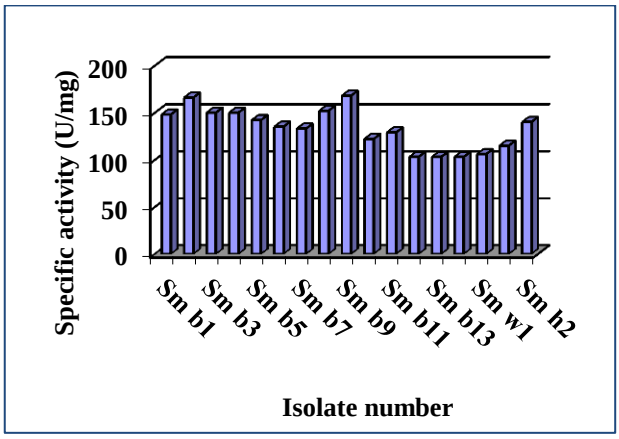



Figure 4: Specific activities for all *Serratia marcescens* isolates.

## Optimization of growth conditions for tannase production from *S. marcescens*

### *1- Effect of culture medium on tannase production from S. marcescens*

Sixteen different culture media were used for determining the optimal culture medium for tannase production by *S. marcescens* b9. The result showed that among thirteen various tannin-rich agro-residue wastes the ber leaves was the best medium and gave higher tannase activity 39U/ml followed by tannic acid that gave tannase activity 37 U/ml in comparison with the other used agro-residue wastes. In contrast, the combination of nutrient broth with tannic acid and ber leaves, separately, led to increase the tannase activity to 42 and 48U/ml, respectively (Figure 5). Thus we can conclude that nutrient broth supplemented with ber leaves was the best medium for tannase production followed by nutrient broth supplemented with tannic acid. While the remaining media gave tannase activity ranged between (32-39U/ml). On the other hand, amla bark revealed lower tannase activity 27U/ml.

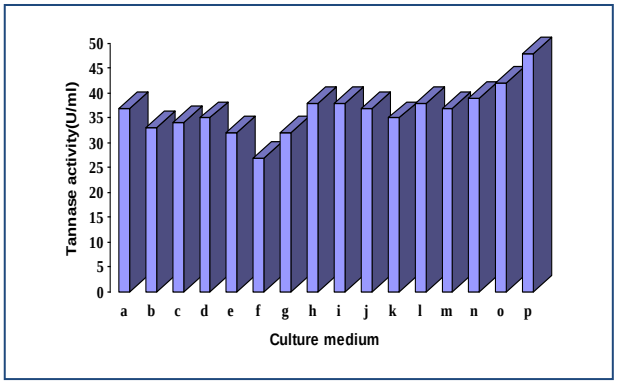



Figure 5: Tannase production by *Serratia marcescens* b9 cultured in different culture media
a:tannic acid, b:tamarind seed, c:mango leaves, d:guava leaves, e:eucalyptus bark, f:amla bark, g:jamun leaves,h:pomegranate rind, i: amla fruit,j: mulberry leaves, k:keekar leaves, l: amla leaves, m: amaltash leaves,n:ber leaves,o: nutrient broth with tannic acid and p: nutrient broth with ber leaves

### *2- Effect of pH on tannase production from S. marcescens*

The productivity of tannase was variable at different pHs. The results showed that the optimal pH for tannase production by *S. marcescens* b9 was 5.5 with tannase activity 52U/ ml than other pH values that led to reduce the activity since reduced to 24 and 28 U/ml when the pH of the medium was 2 and 8, respectively (Figure 6).

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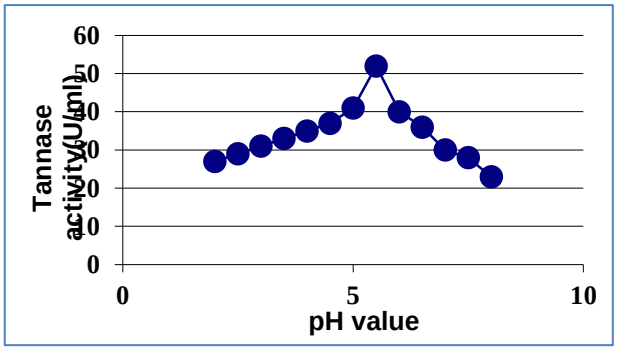



Figure 6: Tannase production by *Serratia marcescens* b9 at different pHs.

