Detection of the Optimal Conditions for Pectate lyase Productivity and Activity by Erwiniachrysanthemi

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Abstract*—Pecate lyase has great commercial significance in the feed and drink industries, pectate lyase is an enzyme catalyzing the hydrolysis of pectin, a plant polysaccharide that contributes to the structure of plant tissues, into galacturonic acid. Although several substrates for the production of pectate lyase have been reported as being economically effective such as utilization of pectin-rich substrate rather than pure pectin, however, there is still need to develop the substrate to make the entire process much cheaper and more effective. The present work deals with physiological studies on bacterial pectate lyase. Out of 50 spoilt cucumber and tomato samples from local markets in Baghdad city. Eighteen isolates from Erwiniachrysanthemi were obtained (36%). Pectate lyase activity was found in all isolates in primary and secondary screening and the isolate Erwinia chrysanthemiEc9 yielded the highest pectate lyase production. The enzyme activity was increased to 75.31U/ml when this isolate was cultivated under the optimal conditions which consisted of using basal medium containing (1.5%) (w/v) lemon peel extract and 0.02% (w/v) yeast extract with pH 6.0 at 25℃ for 24 hour. The enzyme revealed maximum activity (88.34U/ml) with lemon peel powder (0.9% w/v), followed by orange peel and apple pomace powders with relative activities of 117,105 and 102%, respectively, as substrates. The best cofactors for this enzyme were Ca2+and Fe2+ with relative activities of 138 and 112%, respectively, and severely inhibited in presence of Hg2+and Cu2. Consequently, lemon peel has a potential as an effective and much cheaper (economical) substrate for pectate lyase production and pectate lyase activity in comparison with traditionally used substrates like polygalacturonic acid and other saccharides.

***Index Terms*—pecata lyase, erwinia chrysanthemi**

I. INTRODUCTION

Manuscript received April 15, 2014; revised July 20, 2014.

Erwiniachrysanthemi is a gram-negative, facultatively anaerobic, rod- shaped bacteria and belongs to family Enterobacteriaceae[1], [2]. This bacterium is a soft rot pathogen degrading succulent fleshy plant organs such as roots, tubers, stem cuttings and thick leaves. It is also a vascular wilt pathogen, colonizing xylem and becoming systemic within the plant [3], [4], also it is able to survive in the soil [3].

Erwiniachrysanthemi is a plant pathogen which can cause disease in a wide range of plants, including bananas, maize, onions, lettuce, garlic, potato, sweet-potato, eggplant, zucchini, onion, carrot, cauliflower, arracacha, melon, cucumber, sweet-pepper, okra, cabbage, tomato but is best known in temperate regions for causing blackleg (stem rot) and tuber soft rot in fruits and vegetables its success partly lies in its ability to produce many pectinases that are able to macerate and break down the plant cell wall material. This exposed part of the plant releases nutrients that can facilitate bacterial growth [1], [3]. Fruit softening is associated with cell wall disassembly and modifications to the pectin fraction are some of the most apparent changes that take place in the cell wall during ripening[5].Soft rot caused by several types of bacteria, such as *Erwinia*, *Pectobacterium*, *Bacillus*, *Enterobacter* and *Pseudomonas*, but primarily subspecies of *Erwinia carotovora* and *E. chrysanthemi*, are a widespread and destructive disease of fleshy fruits, vegetables, and ornamentals throughout the world[6], [7].

Pectin is a plant polysaccharide that contributes to the structure of plant tissues as a component of the middle lamella and primary cell wall. Pectin are high molecular weight acid polysaccharides primarily made up of α-(1→4) linked D-galacturonic acid residues with a small number of rhamnose residues in the main chain and arabinose, galacose and xylose on its side chain [8], [9].

doi: 10.12720/jomb.4.3.184-191

Pectin is present in all plants but the content and composition varies depending on the species, variety, maturity, plant part, tissue, and growing condition. Pectin is higher in legumes and citrus fruits than cereals. Apple (*Malus domestica*), grapefruit (*Citrus paradise*), lemon (*Citrus limon*), orange (*Citrus sinensis*), mango (*Mangifera indica*) and apricot (*Prunus armeniaca*) are known to have high levels of pectin. Grapes, Lemon and Sweet orange are citrus fruits which consist of two parts namely the peels (rind skin) and pulp. These two parts are easily separated from each other with the pulp serving as the edible parts of the fruit while the peels as a good source of pectin, a whole mature fruit contains 3-7% pectin substances on a dry weight basis and 0.1-1.1% on a fresh weight basis [8].

