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Effect of Various Parameters on Pectinase Producing Activity of Two Bacterial Isolates and Application of Crude Enzyme in Apple Juice Extraction

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Abstract: ***Two pectinase producing bacterial strains were isolated from decayed oranges and tomato. Preliminary identification of two potential isolates was carried out by Gram's staining and biochemical tests. Based on Gram's staining and microscopic examination, two bacterial strains, O6 and T2, are Gram positive Cocci shaped bacteria. Both strains showed different pectinolytic zones depending on the concentration of inoculum and the larger pectinolytic zones were observed by O6. The investigation of optimum crude enzymes producing activity of isolated strains was done at different temperature, pH, reaction time and substrate concentration. The best enzyme activity was observed at seven days incubation period and it was found that the optimum reaction temperature were 40°C and 45°C for isolate O6 and T2 respectively. Both two isolates showed maximum activity at pH 5 and the optimum reaction time at 35mins as well. The optimum pectinase producing activity of isolate o6 and T2 occur at 1% pectin and then remain constant for higher than 1%. The highest protein content of the cell free supernatant was found that the isolate O6 is 0.514 mg/ml followed by T2 which is 0.453 mg/ml. Between two bacterial isolates, O6 showed better pectinase activity than T2 at all optimum conditions except reaction temperature.***

Keywords: pectinase, pectin, bacteria, decayed fruits and vegetables

1. Introduction

Pectinase, that break down pectin is a well-known term for commercial enzyme preparation; a polysaccharide substrate, found in the cell wall of plants. This enzyme splits polygalacturonic acid into monogalacturonic acid by opening glycosidic linkages. Through this process, it softens the cell wall and increase the yield of juice extract from the fruits [1].

Plant and microorganisms are two major sources of the pectinase enzyme. But, microbial source of pectinase has become increasingly important for both technical and economic point of view. A great variety of microorganisms such as bacteria [2] yeast [3] and mold are capable of producing pectic enzymes. The composition of pectic enzymes varies among species of microorganisms. It has been reported that microbial pectinases account for 25% of the global food enzyme sales [4].

Bacterial extracellular pectinase are the most significant not only among all the microbial pectinases but also compared with animal, plants, viruses and fungal extracellular pectinase. Extracellular pectinase produced by Bacillus and Cocci species are of main interest from a biotechnological perspective, and are not only in scientific fields of protein chemistry and protein engineering but also in applied fields such as foods, pharmaceutical and paper industries. These pectinases account for 10% of the total worldwide production of enzymes [5].

Microbial pectinase can be shown as the most important enzyme for the fruit juice industry. Although most of the microorganisms can produce pectinase, only microbes that produce extracellular pectinase are of great importance in industrial application and have been commercially applied. Among these, strains of Cocci species dominate the industrial sector.

Pectinases are widely used in food industries for fruit juices extraction and clarification. And it's also used removal of pectin in fruit juices, in maceration of vegetables to produce pastes and purées and in winemaking, is often produced by Cocci species [6]. In extraction process, pectinase increases the fruit juice yield, decreases the viscosity of juice and degrades the gel structure, thus improves the juice concentration capacity [7]. In fruit juice extraction, enzymatic maceration can develop yields by more than 90% compared to conventional mechanical juicing, besides improving the organoleptic (color, flavor) and nutritional (vitamins) properties and technological efficiency (ease of filtering). In several processes, pectinolytic enzymes can be applied in associated with other cell wall degrading enzymes such as cellulases and hemicellulases [8]. The pectinases and cellulases mixture has been reported to improve more than 100% juice extraction yields. Soares reported that an improvement between three and four times in fruit juice yields from papaya, banana and pear using enzymatic extraction instead of the conventional pressing process [9]. The treatment of enzymatic can be help in decrease of 62% of the apple juice viscosity. Pectinase preparations which are commercially available to be used in food processing are traditionally associated with polygalacturonases, pectin

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lyases and pectin methyl esterases.

A large number of strains of microorganisms such as bacteria, fungi, yeast and actinomycetes have ability to degrade pectin. However, Bacterial strain are always preferred over fungal strain because of ease in fermentation and strain improvement which can be carried out easily in bacterial strain to improve the yield.

The present study was carried out for pectinolytic enzyme production and selection of more efficient isolates for industrial applications. The objective of this work consists of determining the optimum parameters for maximum activity of pectinase enzyme and detection of clarification of fruit juice by crude enzymes of two potential isolated strains.

2. Material and Methods

2.1 Collection of Samples

Fruit and vegetable samples were collected from Mandalay Region and Shan State in Myanmar. The collected samples were taken with the help of sterile plastic bags and transported to the laboratory for bacterial isolation. The samples were labeled and stored at 4 °C for further investigation.

