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Screening of bacteria from vegetable waste for evaluation of pectinase activity and its application in juice clarification

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Abstract

The aim of the current study was to identify pectinase-producing bacteria from vegetable wastes and use them to clarify juices. Vegetable waste was gathered from several locations. Using standard isolation techniques, 20 colonies with different morphologies were isolated from vegetable waste. After that, it was spotted on pectin media to expose it to pectinolytic activity. Ten of these cultures responded significantly to the pectinolytic activity. When compared to other cultures, K2, K4, and K10 cultures exhibited higher pectinase activity. The ideal temperature and pH for all three isolates were discovered to be 60°C, 6 and 7, respectively. Juice clarity was assessed using pectinase from the three isolates that were chosen by treating the carrot, beetroot, and cucumber with cell free supernatant of pectinolytic cultures before juice extraction. Comparatively pectinase from K4 culture treated carrot, showed maximum juice extract which was 1.94 folds higher than control. Similarly, beetroot & cucumber treated with pectinase from K2 culture gave maximum juice which was 1.22 & 1.3 folds higher than control. Accordingly, pectinase from K4 culture significantly hydrolyzed the treated juices & produced highest yield at 51.5, 55 & 65.5% with carrot, beetroot & cucumber respectively. The results demonstrated that effectiveness of the pectinase enzymes in reducing cloudiness and improving the clarity and stability of vegetable juices. In summary the evaluation of pectinase from bacteria isolated from vegetable waste for juice clarification shows promising potential for application in the juice industry and highlights the potential of using microbial enzymes as a sustainable and efficient alternative for juice processing.

Keywords: Pectinase, Bacteria, Vegetable waste, Juice Clarification

1. Introduction

Pectinases are a group of enzymes that breakdown pectin, a complex polysaccharide found primarily in the walls of plants[1]. Pectin acts as a carbohydrate structure that provides rigidity and stability to tissues. Pectinases play an important role in the degradation of pectin and contribute to many biological and economic processes, including plant growth, fruit ripening, and juice production[2]. Pectinase production is a vital process in biotechnology because of the demand for this enzyme for many industrial applications[3]. Pectinase, an enzyme complex that breaks down pectin, is important in industries such as food and beverages, textiles, and paper. Pectinases are a group of enzymes that breakdown pectin, a complex polysaccharide found in the cell walls of plants[4]. Pectin functions as a structural element that provides rigidity and stability to tissues. The hydrolysis of pectin is important in many industries, particularly the food and beverage industry, where pectinases are widely used to clarify fruit juices and wines and enhance the extraction of fruit and vegetable juices[5]. The growth in the fruit and vegetable juice industry has led the food industry to further explore juice processing[6]. One of the most important steps in making juice is removing pectin, the polysaccharide layer that causes the juice to become cloudy[7]. Pectinase is an enzyme that breaks down pectin and is widely used in the refining process[8]. Pectinases can be produced by many organisms, including plants, nematodes, insects, bacteria, fungi, yeasts, actinomycetes, and protozoa[9]. Bacterial isolates that produce important pectinases include bacteria such as Bacillus, Pseudomonas, and Staphylococcus[9]. Among these, Bacillus species are important for pectinase production[10]. Traditionally, pectinases are derived from bacteria, but recent research has shown that bacterial pectinases have similar effects, sometimes providing advantages in terms of production costs and safety[11]. Vegetable waste is the best source of many microorganisms that can produce enzymes during the natural decomposition process[12]. The use of these organisms not only ensures good and environmentally friendly enzyme production but also reduces waste and resource recovery. This study focused on the isolation of pectinase-producing bacteria from vegetable waste, which is a ready-made and sustainable product.

2. Methodology

2.1 Vegetable sample

Mixed vegetable waste samples were collected from five different localities of Mysore and T-Narsipura, including market vegetable waste, roadside vegetable waste, and households. These samples were named K1, K2, K3, K4, and K5. The sample was collected in sterile polythene zip lock covers and processed in the laboratory[8, 13].