Pectinase is a generic name for a family of enzymes that catalyse hydrolysis of the glycosidic bonds in the pectic polymers-pectinases are one of the most widely enzymes in bacteria, fungi and plants [9]. Pectate lyases are widely distributed among microbial plant pathogens, where they play an important role as virulence factors. They have also been found in saprophytic micro-organisms, including members of the genus *Bacillus* and *Erwinia*, *Pseudomonas* and in some thermophilic bacteria[10]. Pectate lyasefrom *Erwinia chrysanthemi*, causes devastating diseases involving maceration of parenchymatous tissues of various dicot plants. These enzymes act by depolymerizing cell-wall polygalacturonides in the presence of calcium ions, thus destroying the integrity of the plant tissues [5]. *Erwiniachrysanthemi* pectinases include pectin esterase (E.C.3.1.1.11), polygalacturonase (E.C.3.2.1.15), galacturan 1,4-α-galacturonase (E.C.3.2.1.67), exopoly-α-galacturonosidase (E.C.3.2.1.82), endo-pectate lyase(E.C.4.2.2.2),exo-pectate lyase (E.C.4.2.2.9) and endo- pectin lyase (4.2.2.10), classified on the basis of their mode of action [4], [7], [8]. Pectate lyase is an enzyme involved in the marceration and soft rotting of plant tissue. Pectate lyase is responsible for the eliminative cleavage of pectate, yielding oligosaccharides with 4- deoxy-alpha-D-mann-4-enuronosyl groups at their non-reducing ends [11].

Pecate lyase has great commercial significance in industrial applications, such as extraction and clarification of fruit juices, maceration of vegetables, scoring of cotton fabric, retting of flax, degumming of plant fibers, waste water treatment, oil extraction, eat and coffee fermentations, bleaching of paper, in poultry feed additives and in the alcoholic beverages and food industries[7], [12], [13]. For these reasons, the aim of this study was to investigate pectate lyase production by *Erwiniachrysanthemi* besides to optimize medium conditions for increasing the activity of pectate lyase besides to detection of substrate specificity and effect of metal ions.

II. Materials and Methods

A. Collection of Samples

The study included a total of 50 spoilt vegetables samples of which 25were from cucumber and 25 were from tomato collected local markets in Baghdad city. These samples were analyzed according to the method that described by [14]. Briefly, 25 g of sample (cucumber or tomato) was blended with 200 ml peptone water 0.1% by using a blender for 2 min and incubated at 30 ℃ for 18-24hour.

B. Isolation and Identification of Erwiniachrysanthemi

One loopfull of each sample was streaked on blood agar and MacConkeys agar, then incubated at 30 ℃ for 18-24 h. For isolation of *Erwinia* spp., cells were grown on selective medium that containing the followingper500ml:(1N NaOH,4.5ml; 10%$CaCl_2.H_2O$,3.0ml; $NaNO_3$,1.0g; agar,1.5g; sodium polypectate,10g;10%SDS,0.5ml and 0.075%crystal violet,1.0ml)[15]. Several biochemical tests were done to differentiate *Erwiniachrysanthemi* from the other species. These include the following tests: a negative indol test, a positive lipase test, ability to phosphatase and lecithinase production and inability to produce acid from trehalose and maltose [16-18]. In addition to these biochemical tests, API 20E identification was used to differentiate *Erwiniachrysanthemi* from the other species.

III. Screening for Pectate Lyase Production

A. Quantitative Analysis

All bacterial isolates were evaluated for their ability to produce pectate lyase by placing10 µl of cultures into wells (5 mm in diameter) on PGA solid medium which contained basal medium(per liter)($(NH_4)_2SO_4$ 2.0g, $MgSO_4.7H_2O$: 0.3g, $CaCl_2.2H_2O$: 0.3g, $FeSO_4.7H_2O$: 0.5g, KH_2PO_4: 10.0g) and 4g of polygalacturonic acid(pH7)[19]; and incubation at 30 ℃ for 24 hour, then the plates were flooded with a cetyl-trimethyl ammonium bromide solution(10g/ liter). The colonies that produced pectate lyase were surrounded by clear haloes as a result of substrate degradation.