2.2 Isolation of the bacteria

1 g of fruit and vegetables samples were added into 100 ml of 0.9% normal saline as a diluent and made 10 fold serial dilutions. 100 µl from each diluted sample were streaked into sterile pectinase screening agar media (PSAM) plates in triplicate and were incubated at 37 °C for 2–3 days in order to obtained colonial growth. The media composed of (% w/v): pectin (1.0)gm, $(NH_4)_2HPO_4$ (0.3)gm, K_2HPO_4 (0.1)gm, KH_2PH_4 (0.3)gm, $MgSO_4.7H_2O$ (0.02)gm, Agar (2.5)gm, the initial pH of the medium was adjusted to 7. The colony grows well on the agar media were selected and subsequently subculture for pure isolates. Pure cultures were maintained on pectinase screening agar media slants at 4 °C, and also stored as glycerol stocks at −20 °C for further use [10].

2.3 Screening of the bacterial isolates for Pectinolytic Activities

The screening of the bacterial isolates for pectinase activities was carried out by using pectinase screening agar media (PSAM) plates. The initial pH of medium was adjusted to 7 and incubated at 37 °C for 7 days. Plates were flooded with 50 mM potassium - iodide solution and incubated for 15 min at 37 °C and pectinase producing colonies were detected by the appearance of a clear halozone around them. A clear zone around the growth of the bacteria was indicated to pectinase activity [11].

2.4 Identification of the Bacterial isolates

The bacterial isolates which hydrolyzed pectin was characterized by morphological and biochemical tests. The identity of the bacterial isolates was determined with reference to Bergey's Manual of Determinative Bacteriology.

2.5 Submerged fermentation Condition for Production of Pectinase

(1) Inoculum Preparation

A loopful of the bacterial isolates (O6 and T2) showing the halozone of hydrolysis on pectinase screening agar plates was inoculated on liquid medium contained (% w/v): peptone (1.5), yeast extract (0.3), glucose (0.2), NaCl (0.2), K2HPO4 (0.12), Na2CO3 (0.3), pH 7. Medium (60 ml) contained in 250-ml Erlenmeyer flasks were inoculated and cultivated at 37°C by shaking at 100 rpm for 24 hr.

(2) Production of Pectinase

The pectinase production of submerged fermentation was carried out in 250-ml Erlenmeyer flasks, each with 90 ml of the fermentation (the same medium used in qualitative screening without agar-agar). The sterilized medium was inoculated using 10 ml of a 24h-old of the prepared inoculum culture. Fermentation was carried out on a rotary shaker at 100 rpm for 7days at 37 °C. After 7 days incubation, the culture broth was centrifuged at 5000 rpm for 20 min to remove the cells and debris. The supernatant was designated as the crude enzyme which then used for enzyme assay and characterization [12].

2.6 Enzyme Essay

The pectinase activity of crude enzyme (O6 and T2)s measured based on the determination of reducing sugars produced as a result of enzymatic hydrolysis of pectin by dinitrosalicylic acid reagent (DNS) Miller (1959) method. The reaction mixture consisted of 0.2ml of 1% pectin in 2.0ml of sodium citrate buffer of pH 5 and 1.0 ml of enzyme extract. The reaction mixture was incubated at 35ºC for 30 min. After 30 min, 1.0ml of this reaction mixture was withdrawn and added to test tubes containing 0.5ml of 1M sodium carbonate solution. To each test tube, 3.0ml of DNS reagent was added and the test tubes were shaken to mix the contents. The test tubes were heated to boiling on water bath for 10 min and cooled 20ml of distilled water was added to contents of each tube and the absorbance was measured at 540 nm using spectrophotometer. The enzyme and the substrate were run parallel. The standard curve was prepared with galacturonic acid [13].

2.7 Total protein estimation of crude enzyme

The protein concentration of crude enzyme isolated from two potential isolated bacteria was determined by the Lowry's method, as described by Lowry's (1951). Standard graph was plotted between concentration of Bovine Serum Albumin (BSA) and optical density. The protein content of crude enzyme sample was calculated from standard graph by comparing the O.D with standard graph.

2.8 Optimization of incubation periods

The isolated bacterial strains O6 and T2 were subjected to different culture conditions to derive the optimum conditions

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for pectinase activity. Growth and pectinase activity were estimated at regular day's intervals (3, 5, 7, 9, 11, and 13 days). The experiments were carried out (temperature 35°C , pH 5 and reaction time 30 min) in 250 ml Erlenmeyer flask containing 100 ml of basal medium.

2.9 Screening of reaction parameters of crude enzyme

The characterization of crude enzyme was done for the effect of temperature, pH, reaction time and substrate concentration. One parameter was being screened the other two parameters were kept constant (Temperature-35˚C, pH-5, Reaction time-30min).

(1) Effect of temperature on pectinase activity

For determination of different temperature, 0.2 ml of (1%) pectin, 2 ml buffer solution and 1ml of crude enzyme was added and mixed in different test tubes. Tubes were kept at different temperature ranging from (20°C, 25°C, 30°C, 35°C, 40°C, 45°C, 50°C, 55°C, 60°C, 65°C and 70°C) for 30 minutes. After 30 minutes 3, 5- dinitrosalysilic acid was added in all tubes. All tubes were mixed and kept in boiling water bath for 10 minutes. 8 ml distilled water was added. Absorbance was read at 540 nm.