### *3-Effect of the temperature on tannase production from S. marcescens*

*S. marcescens* b9 revealed higher tannase activity at 37°C with 52 U/ml, and then the activity was decreased to 33, 46 and 44 U/ml at 20, 40 and 50°C, respectively. While lower level of tannase production was obtained at 15°C with tannase activity 23 U/ml (Figure 7). We can conclude that 37°C is favored for tannase production.

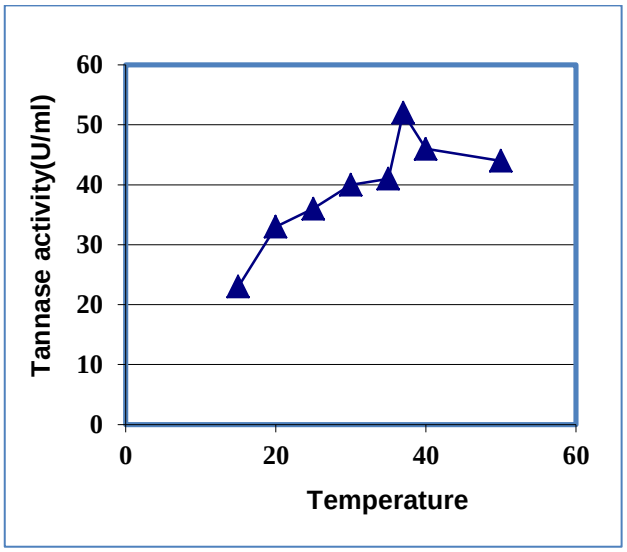


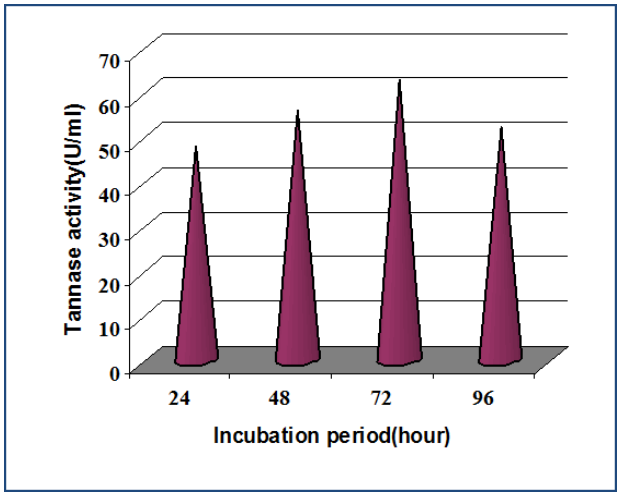

Figure 7: Tannase production by *Serratia marcescens* b9 at different temperatures

### *4-Effect of different incubation periods on tannase production from S. marcescens*

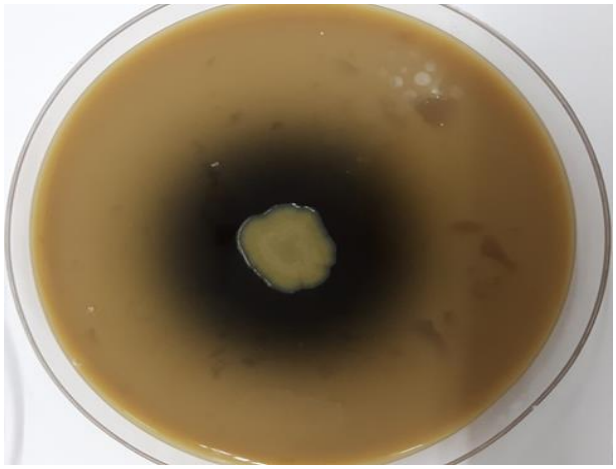
The results revealed that *S. marcescens* b9 after 24 h of incubation gave 48 U/ml of tannase activity and the activity increased with increasing the time of incubation and reached to the maximum value(63U/ml) after 72 h of incubation (Figure 8). While after 96 h it tarted tannase activity with decrease.