B. Quantitative Analysis

The selected bacterial isolates were grown in PGA medium without addition of agar and incubated on the rotary shaker at 150 rpm at 30 ℃ for 24 h. After removal of cells by centrifugation at 8000 xg for 10min., the clear supernatant was used as the crude extra cellular enzyme source and the amount of pectate lyase produced was assayed.

C. Pectate lyase Assay

Pectate lyase activity was assayed by adding 0.3 ml of diluted sample to a solution containing 1 ml of 0.9% polygalacturonic acid and 0.7 ml of 50 mM glycine buffer pH 8.5 containing 0.5mM $CaCl_2$. The mixture was incubated at 30 ℃ for 60 min. After incubation, the reaction was terminated by adding 4 ml of 0.01 M HCl to

the mixtures. Inactivated crude enzyme in boiling water for 10 min was used as control in the reaction. The absorbance was measured at 232 nm and the galacturonic acid content was obtained by using calibration curve relating galacturonic acid concentrations (0-2.5 mM) to 232 nm. One unit of pectate lyase activity was defined as the amount of enzyme that released 1 μmol of reducing sugars (galacturonic acid) from polygalacturonic acid per minute [13], [19].

D. Preparation of Substrate and Crude Pectin Extract from Different Plants

The raw material was prepared for the experiments in the following way: Lemon, orange, apple and mango fruits were first washed and the pulp was then separated from the fruit flesh. The peels of lemon and orange and pomaces of apple and mango were dried in an air-forced oven at 55℃ until constant weight. The dried passion fruit peels and pomace were then milled to a dry powder. The ground powders were packaged in polyethylene bags and stored at refrigerator temperature until required as substrate. The pectin extracts were prepared with modification the method that described by [20,21] as follow: A dry mass (1 g) was subjected to extraction by adding 100 ml of water. The pH was adjusted to 1.2–2.6 with0.5 M HCl, 0.5 M HNO_3 or citric acid. The mixture was then heated to 65℃ and the extraction was carried out with continuous stirring for 45 min. The hot acid extract was filtered through the ordinary screen with 1-mm mesh size equipped with two-layer cheesecloth, and the filtrate was cooled down to 4℃ and used as a sole carbon sources throughout the study.

IV. Studying of Growth Parameters for Pectate Lyase Production

A. Effect of Carbon Sources on Pectate lyase Production

Mixtures of basal medium were mixed with 25 ml of1% lemon and orange peels and apple and mango pomaces extracts(pH7.0) besides to 1%pure polygalacturonic acid, polygalacturonic acid plus glucose, glucose, sucrose, lactose, ethanol, maltose and milled wheat bran were used as carbon sources for pectate lyase production. The selected isolate was inoculated to these media and incubated at 30℃for 24 hour. The fermented broth was centrifuged at 3500 rpm for 10 minutes and the cell free supernatant obtained was collected and used as crude pectate lyase enzyme, then pectate lyase activity was determined.

B. Effect of Nitrogen Sources on Pectate lyase Production

Effect of different nitrogen sources including peptone, treptone, beef extract, yeast extract, casein and urea (organic nitrogen sources) and ammonium chloride, sodium nitrate, potassium nitrate and ammonium sulfate a(inorganic nitrogen sources) was studied by incorporating 0.02% (w/v) of each nitrogen source in lemon peel extract medium. The selected isolate was inoculated to these media and incubated at 30℃ for 24 hour, then pectate lyase activity was determined.

C. Effect of Different Concentrations of Lemon Peel Extract on Pectate lyase Production

Mixtures of basal medium with lemon peel extract of different concentrations (0.5,1,1.5, 2, 2.5, 3, 3.5and 4%) were prepared. The pH was adjusted at 7.0, inoculated with selected isolate and incubated at 30℃for 24 hour, then the pectate lyase activity was determined.

D. Effect of pH on Pectate lyaseProduction

Lemon peel extract medium was prepared at different pH values (5-9).This medium was inoculated and incubated at 30℃ for 24hours. The pectate lyase activity was determined.