(2) Effect of pH on pectinase activity

Citrate buffer of different pH were prepared with 0.2 ml of (1%) pectin, 2 ml buffer solution and 1ml crude enzyme solution was added in test tubes of various pH (2, 3, 4, 5, 6, 7 and 8) and kept for incubation at 35°C for 30 minutes. After 30 minutes, 3 ml of 3, 5- dinitrosalysilic acid was added in all tubes. All tubes were mixed and kept in boiling water bath for 10 minutes and cooled. 8 ml distilled water was added. Absorbance was read at 540 nm.

(3) Effect of reaction time on pectinase activity

For determination of different reaction time, 0.2 ml of (1%) pectin, 2 ml buffer solution and 1 ml enzyme solution was taken and mixed in different test tubes. Tubes were kept for incubation at different reaction time ranging from (15, 20, 25, 30, 35, 40 and 45) min. After incubation 3, 5-dinitrosalysilic acid was added in all tubes. All tubes were mixed and kept in boiling water bath for 10 minutes. 8 ml distilled water was added. Absorbance was read at 540 nm.

(4) Effect of substrate concentration on pectinase activity

For determination of different substrate concentrations (pectin) from (0.1%, 0.2%, 0.5%, 1%, 1.5% and 2 %) was taken, 2 ml buffer solution and 1 ml crude enzyme solution was added and mixed in different test tubes. Tubes were incubated at 35°C for 30 minutes. After 30 minutes, 3 ml of 3, 5- dinitrosalysilic acid reagent was added in all tubes. All tubes were mixed and kept in boiling water bath for 10 minutes and cooled. 8 ml distilled water was added. Absorbance was read at 540 nm.

(5) Determination of pectinase activity at optimum condition

To determine the effect of optimum pectinase activity were also studied. The optimum temperature, pH, reaction time and substrate concentration of both two isolates were chosen and absorbance was read as 540nm.

2.10 Growth curve of the bacterial isolates

In order to detect the bacterial growth curve, 100ml of pectin broth was prepared and autoclaved. Loopful of culture was inoculated in it and incubated in shaker for 0 to 96hrs.

2.11 Application of pectinase in fruit juice Extraction

The aim of this study is to monitor the activity of enzyme measuring the amount of apple juice released by pectinase use and compare it with the normal protocol in the absence of the enzyme and crude enzyme of isolates (O6 and T2).

Apples purchased from local market were washed, peeled and cut into cubes that are roughly 5 mm on a side. It is important to chop the apple into very small pieces added surface area helps the enzyme break down the pectin in the plant cell walls, releasing more juice. The chopped apples were divided equally between the three beakers. Analytical balance was used to weigh equal amounts of chopped apple (20gms.) into each beaker. 3 ml of the enzyme Pectinase (ANC Enzyme solution PTE Ltd, Singapore) was added to one beaker was used as positive control and 3 ml of the crude enzyme and 3ml of water was used as negative control to the other. Chopped apple pieces were stirred in each beaker with a glass-stirring rod. Be sure to wet all of the pieces. The beakers were covered with aluminum foil and were incubated at a 40°C for isolate O6 and at 45°C for T2 in water bath for 2hr. Followed by incubation, boiling water bath for 5 min to inactivate the enzyme. After incubation from boiling water bath, a wooden spoon was used to gently stir/squeeze the apple pieces in each. Juice from the mash was separated by centrifugation at 3000 rpm for 20 min and was discarded. To filter the juice from the filter paper was used in the funnels. Percent transmittance was determined at 660 nm using a UV-Vis spectrophotometer.

A graph was made using the total volume of juice produced by each treatment and the weight of apples used.

3. Results

3.1 Isolation of the bacteria

In the present study, a total of 82 bacterial strains were isolated from decaying fruit and vegetable samples by using pectin screening agar medium (PSAM). The number of isolates and sources were shown in Table 1.

Table 1: The native bacterial isolates and their sources

Sr. No.	*Samples*	*Number of Isolates*
1.	Oranges	36
2.	Guava	7
3.	Pears	8
4.	Apple	16
5.	Banana	6
6.	Mangoes	-
7.	Tomato	9

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3.2 Screening of pectinase activity of bacterial isolates

A total of 82 bacterial strains were screened for their pectinolytic activity which was observed by the formation of the clear zone around the colonies. Among these 82 isolates, only two bacterial strains (O6 and T2) showed halozone formation of pectinase activity. The results are presented in (Fig 1).

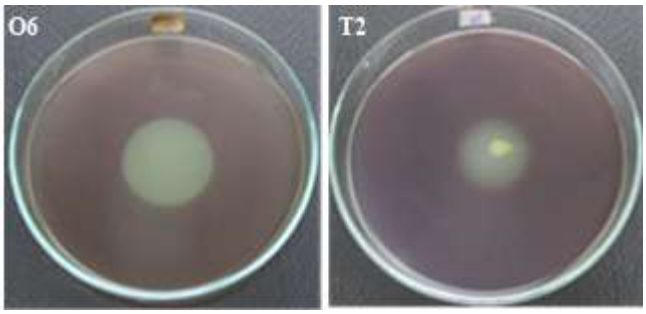

Figure 1: Clear zone formation of isolated bacteria, T2 and O6, on pectinase screening agar media after 7 days incubations

3.3 Biochemical Characterization of the Bacterial isolates

The selected isolated strains (O6 and T2) were further studied for morphological, cultural and biochemical characteristics according to standard techniques and the details are presented in (Table 2, 3 and 4).The two isolates were tentatively identified by following the Bergey's Manual of determinative bacteriology and methods stated in Microbiology –Laboratory Manual. The antibiotic sensibility pattern of isolated strains was shown in Table 5 and (Fig 2).