### Extraction and partial purification of tannase from *S. marcescens*

*S. marcescens* b9 tannase was partially purified by two precipitation methods; the first with ammonium sulfate and the second with organic solvents such as ethanol, acetone and isopropanol. Ammonium sulfate was more efficient than organic solvents, since it was found that 70% saturation of ammonium sulfate led to precipitate of tannase with tannase activity of 80U/ml and specific activity of 320U/mg. In contrast, 30% of ethanol, acetone and isopropanol led to precipitate of tannase with different levels of activity ranged between 45-47 U/ml and the maximal level of specific activity was 300U/mg in case of acetone using as shown in Figure 9 and Table 2.




A

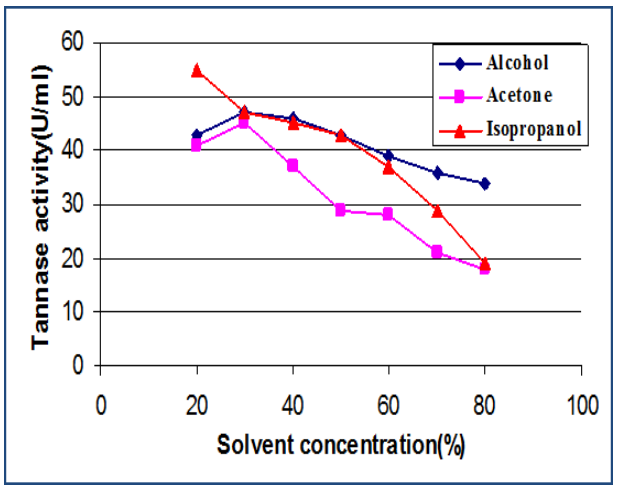


B

Figure 8: A) Tannase production by *Serratia marcescens* b9 at different incubation periods B) tannase production after 72 hour.

*S. marcescens* are opportunistic pathogens that responsible for 2% of nosocomial infections) such as septicemia, urinary tract infection, wound infection, respiratory tract infection, meningitis, sepsis, and endocarditis [18]. In the hospital acquired infections the mortality rate caused by *Serratia* in the blood stream infections reached to 26%, in contrast, this rate was very low in case of the urinary tract infections [19].

*S. marcescens* was rarely isolated from clinical specimens [20]. In a research reported by [21] found that in chronic surgical site infections the sixth and seventh most common organisms were *E. coli* and *Serratia* spp., respectively. Also [22] revealed that out of 32 different Gram- negative isolates mostly from urine, pus, throat swabs and blood specimens, the *Serratia marcescens* isolates were six

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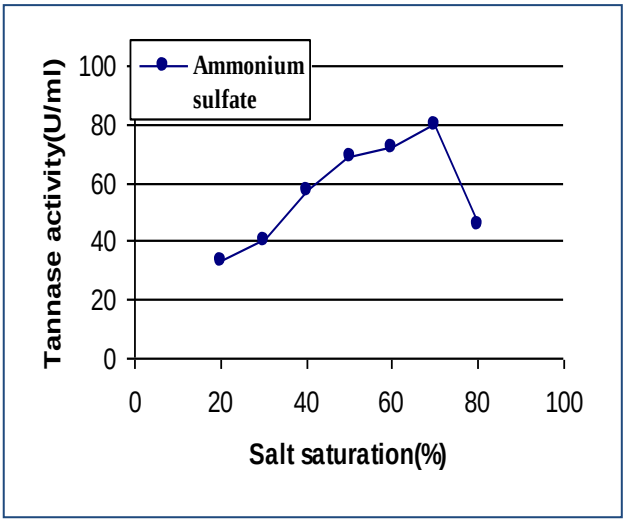


B

Figure 9: Extraction of tannase by using (A) ammonium sulfate (B) organic solvents.