E. Effect of Incubation Temperature on Pectate lyaseProduction

Lemon peel extract medium was incubated with selected isolate at different temperatures (10, 15, 20, 25, 28, 30, 35, 37 and 40℃) for 24 hours. The pectate lyase activity was measured.

F. Effect of Different Incubation Periods onPectate lyase Production

Lemon peel extract medium was incubated with selected isolate at25℃ for different periods (12, 18, 24, 26, 30 and 36 hours). The pectate lyase activity was determined.

G. Substrate Specificity

A study of substrate specificity for the pectate lyase was made by using lemon and orange peels, apple and mango pomaces and wheat branas powders. The pectate lyase assay was done by using these plant powders at concentration 0.9% instead of polygalacturonic acid.

H. Effects of Monovalent and Divalent Cations on Pectate Lyase Activity

The influences of divalent cations (Ba^{2+}, Ca^{2+}, Cu^{2+}, Fe^{2+}, Hg^{2+},Na^{+}, $K^{+}Mg^{2+}$, Mn^{2+}, Ni^{2+}, Se^{2+} and Zn^{2+})on pectate lyase activity were determined with 2 mM concentrations of the corresponding chloride or sulfate form. 1 ml of crude enzyme was mixed with 1 ml of eachmonovalent or divalent cation and incubated at 30℃ for 1 hour, then pectate lyase activity was measured.

V. Results and Discussion

A. Isolation of Erwiniachrysanthemi

Out of 50 plant samples collected, 18(36%) *Erwiniachrysanthemi* was isolated. Among which 11/18 (61%) were from cucumber, 7/18(39%) were from tomato (Fig. 1). This bacterium causes blackleg of potatoes of which it is the chief, if not the only cause, in cold climates. However, in warmer climates *Erwiniachrysanthemi* and *Erwiniacarotovora* can cause

similar or identical symptoms [22]. *Erwiniachrysanthemi* survived for 5 months at temperatures of 10 ℃ and 20 ℃ and relative humidities of 81 and 93%, and temperatures of 30 ℃ and 35 ℃ [23]. Uesug *et al.*, [23] were reported that *Erwiniachrysanthemi* strains were isolated from potatoes, cucumber, broccoli, radish, tomato and sweet pepper. *Erwiniacarotovora* and *Erwiniachrysanthemi* can cause symptoms similar to blackleg and contribute to tuber rotting, also *Erwiniachrysanthemi* can cause a slow wilt in the field with darkened vascular tissue and a brown discoloration in the stems [24], [25].The insoluble plant cell wall polymers are significant barriers to microorganisms, yet represent an abundant source of carbohydrates. The disease process in general requires pectin degradation and is often dependent on plant sensitivity and environmental conditions such as temperature, nitrogen starvation, osmolarity and oxygen limitations [26]. In cucumber plants fed unbalanced solutions, bacterial wilt was increased significantly in low N and low K solutions, while the disease indices of plants grown in low P, high N, high P, and high K solutions were either not significantly or not consistently different from those in the balanced solution [24].

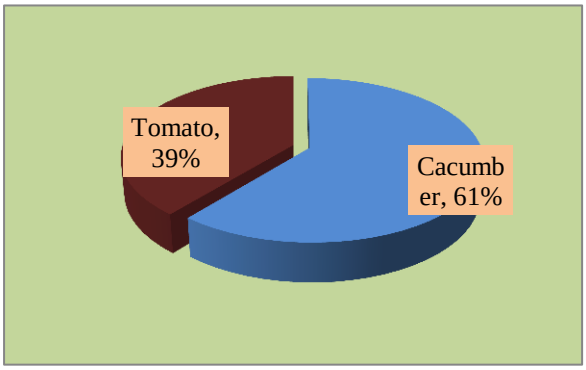

Figure 1 Percentage of erwiniachrysanthemi from different plant sources

VI. Screening for Pectate Lyase Producing Isolates

A. Qualitative Analysis

Among eighteen *Erwiniachrysanthemi* isolates subjected to rapid screening for extracellular pectate lyase production by using PGA solid medium plates, All *Erwiniachrysanthemi* isolates were found to be positive for pectate lyase activity by growth on this medium and production of clear zones. *Erwiniachrysanthemi*Ec9 gave higher clear zone around the colony in comparison with other isolates (Table-I).In bacteria, the major end products of polygalacturonases and pectate lyases are saturated and unsaturated di-galacturonate, respectively, which enter cells and are further metabolized intracellularly. Extracellular galacturonate enters the cell through active transport in *Erwiniachrysanthemi* or is produced intracellularly by the action of oligo-galacturonate lyase [26]