Table 2: The results of colony characteristics which shows Pectinase activity

Sr no	*Colony Characteristics*	*Bacterial Isolates*	
		O6	*T2*
1	Colony Surface	Smooth	Smooth
2	Colony Colour	Pae Yellow	Brown
3	Visual Characteristics	Translucent	Translucent
4	Shape of the Colony	Circular	Circular
5	High of the Colony	Raised	Raised
6	Pectinase Activity	Positive	Positive

Table 3: The results of Various Biochemical tests

Sr no	*Biochemical tests*	*Samples*	
		O6	*T2*
1	Motility	-Ve	-Ve
2	Catalase	+Ve	+Ve
3	Citrate Utilization	-Ve	+Ve
4	Nitrate Utilization	-Ve	-Ve
5	Starch Hydrolysis	+Ve	+Ve
6	Gelatin Hydrolysis	-Ve	+ve
7	Methyl Red Test	-Ve	+Ve
8	Voges Proskauer Test	-Ve	+Ve
9	Mac Conkey's Plate	-Ve	-Ve
10	Mannitol Salt Agar	-Ve	-Ve
11	Pectnase Activity	+Ve	+Ve
12	Protease Activity	-Ve	+Ve

Table 4: The results of Gram's Staining

Strain No	*Gram Staining*	*Morphology (Bacillus/ Cocci)*
O6	Positive	Cocci
T2	Positive	Cocci

Table 5: Antibiotic Susceptibility Patterns of pectinolytic Bacterial isolates

Sr No.	*Antibiotics*	*Well Concentrations*	*Inhibition zone diameters (mm) of isolated strains*	
			T_2	O_6
1.	Gentamycin	10µg	24(S)	22(S)
2.	Ampicillin	10µg	23(S)	24(S)
3.	Kanamycin	30µg	23(S)	24(S)
4.	Doxycycline	30µg	25(S)	26(S)
5.	Penicillin	10µg	25(S)	24(S)
6.	Ciprofioxacin	5µg	26(S)	25(S)
7.	Chlorenphenicol	30µg	31(S)	24(S)
8.	Tetracycline	30µg	25(S)	24(S)

S= Sensitive

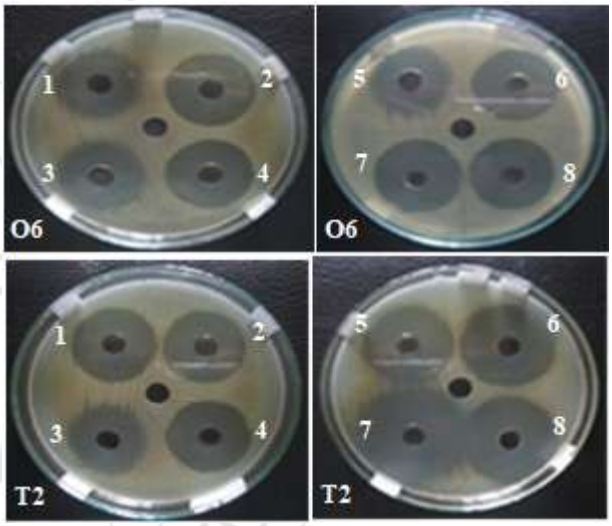

Figure 2: Antibiotic Susceptibility test of Isolated Strains (T2 and O6)

1= Gentamycin 4= Doxycyline 7= Chloremphenicol
2= Ampicilin 5= Penicilin 8= Tetracycline
3= Kanamycin 6= Ciprofloxacin C= Control

3.4 Estimation of protein content

(1) Standard curve

For the determination of concentration of protein in the crude enzyme sample, a standard curve was plotted (Fig 3) with the known concentration of a standard protein bovine serum albumin (BSA).

(2) Protein estimation for crude enzyme

Protein concentration from the crude enzyme sample was determined by Folin-Lowry method. The highest protein content was found in the cell free supernatant of isolate O6 is 0.514 mg/ml followed by T2which is 0.453 mg/ml (Fig 4).

3.5 Effect of incubation period on pectinase activity

The activity was determined by measuring the release of reducing sugar using dinitrosalicylic acid reagent DNS assay (Miller. 1959). The standard graph of galacturonic acid was

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plotted (Fig 5). The effect of incubation period was measured 3, 5, 7, 9, 11 and 13 days incubations. The optimum production of pectinase activity of isolates O6 and T2 occurred at 7 days incubation period (Fig 6).