The growth of bacterial isolates on tannic acid containing nutrient plates indicated that these isolates can degrade of tannic acid to their simpler structures like gallic acid [23, 24]. This formed dark green halo of gallic acid was formed during the breakage of tannin-protein complex by bacteria producing tannase and led to form a brown zone around the colonies after prolonged incubation [19]. Nandini *et al.*, [25] observed that a high correlation between the dark green zone and the quantitative tannase enzyme production.

Table 2: Partial tannase purification from *Serratia marcescens* b9.

| Purification step | Size(ml) | Tannase activity (U/ml) | Protein conc. (mg/ml) | Specific activity (U/ mg) | Total activity | Purification fold | Yield (%) |
|---|---|---|---|---|---|---|---|
| Crude supernatant | 20 | 63 | 0.43 | 146.5 | 1260 | 1 | 100 |
| $(NH_4)_2SO_4$ precipitation (70%) | 10 | 80 | 0.25 | 320 | 800 | 2.18 | 63.5 |
| Ethanol (30%) | 10 | 47 | 0.21 | 223 | 470 | 1.52 | 37.3 |
| Acetone(30%) | 10 | 45 | 0.15 | 300 | 450 | 2.04 | 35.7 |
| Isopropanol (30%) | 10 | 47 | 0.18 | 261 | 470 | 1.78 | 37.3 |

The better zones of hydrolysis indicated for better tannase producing species and their tannin degrading ability as referred by [24].

Few bacterial genera are known with productivity to tannase including *Bacilli*, *Corinebacterium*, *Lactobacillus* and *Serratia* [26]. The tannic acid when used as a sole carbon source revealed notable increasing in the turbidity due to the bacterial growth that complemented with decreasing in brown- black color of the medium [27]. The mechanism in which the stimulation of tannase production in the microorganism still unclear while there was suggestion that intermediate compounds produced from tannic acid hydrolysis since tannic acid cannot penetrate the cell membrane and stimulates tannase production. The presence of glucose in the reaction medium refers to the production of tannase and starting of tannic acid hydrolysis to intermediate structures [27]. In contrast, Riul *et al*., [28] observed that high tannic acid concentrations led to reduction in tannase production as result to gallic acid precipitation on the surface of the cell. In the culture medium the liberated glucose and gallic acid refer to breakdown of tannic acid that added as the sole carbon and energy source [7]. The differences in tannase productivity by bacterial isolates may due to differences in the sources of these isolates or the variation in the gene expression for synthesis of tannase [29].

In Miller method the amount of liberated glucose in the production medium was associated with high values of enzyme activity. This method his method is reliable, economical, time-savers and simple than the other methods. While the limitation to this method it is not specific for liberated glucose and may also give false results when interfered by other reducing sugars [17].

The presence of yeast extract and beef extract in fermentation medium led to enhancement of tannase production by *Serratia ficaria* [30]. The extract of chestnut was used as carbon source for tannase productivity by Bacillus *polymyxa* [31]. While Muslim *et al*., [32] reported that basal medium that containing 5% of pomegranate peels powder was the best medium for enhancement of tannase productivity in *Erwinia carotovora*. Different tannin rich plant extracts such as *Eucalyptus tereticornis Acacia auriculiformis* and *Ficus bengalensis* were used for tannase production as a rich tannin source by *Bacillus licheniformis* KBR6[33]. *Pseudomonas aeruginosa* was also screened for tannase productivity by using four tannin rich leaves plants including *Phylanthus emblica, Eugenia cuspidate, Acacia nilotica* and *Syzygium cumini* [34].

Tamarind seed was used in solid state fermentation as carbon source [6]. Also [35] reported that tamarind seed powder led to maximum tannase production. In contrast, the presence of catabolic repression for biosynthesis of proteins may be referred to an inhibition of tannase production.

There is a certain pH for each microorganism for induction of the gene and subsequent

expression of the enzyme [36]. It is found that maximum bacterial tannase production achieved in very stringent environment since most of the tannase production ranged from acidic pH= 4.5 to neutral pH= 7.0. The tannase biosynthesis associated with tannic acid refraction and formation of gallic acid production, both of them create the acidic environment. So that, fermentation process at lower and higher pH had determined effect in tannase production. The catalytic property of the tannase is strongly affected by the ionic property of the amino groups and carboxylic groups on the protein surface [6].