2.2 Isolation and screening of pectinolytic bacteria:

Vegetable waste collected from the different localities was crushed and ground. The samples were serially diluted in saline (0.85% NaCl) and spread-plated with the appropriate dilution on nutrient agar media. The plates were incubated at 37°C for 24-48 hours until viable colonies appeared. Morphologically distinct colonies were selected and grown in nutrient broth for 24 hours at 37°C[13].

2.3 Screening for pectinolytic activity

Pectin media were prepared according to the composition in Table 1. The isolated cultures were then spot inoculated on inoculated pectin media and incubated on plates at 37°C. After incubation, the plates were stained with Lugol's iodine (5.0 g potassium iodide, 1 g iodine, and 330 ml H_2O) dye and observed for the zone of clearance[14].

Table.1 Composition of pectin media

Chemicals	**g/100 ml**
KCl	0.05
$MgSO_4$	0.1
Trisodium citrate	0.1
Citric acid	0.1
Yeast extract	0.1
Citrus pectin	1.0
Agar	1.8

2.4. Production of pectinase enzyme.

The production medium consisted of 10-20 g/L pectin, 5 g/L peptone, 3 g/L yeast extract, 2 g/L KH2PO4, 0.5 g/L $MgSO_4 \cdot 7H_2O$ (magnesium sulfate heptahydrate), 0.1 g/L $CaCl_2 \cdot 2H_2O$ (calcium chloride dihydrate), and 0.1 g/L $FeSO_4 \cdot 7H_2O$ (ferrous sulfonate heptahydrate), with a pH of 0.01 g/L. Following inoculation with the standard inoculums, the conical flasks were incubated for 48 h at 30°C in a shaking incubator at 150 rpm. After incubation, the cell-free supernatant was collected in a clean vial, and the activity was observed before and after washing[15].

2.5 Determination of enzyme activity and protein concentration

Pectinase activity was evaluated by the dinitrosalicylic acid (DNS) method, and protein content was estimated by the Lowry method[16]**.**

2.6 Enzyme purification

The crude enzyme extract was placed in an ice bath. Then, solid ammonium sulfate was added to the chilled extract to achieve 70% saturation, and the mixture was stirred for 30-60 minutes on ice and then centrifuged at 10,000 rpm for 20 minutes at 4°C to pellet the precipitated proteins suspended in a minimal volume of citrate buffer (pH 4.8). After that, the dialysis tubing was soaked in distilled water and then in citrate buffer (pH 4.8). The resuspended protein solution was placed in dialysis tubing and dialyzed against several changes of citrate buffer (pH 4.8) at 4°C for 24 hours to remove excess ammonium sulfate and small impurities[17].

2.7 Effect of pH and temperature on enzyme activity

Pectinase activity was measured in different environments, such as at different pH values (5.0, 6.0, and 7.0 8.0) and at different temperatures (30 to 60°C)[18].

2.8 Effect of substrate concentration and temperature on bacterial growth

The pectinase-producing culture was optimized under different pH values (5.0 to 8.0) and substrate concentrations of 0.25, 0.75, and 0.1% pectin[19].

3. Results and Discussion

3.1 Isolation of Microbes

Isolation of bacteria from vegetables:

In the present study, a total of five vegetable waste samples from different localities of Mysore and T. Narasipura were screened for pectinolytic bacteria (Figure 1). After serial dilution and plating, the culture showed vigorous growth on nutrient agar (Figure 2).

Figure 1. Vegetable waste samples collected from different localities of Mysore and T. Narasipura.