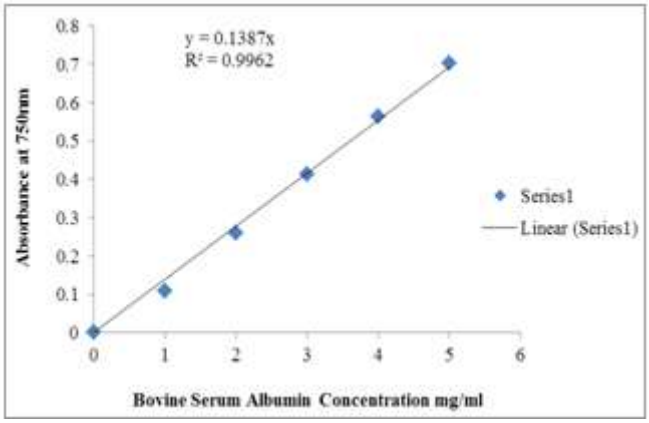

Figure 3: Standard Curve for Bovine Serum Albumin (BSA)

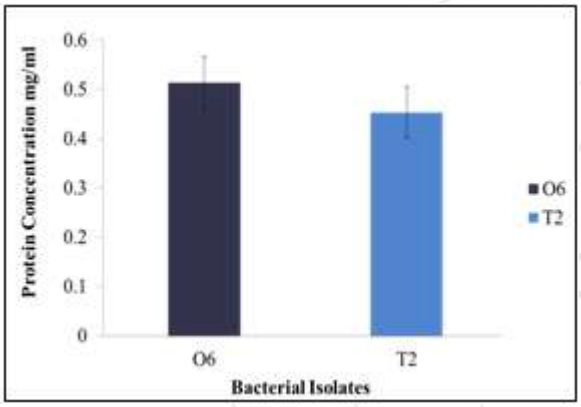

Figure 4: Protein estimation of bacterial isolates O6 and T2

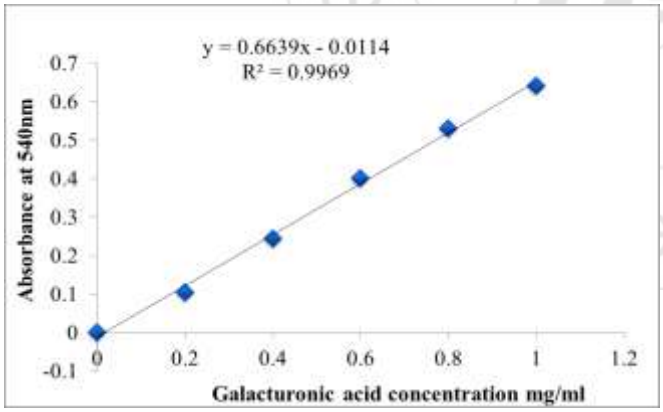

Figure 5: Standard Calibration Curve for Galacturonic acid

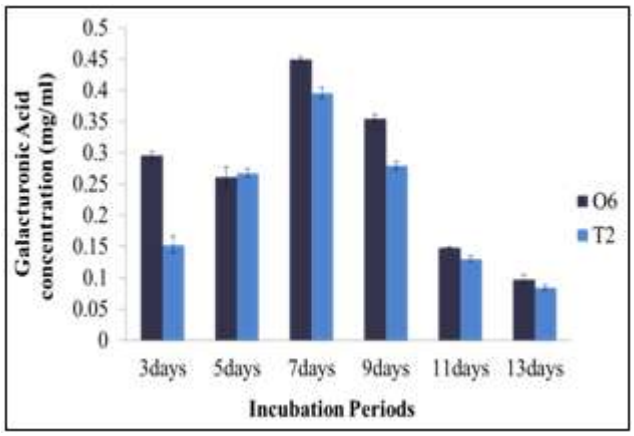

Figure 6: Effect of incubation period on pectinase activity of selected bacterial isolates

3.6 Screening of reaction parameter of crude enzyme

The characterization of crude enzyme sample was done. The effect of temperature, pH, reaction time course and percent of substrate concentration was studied and results were noted as follows.

(1) Effect of temperature on pectinase activity

The effect of temperature on the activity of crude enzyme was studied by DNS method. Different temperatures as 20°C, 25°C, 30°C, 35°C, 40°C, 45°C, 50°C, 55°C, 60°C, 65°C and 70°C were used to determine optimum reaction temperature for enzyme . The influence of temperature on pectinase activity is shown in (Fig 7). The optimum temperature for petinase producing activity was observed at 40°C for O6 isolate and 45°C for T2 isolate.