B. Quantitative Analysis

All *Erwiniachrysanthemi* isolates that exhibited clear zone around the colonies were tested for pectate lyase production in liquid condition by growing in PGA medium without addition of agar. These isolates revealed pectate lyase activity between 5.47-43.53 Unit/ml (Table-I). *Erwiniachrysanthemi*Ec9 demonstrated high pectate lyase activity 43.53 Unit/ml, so that this isolate was selected as the best pectate lyase producer. The pectinases are inducible enzymes that require the presence of the inducer to be synthesized. Although pectin is a natural inducer for pectinases production, its elevated cost makes it difficult to use it at industrial level [9]. The production of pectate lyase by *Pseudomonas fluorescens* is absolutely required Ca^{2+}.It is possible that Ca^{2+}may be required for maintaining the structural integrity and thermal stability of the enzyme protein and the increasing in thermal stability is in part due to the decrease in heat inactivation by raising the Arrhenius energy from 72 kcal $^{-1}$ in the absence of Ca^{2+} to 82 kcal mol^{-1} in the presence of Ca^{2+}[6]. The levels of pectate lyase produced by *Aspergillus nidulans* and *Erwinia carotovora* were similar, but were ~10-fold lower than the level produced by *Erwiniachrysanthemi* [27].

TABLE I Diameter of Inhibition Zone and Pectate Lyase Activities for Erwiniachrysanthemi Isolates

Isolate No.	Diameter of inhibition zone (cm)	Pectate lyase activity (U/ml)	Isolate No.	Diameter of inhibition zone (cm)	Pectate lyase activity (U/ml)
Ec_1	1.4	20.13	Ec_{10}	1.2	7.39
Ec_2	1.7	24.17	Ec_{11}	1.8	26.94
Ec_3	1.1	5.47	Ec_{12}	2.3	39.15
Ec_4	1.9	33.64	Ec_{13}	1.9	30.64
Ec_5	1.8	29.32	Ec_{14}	1.6	25.16
Ec_6	1.6	25.00	Ec_{15}	2.3	41.28
Ec_7	1.2	7.18	Ec_{16}	1.5	20.44
Ec_8	1.5	21.58	Ec_{17}	1.4	19.24
Ec_9	2.6	43.53	Ec_{18}	1.3	12.02

VII. Studying of Growth Parameters for Pectate Lyase Production

A. Effect of Carbon Sources on Pectate lyase Production

Among the various carbon sources tested for pectate lyase production (Fig. 2), pectin was the best carbon source for pectate lyase production yielding the maximum enzyme activities of about 53.07 U/ml with (1%) lemon peel extract followed by about 50.34 U/ml for(1%) wheat bran. While the inhibition in enzyme production was observed when sucrose, lactose, maltose, ethanol and glucose were added to the basal medium as carbon source. Lemon peel is an interesting alternative, since, it is a relatively cheap and easily available

substrate, which can serve as a feedstock for large-scale fermentation, whereas pure ploygalacturonateis only available in limited quantities and at very high cost. An extraction process is the most important operation to obtain pectin from vegetal tissue. Pectin extraction is a multiple-stage physical–chemical process in which hydrolysis and extraction of pectin macromolecules from plant tissue and their solubilisation take place under the influence of different factors, mainly temperature, pH and time [20].

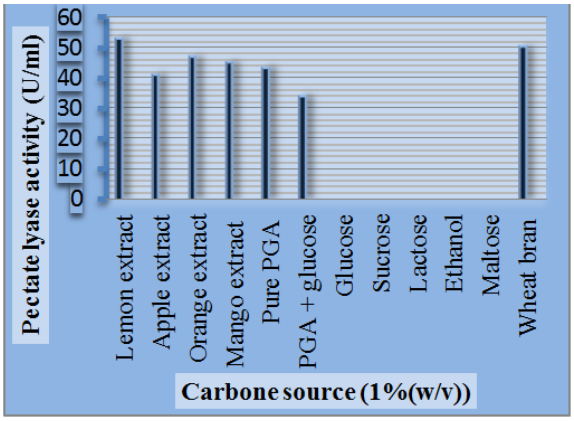

Figure 2 Effect of carbon source on pectate lyase production by ErwiniachrysanthemiEc9