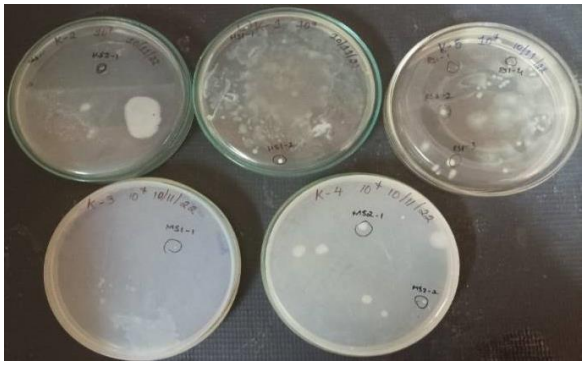

Figure 2. Isolation of microbes from samples on nutrient agar media

The K-5 sample collected from Bannimantap Market had the most colonies, whereas the K-2 (collected from T. Narasipura Household) and K-3 (from Bannimantap Household) samples had the fewest colonies. Each plate showed morphologically distinct colonies of varying sizes, colours, edges, spreadages and textures. About 20 colonies showing distinct morphologies were carefully picked and grown in nutrient broth. Purity was confirmed by repeated streaking.

3.2 Evaluation of pectinolytic activity

The 20 isolates from different vegetable wastes were subjected to pectinolytic activity by spotting on pectin media. Among the 20 isolates, 10 cultures showed a positive reaction with hydrolysis/clearance after staining with Lugol's iodine. Figure 3 displays representative plates showing pectinolytic activity.

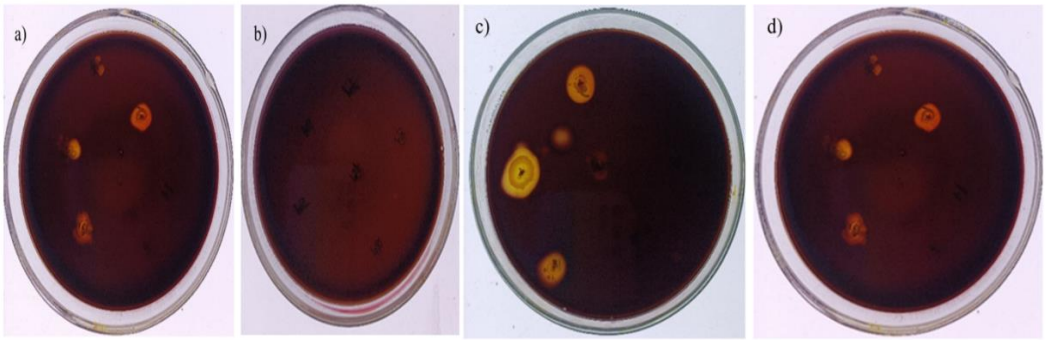

Figure 3. Evaluation of pectinase activity.

3.3 Quantification of pectinase activity in selected cultures

The pectinase activity of approximately 10 selected isolates was quantified and is expressed relative to the total protein content. Initially, a standard graph was generated using BSA as a standard. The standard curve for BSA was found to be linear in the range of 10 to 100 µg/ml. The correlation coefficient R^2 = 0.9885 indicates a strong correlation between concentration and absorbance, and a correlation equation y=0.0048 was generated, which was used to calculate the protein content in the sample. The total protein concentration was estimated in CFSs (cell-free supernatant) and selected cultures. The protein content was high in the K9 culture and very low in K1(Figure 4).