Maximum tannase production by *Klebsella pneumoniae* was obtained at pH5.2 and tannase activity was appeared to be low at lower and higher pH as reported previously by [37], who also reported that the acidic pH was favored for tannase activity whereas it decreased in the alkaline ranges and this may increase the benefit with using tannase in food-processing industry such as clarification of acidic beverages. The effectiveness of pH on tannase production results from the protonation or deprotonation of its amino acids and their active sites, it may also leads to ionization of amino acids and formation of structural changes [38].

A similar observation was found by [39] who reported that *Serratia* showed a good tannase activity at 37°C. Another study by [40] on the tannase produced by *K. pneumoniae* MTCC 7162 revealed that higher tannase production was reached at 37°C. While [37] showed higher level of tannase production at 35°C and the tannase activity was decreased at the temperatures that lower or higher than this value.

Different biosynthetic processes in microorganisms and transportation of various components through the cell membrane affected with culture temperature [36]. Earlier studies on bacterial tannase production reported that at low temperature the tannase yield was decreased due to their lower transport of substrate through the cell membrane and mesophilic temperature range led to optimum tannase yield since tannase production stated with increasing of kinetic energy of reacting molecules and increasing of the reaction rate [41]. On the other hand, the higher temperatures caused thermal denaturation of metabolic pathway which increased the maintenance energy requirement of cellular growth and leading to poorer metabolites production [6].

Kumar *et al*., [37] demonstrated that the increasing in incubation period caused an increasing in tannase activity by *K. pneumoniae* MTCC 7162 up to 92 h and after this time the activity started with decreasing and this may be because of consumption of nutrients that found in the production medium. Five different bacterial isolates collected from the tannery effluent and screened for tannase production by plate assay method and showed high level of tannase productivity after 96 hours [17]. Also Kuppusamy *et al.*[35] revealed that maximum production of tannase was at 96 h and after this time of fermentation the enzyme production decreased because of lacking of the substrate. *Citrobacter* sp isolated from water and soil specimens gave maximum tannase production after 48 hours [42]. After 24h of incubation all strains of *Serratia* sp. and *pantoea* sp. that isolated from olive mill by products completely degraded all the added tannic acid and depletion of the tannic acid content [7].

Maximum bacterial tannase production reported after 24h of incubation and maximum productivity of tannase was at the stationary phase of growth [43, 34]. Deschamps *et al.* [31] reported that optimum production of tannase from *Bacillus plumilus*, *B. polymyxa*, *Corynebacterium* sp. and *K. pneumoniae* was at early exponential phase of growth. This phenomenon may be due to prolonged incubation period of the cells in presence of tannic acid and accumulation of gallic acid in the production medium, both of them formed acidic environment and slowing the biosynthesis of enzyme [41].

The initial partial purification with ammonium sulfate precipitation showed a very low

recovery of tannase [44]. 80% saturation with ammonium sulphate led to kept 34% of the total tannase and the removal for some contaminant proteins [45]. On the other hand, [46] reported that at low concentration of isopropanol, acetone and ethanol the tannase activity increased but with increasing of these compounds in the reaction medium led to reduce of tannase activity since about 30% of tannase activity lost in the presence of these solvents. The precipitation with ethanol and acetone resulted in a loss of enzyme activity (completely inhibited) as mentioned by [43]. For partial purification of tannase from *Citrobacter freundii* an ammonium sulphate at 65% saturation gave a yield of tannase reached to 60.7 % [37].

## Conclusions

*S. marcescens* is caused *to* septicemia infections in higher rate. All *S. marcescens* isolates were able to produce tannase and *S. marcescens* b9 was a better producer. The optimum conditions for tannase productivity included of using nutrient broth supplemented with ber leaves at pH 5.5 and a temperature of 37°C for 72 hour and In partial purification of tannase the ammonium sulfate was more efficient than organic solvents.

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