Similar result was reported by Reda *et al*. [28], in accordance to this study where the pectin produces the highest pectate lyase compared to other carbon sources substituted (12.19 U/ml). There was an inhibitory effect on the synthesis of pectate lyase. This result is in agreement with the study of [7] where the production of pectate lyase was lower when free sugars were added to the medium compared to the presence of pectin as the sole carbon source in submerged fermentation. According to [10], low enzyme production with other carbon sources is maybe because of catabolite repression. It was also evidenced by [7], that there was a catabolite repression of pectic enzymes in the presence of glucose and other sugars.

B. *Effect of Nitrogen Sources on Polygalacturonase Production*

Various organic and inorganic nitrogen sources were tried in pectate lyase production by *Erwiniachrysanthemi*Ec9 (Fig. 3). The best nitrogen source was yeast extract (57.24U/ml) followed by peptone (54.12 U/ml) and ammonium sulphate (53.27 U/ml). On the other hand, urea, ammonium chloride, sodium nitrate, potassium nitrateseem to inhibit pectate lyase production. Urea was a very poor nitrogen source for pectinase production. Yeast extract have been reported to give maximum pectinase yield by *Bacillus* sp. DT7 [29] when it was combined with pectin. Phutela *et al*. [30], found that ammonium sulphate stimulated pectinase production and lack of this nitrogen source resulted in absence of extracellular pectinases by *Aspergillus fumigatus*. These findings suggested that ammonium sulphate may be suitable for fungal pectinase whereas yeast extract was suitable for bacterial pectinase production.

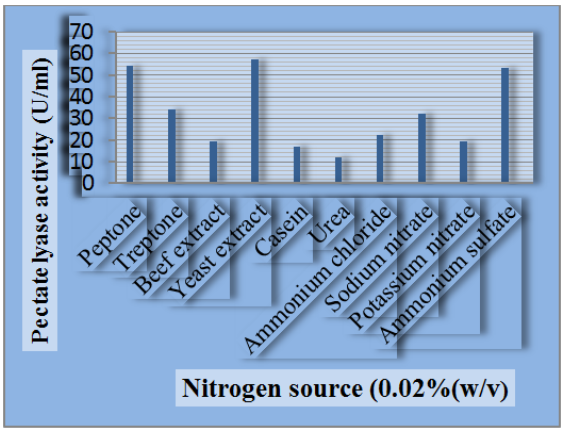

Figure 3 Effect of nitrogen source on pectate lyase production byErwiniachrysanthemiEc9

C. *Effect of Different Concentrations of Lemon Peel Extract on Pectate lyase Production*

The best concentration of lemon peel extract in media for pectate lyase production was 1.5% with 61.13U/ml pectate lyase activity (Fig. 4) and the enzyme production decreased with further increased in substrate concentration (2-4%).Probably, this is due to the presence of a high galacturonic acid concentration from pectin degradation. Increased in enzyme production by *Debaryomyces nepalensis* was observed with lemon peel at 2.3% and further increased in the concentration, resulted in slight decreased in enzyme activity [31]

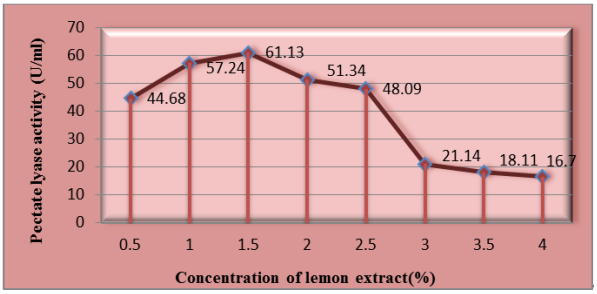

Figure 4 Effect of different concentrations of lemon peel extract on pectate lyase production byErwiniachrysanthemiEc9