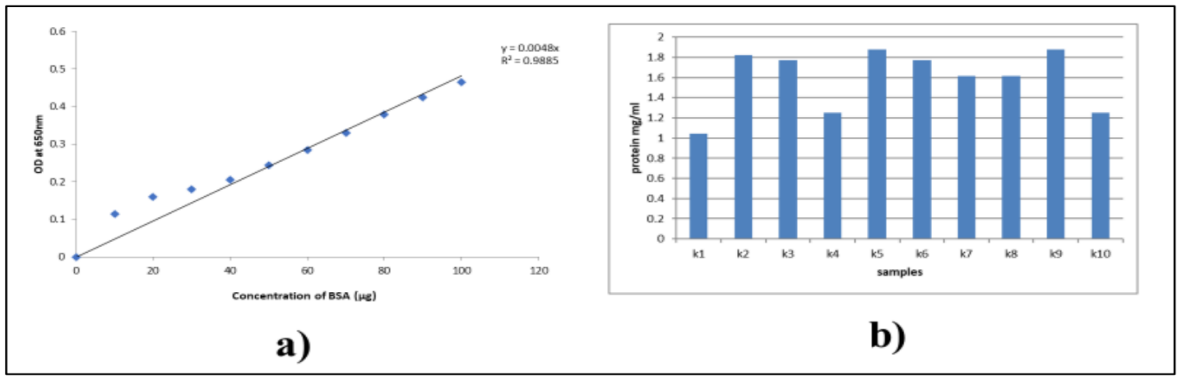

Figure 4. a) The standard graph for protein b) Total protein content in cell-free bacterial culture supernatant from vegetable waste

3.4 Quantification of pectinase activity by estimation of reducing sugars

Furthermore, pectinase activity was estimated by measuring the amount of reducing sugars formed by the strains. The amount of reducing sugars was analysed by the DNS (3,5-dinitrosalicylic acid) method and compared with the standard graph generated with galactose.

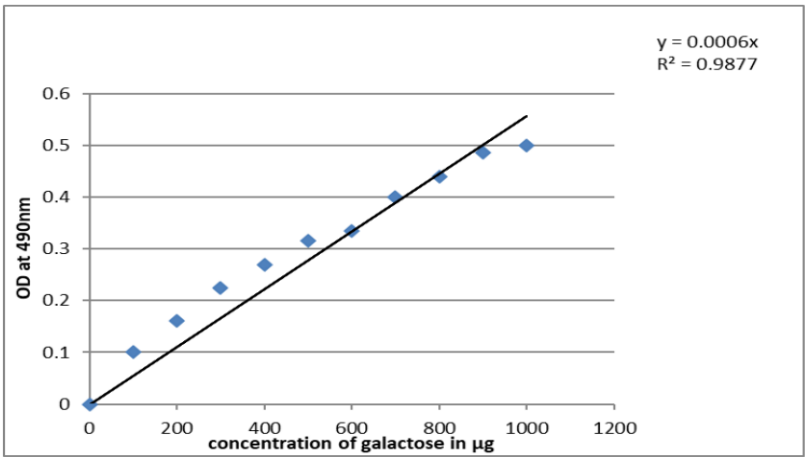

Figure 5. Standard graph for galactose estimation.

The standard graph for galactose was found to be linear in the range of 100 to 1000 µg. The correlation coefficient R^2 = 0.943 indicates a strong correlation between concentration and absorbance, and a correlation equation y=0.0006 was generated, which was used to calculate galactose activity in the sample (Figure 3.4). The pectinase activity of selected isolates is presented in Figure 3.4.1 Accordingly, K10 culture showed a maximum specific activity of 0.8617 U/mg. However, the K3 culture showed the least activity at 0.202 µg/mg.

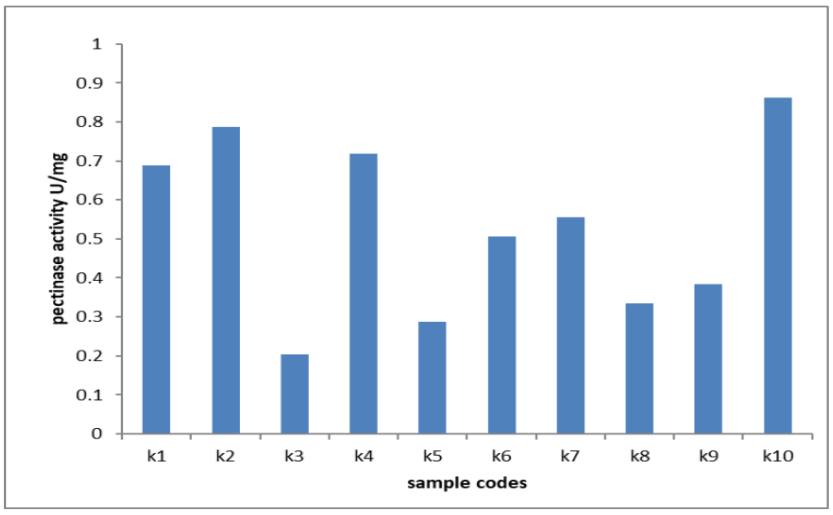

Figure 6. Pectinase activity of isolated bacterial cultures.