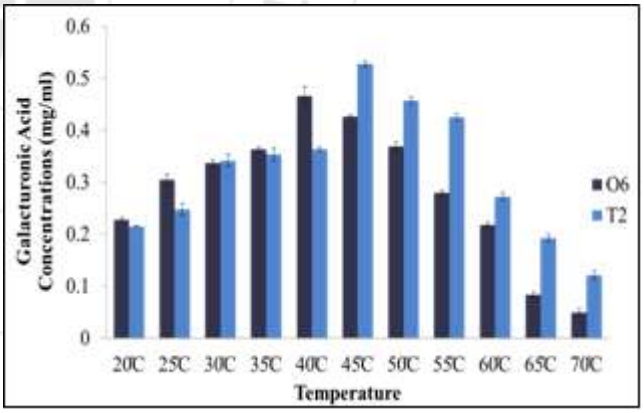

Figure 7: Effect of reaction temperature on pectinase activity of selected bacterial isolates

(2) Effect of pH on pectinase activity

The effect of pH on the activity of crude enzyme was studied by DNS method. The effect of pH on pectinase activity of O6 and T2 were determined at different pH ranging from 2 to 8. The results are shown in (Fig 8).

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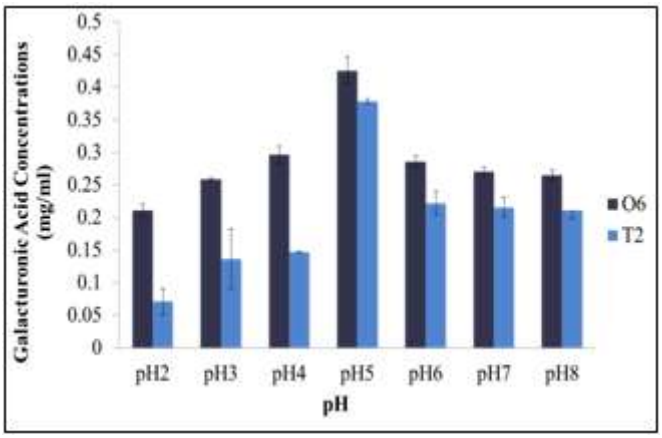

Figure 8: Effect of reaction pH on pectinase activity of selected bacterial isolates

(3) Effect of reaction time on pectinase activity

The DNS method was employed for studying effect of reaction time on pectinase activity. The optimum reaction time for the pectinase activity of isolates O6 and T2 was found to be 35 minutes and show in (Fig 9).

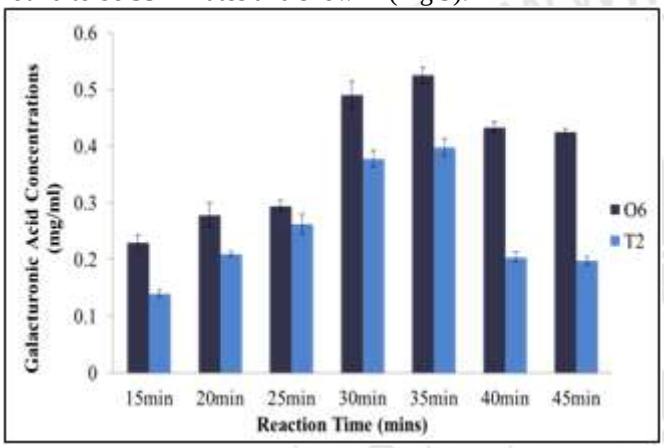

Fig 9: Effect of reaction time on pectinase activity of selected bacterial isolates

(4) Effect of substrate (pectin) concentration on pectinase activity

To determine the effect of substrate (pectin) concentration for pectinase production O6 and T2 were incubated at different concentration of pectin as 0.1%, 0.2%, 0.5%, 1.0%, and 1.5% (Fig 10).

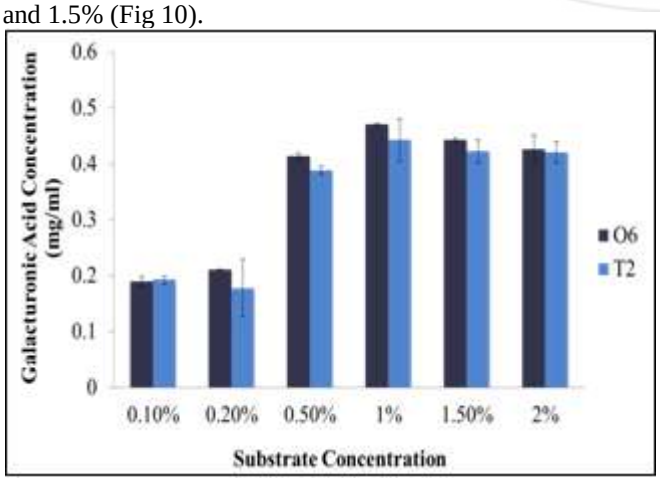

Figure 10: Effect of substrate concentration on pectinase activity of bacterial isolates

(5) Determination of pectinase activity at optimum condition

The effect of optimum reaction temperature, pH, reaction time and substrate concentration (pectin) of pectinase activity of isolated strains (O6 and T2) were studied. The results are shown in (Fig 11).