D. *Effect of pH on Pectate Lyase Production*

Experiments were executed to find out the optimum pH in order to maintain the favorable conditions for increasing pectate lyase production. The fermentation medium pH was adjusted accordingly with 1N HCl /NaOH from 5-10. The significance of pH on the production of pectate lyase was observed. The maximum pectate lyase production of 67.0U/ml was obtained at pH 6.0(Fig. 5). This may be attributed to the balance of ionic strength of plasma membrane. The pH affects in enzyme production because of its role in the solubility of medium

substrates and its effect on the ionization of the substrate and it's availability for the bacterial growth. Moreover, the pH affects the productivity and enzyme stability [32].This could also be explained by the fact that *Erwiniachrysanthemi* was isolated from the spoilage vegetables such as tomato which was an acidic by nature. Moreover, the optimal initial medium pH for pectinase production by bacteria in submerged fermentation has also been reported in the acidic range of 6.0 [28] and 6.5 [33]. The optimal pH for pectinase production by *Enterobacter aerogenes* NBO2 was 6.5[7]. In contrast, the pectenase from fungal isolates shows pH optima between 4 and 6.5[34].

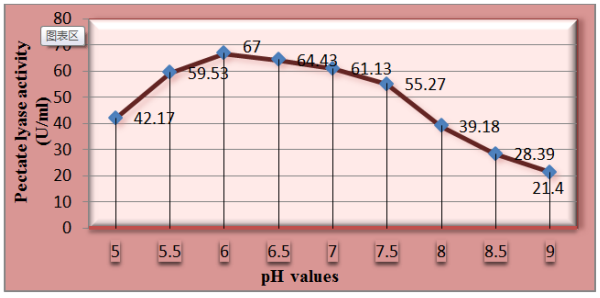

Figure 5 Effect of pH value on pectate lyase production byErwiniachrysanthemiEc9

E. *Effect of Temperature on Pectate Lyaseproducton.*

Incubation temperature has a profound effect on enzyme production. So the fermentation was carried out at different temperatures ranging from 10 to 40℃ by *Erwiniachrysanthemi* Ec9 under submerged culture conditions. The maximum enzyme activity of 75.29U/ml was obtained at 25℃ (Fig. 6).

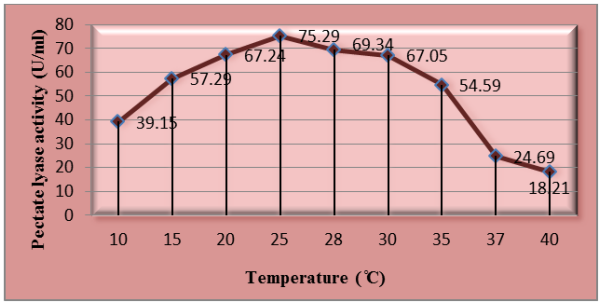

Figure 6 Effect of temperature on pectate lyase production byErwiniachrysanthemiEc9.

The enzyme production reduced gradually with further increase in incubation temperature. This may be due to the denaturation of microbial strain at higher temperatures. Lower and higher temperatures decreases the specific activities because of the thermal effects of these temperatures on the microorganism growth and on the enzymatic reaction rate inside the cells which reflects on the vital creation of the enzyme [32].Therefore, the data obtained indicated the enzyme production was not growth dependent. The optimum temperature for pectate lyase by *Chryseomonas luteola* is at around 20 to 24℃ in nutrient broth [35]. Maximum pectate lyase production at 84.5 U/ml was achieved at temperature 35℃ by *Paenibacillus polymyxa* N10 in Submerged Fermentation[12].

F. *Effect of Different Incubation Periods on Pectate Lyase Production*

Fig. 7 shows a rapid increased in pectate lyase production by *Erwiniachrysanthemi* Ec9 until it achieved maximal production at 24 hours of cultivation (75.31U/ml). The enzyme production decreased slowly after achieving its maximal production. This might be due to sugar utilization or enzyme denaturation as a result of interaction of other compounds in the medium [28]. When compared to previous findings, the cultivation time by *Erwiniachrysanthemi* (24 hour) was shorter compared to cultivation time showed by *Bacillus* sp.DT7 and *Bacillus firmus*-I-10104 which yield maximum pectinase activity at 36and 98 h of cultivation, respectively [28, 29]. Hence, the shorter fermentation cycle will make the strain cost effective for commercial exploitation. The highest pectinase production by *Enterobacter aerogenes* of about 18.54 U/ml at 24 hours incubation time [7].