3.5 Optimization of pectinase production from isolated culture

3.5.1 Optimization media pH

The media were adjusted to pH 5, 6, 7, and 8 and supplemented with 0.5% pectin. The K2, K4, K10 culture are grown for 24 hours, and the total protein content and pectinase activity were analysed at pH 6 & 7 was found to be optimal (Figures 7 and 8).

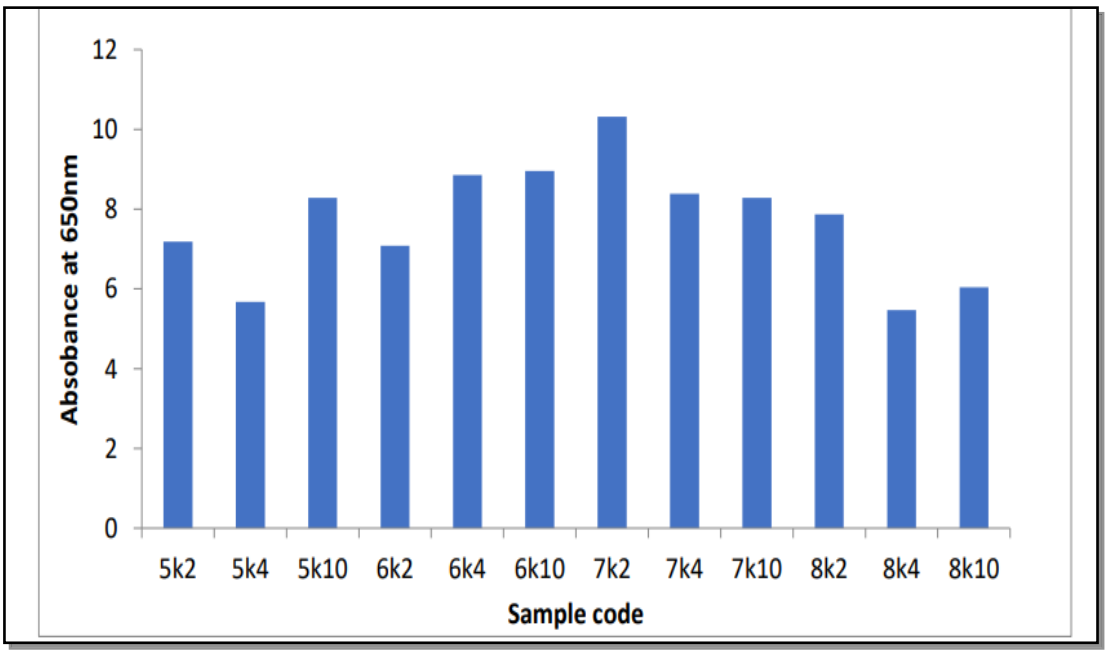

Figure 7. Optimization media pH (protein content).