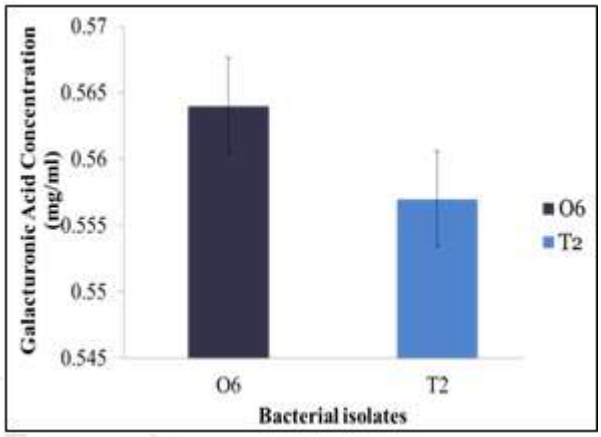

Figure 11: The reducing sugar (galacturonic acid) concentration of optimum reaction temperature, pH, reaction time and substrate concentration (pectin) of two isolates, O6 and T2

3.7 Growth Curve of the bacterial isolates

The bacterial growth curve of bacterial isolates (O6 and T2) was shown in (Fig 12 and 13).The optimum incubation period of both two isolates occurs in 48hr.

3.8 Application of pectinase in fruit juice extraction

The application of pectinase from commercial pectinase enzyme, crude enzyme and water was studied for apple fruit juice extraction and were shown in Fig 14.

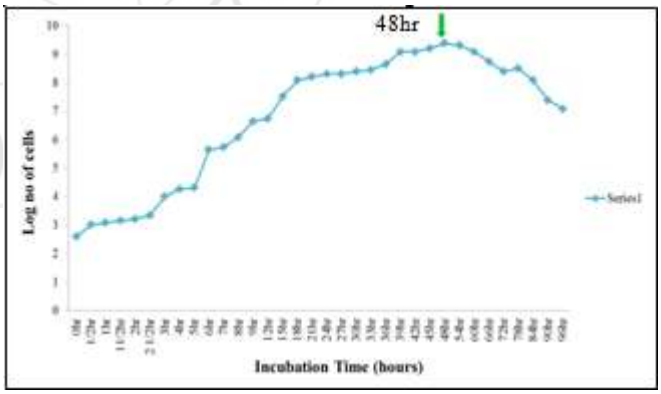

Figure 12: Bacterial growth of isolated strains O6

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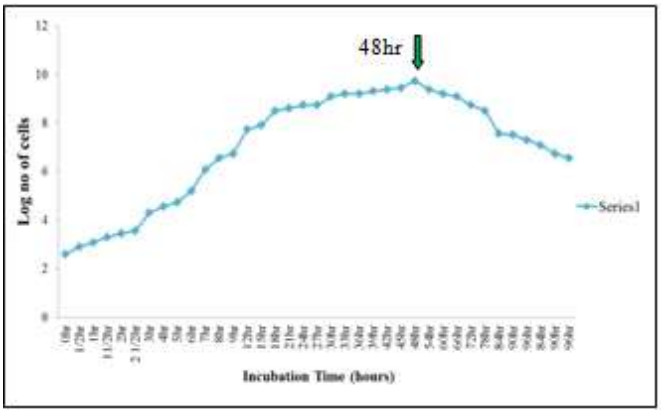

Figure 13: Bacterial growth of isolated strains T2

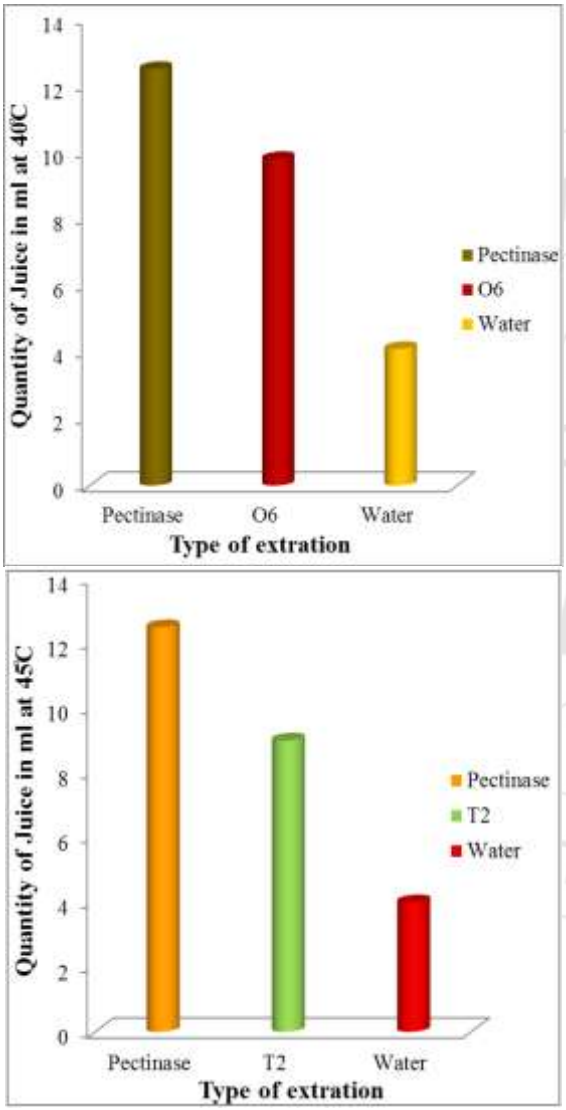

Figure 14: Extraction of apple juice with crude enzyme, purified pectinase and water

4. Discussions

Enzyme production is one of the broad areas of biotechnology which accounts for about 1.5 billion of the world market. The enzymes of microbial origin were found to be more advantageous than others. In present investigation the effect of various parameter studies of pectinase were done. The extracellular pectinase was isolated from bacterial source.