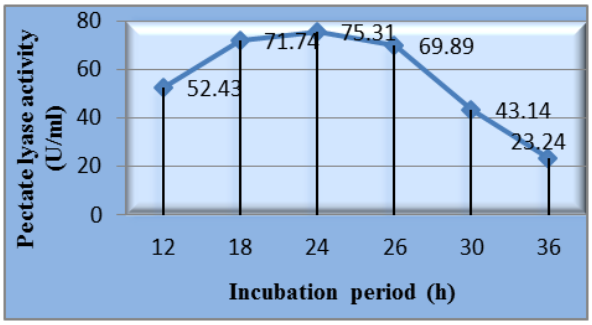

Figure 7 Effect of different incubation periods on pectate lyase production byErwiniachrysanthemiEc9

VIII. Substrate Specificity

The crude pectate lyase from *Erwiniachrysanthemi* Ec9 showed maximum activity(88.34U/ml) with lemon peel powder(0.9% w/v), followed by orange peel and apple pomace powders, respectively(Table II), with relative activities of 117,105 and 102%, respectively, but it decreased with all other substrates such as mango peel and wheat bran powders. This indicates that lemon peel powder might be the most suitable substrate for maximum pectate lyase activity. It is interesting to note that although plants considered in this study all contain pectin but there is distinct variable differences in pectate lyase activity. The possible differences in pectin synthes are in these species**.** Citrus peel contains relatively higher i.e.20-30% of pectin as compared to the apple, since apple pomace contains 10-15% of pectin on a dry matter basis. Apple, quince, plums, gooseberries, oranges and other citrus fruits contain much more pectin as compared to soft fruits like cherries, grapes and strawberries contain little pectin [36]. The use of inexpensive substrates can economize the process of production and there were many of agriculture wastes as orange pulp,

pectin containing compounds and malt sprouts, lemon and orange peels, and many other materials [36], [37].

TABLE II EFFECT OF SUBSTRATES ON PECTATE LYASE ACTIVITY

Substrate	Pectate lyase activity (U/ml)	Remaining activity (%)
Lemon peel	88.34	117
Orange peel	79.12	105
Mango pomace	72.84	97
Apple pomace	77.09	102
Wheat bran	71.18	95
Polygalacturonic acid (control)	75.31	100

IX. EFFECT OF MONOVALENT AND DIVALENT CATIONS ON PECTATE LYASE ACTIVITY

The influences of some metal ions on pectate lyase activity is presented in table-3.Pectate lyase activity was significantly increased in presence of Ca^{2+}, Ba^{2+} Mg^{2+},Na^{+}, or K^{+} .The best cofactors for this enzyme were $Ca^{2+}$and Fe^{2+} with relative activities of 138 and 112%, respectively. In contrast, pectate lyase activity was inhibited in presence of Mn^{2+}, Ba^{2+}, Ni^{2+}, Se^{2+}, or $Zn^{2+}$and severely inhibited in presence of $Hg^{2+}$and Cu^{2+}. Ca^{2+} appears to play a role in maintaining the structural integrity required for catalytic activity of pectate lyase [6], [37]. Potassium ions were found to stimulate pectate lyase secretion and substrate binding [37]. Pectate lyase can be distinguished by the absolute Ca^{2+} requirement for catalysis its activity. In a study done by[38]reported that three extracellular pectate lyases (Pels) were produced by *Bacillus* isolated from fermenting cocoa beans and demonstrated that Fe^{2+} was found to be a better cofactor than Ca^{2+} for activity one of them, while Ca^{2+} was the best cofactor for activity of the others.

TABLE III EFFECT OF SOME METAL IONS ON PECTATE LYASE ACTIVITY

Metal ion	Pectatelyase activity (U/ml)	Remaining activity (%)
Ba^{2+}	83.92	95
Ca^{2+}	121.90	138
Cu^{2+}	57.10	42
Fe^{2+}	98.94	112
Hg^{2+}	37.98	43
Na^{+}	91.87	104
K^{+}	92.75	105
Mg^{2+}	90.10	102
Mn^{2+}	85.68	97
Ni^{2+}	76.85	87
Se^{2+}	74.20	84
Zn^{2+}	68.02	77
control	88.34	100

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