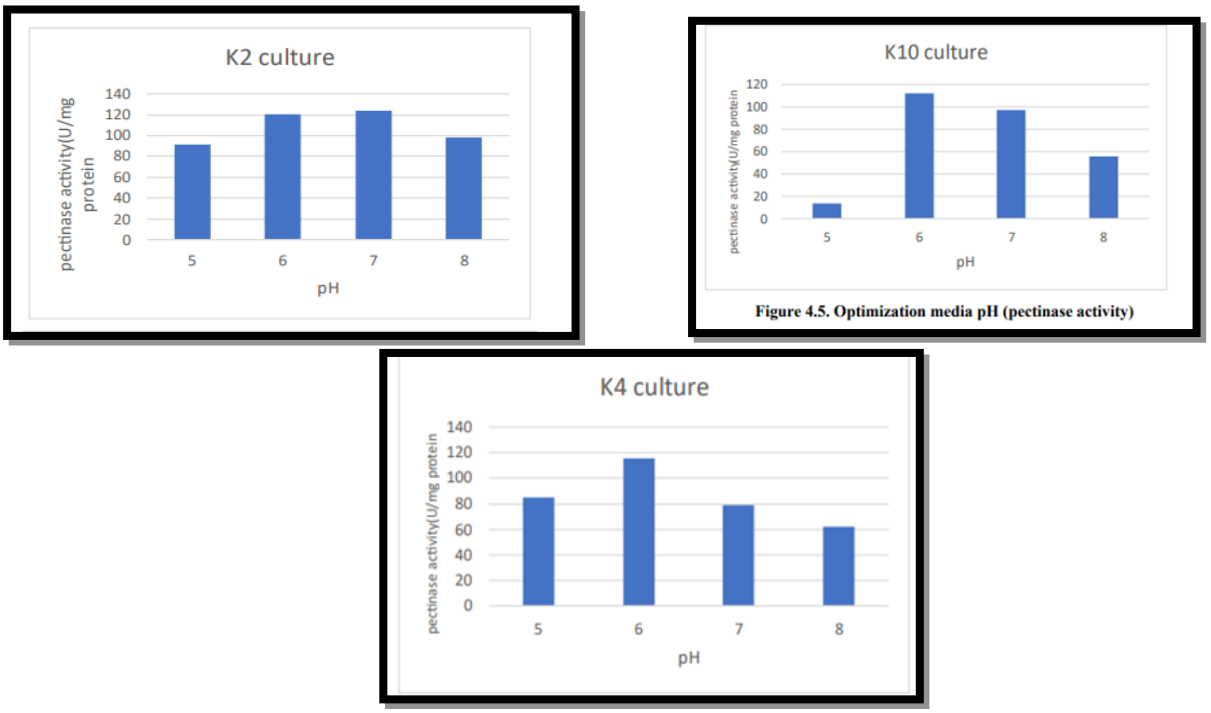

Figure 8. Optimization media pH (pectinase activity).

3.6 Optimization of growth temperature

The media were prepared at the optimum pH, and the K2, K4, and K10 cultures were inoculated separately in tubes, grown at different temperatures (30°C, 40°C, 50°C, and 60°C) and allowed to grow for 24 hours. According to the results the optimum temperature was 60°C for all three isolates (Figure 9).

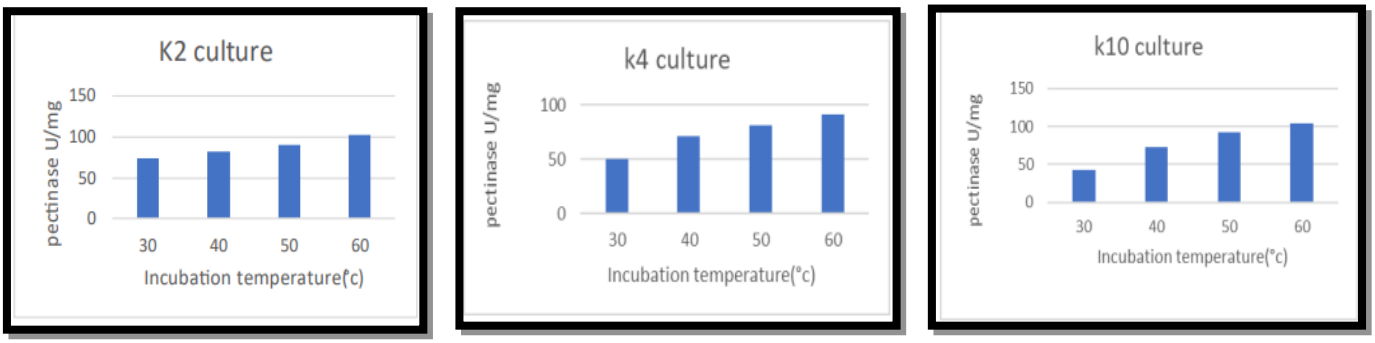

Figure 9. Optimization of growth temperature (protein content).