In the present study we made a successful primary attempt to isolate the potential (82) bacterial strains from decaying fruits and vegetables samples producing industrially important pectinase enzyme. Among all, the isolates from decaying orange (O6) and decaying tomato (T2) showed the halozone formation on pectinase screening agar media (PSAM) plates. Therefore; confirmation could be made for the production of pectinase enzyme from these two bacteria according to method described by E. Venkata Naga raju. et.al [14].

Microscopic morphology of two bacterial strains was analyzed as Gram positive cocci shape bacteria in Gram's staning. In biochemical tests, the isolates O6 and T2 were positive for Catalase and Starch hydrolysis tests. T2 strain was positive for Methyl red, Citrate utilization, Gelatin hydrolysis, and Voges Paskauer test and O6 strain show negative. These two strains negative results for nitrate reduction test and MacConkey's Plate. The T2 isolate was positive for glucose, maltose, galactose, and fructose and mannitol fermentation. And O6 was also positive for glucose, lactose, maltose, galactose, fructose except sorbitol. Some researchers have also been studied that the gram positive cocci could produce the production of pectinase enzyme [15, 16 and 17]. And the two isolated strains were sensitive in all eight types of antibiotics. Therefore, these selected two isolated strains (T2 and O6) can be applied in fruits juice preparation and possible outbreak of these microorganisms would be controlled easily.

Enzyme assay of crude enzyme was done 13 days incubations and the optimum incubation period was found 7days incubation with the galacturonic acid concentration of 0.449mg/ml for O6 and 0.396mg/ml for T2 respectively. The isolate O6 is to be higher pectinase activity than other strain T2. The protein content of the crude enzyme was quantitatively determined by Folin-Lowry method. The highest protein content was found in crude extract of O6 (0.514mg/ml) and lowest protein content was found for T2 (0.453mg/ml).

The effect of temperature on pectinase activity was studied at different temperature at 20°C to 70°C with increases 5°C intervals. Optimum temperature for the extracellular pectinase production activity is 40°C for O6 and 45°C for T2 as can be seen from Figure 7 with the galacturonic acid concentration of O6 occurs 0.467mg/ml and T2 occurs 0.528mg/ml. However, it was observed that the pectinase activity decreased as the temperature was increased at 45°C for O6 and 50°C for T2 as compared to the production at 40°C and 45°C. This may be an indication that temperature above 40°C for O6 and 45°C for T2 is not suitable for pectinase production.

The effect of pH on pectinase activity was also studied by conducting experiment at different pH levels as 2 to 8 keeping temperature at 35°C. Figure 8 show that pH 5.0 is optimum for maximum yield of the enzyme activity of both two isolates (O6 and T2) with the galacturonic acid concentration of 0.426mg/ml for O6 and 0.378mg/ml for T2 respectively. Similar results of higher pectinase production

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activity under acidic condition were reported also by Vasanthi and Meennakshisundaram 2012.

In addition to pH and temperature effect of reaction time on pectinase activity was also studied. It was observed that for (O6 and T2) the reaction time was 35 minutes. At these reaction times pectinase activity reached maximum product and enzyme was saturated with substrates for these isolates. This fact is also supported by other studies (Torimiro and Okanji 2013).

Effect of substrate concentration was also study with different pectin (0.1%, 0.2%, 0.5%, 1%, 1.5% and 2%) for pecctinase activity. Enzyme production increases with increase in concentration till 1% then it remain constant. The optimum production of pectinase activity of both two isolates occurs at 1% concentration. O6 produced 0.47mg/ml and T2 produce 0.442mg/ml of enzyme respectively.

The application of pectinase from the isolates was studied for apple fruit juice clarification. In the present study, the apple juice extraction was determined by purified pectinase enzyme, crude enzyme of isolates (O6 and T2) and water. In this extraction application, the optimum temperature, 40°C for O6 and 45°C for T2, was applied. The activity of crude enzymes from isolates (O6 and T2) showed comparative activities with purified pectinase.

5. Conclusions

Most of the microorganisms are capable of producing various extracellular and intracellular enzymes using various economical substrates. Pectinase is an extracellular enzyme, which is produced from various microorganisms including bacteria, Fungi and some actinomycetes. In this study, pectinase enzyme was produced by Cocci species which was obtained from decaying fruit and vegetable samples. The isolate O6 showed highest activity than others isolate T2. The pectinase activity of isolated strains O6 and T2 showed activity at pH 5 which is slightly acidic pH and the optimum temperature of 40 and 45 °C. Therefore it is possibly a better enzyme source for industrial purpose. In juice extraction, O6 and T2 (crude form) gave about 80% of juice yield compared by commercial pectinase enzyme (pure form). Therefore, these enzymes may be scaled up for juice production after careful investigation.

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