3.7 Optimization of substrate concentration:

The media were prepared at the optimum pH and supplemented with 0.25, 0.75, and 0.1% pectin, and Cultures K2, K4, and K10 were inoculated separately, and the cultures were grown at an optimum temperature of 60°C for 24 hours. According to the obtained results, the optimum substrate concentration was 0.75% for all three isolates (Figure 10).

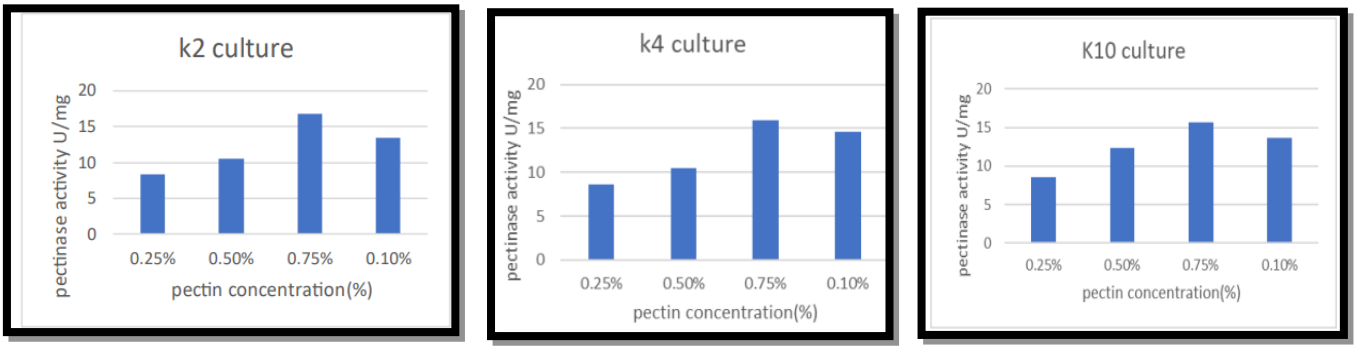

Figure 10. Optimization media pH (pectinase activity).

3.8 Optimization of production time

The media were prepared with the optimum pH and pectin concentration, followed by incubation at optimum temperature for 3 days. Protein content and pectinase activity were checked after every 24 hours. The results confirmed that 48 h is optimal for maximum production of pectinase (Figure 11). A further increase in the incubation time slightly reduced the activity.

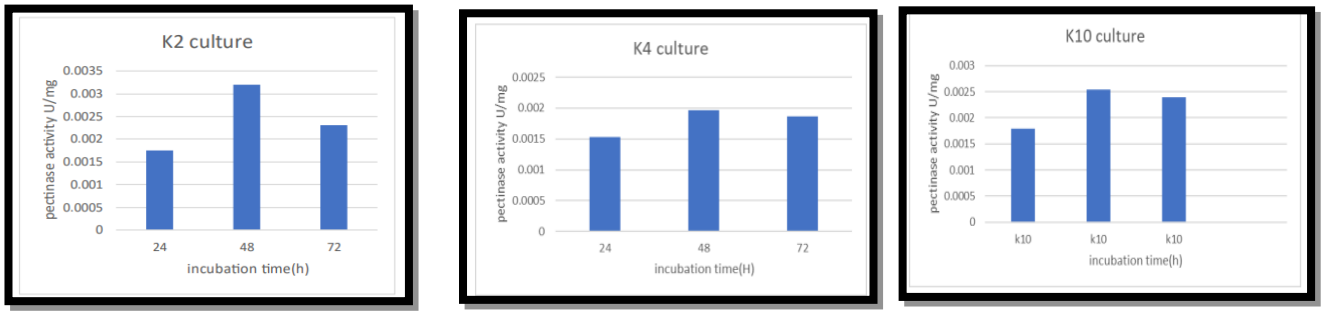

Figure 11. Optimization of production time (pectinase activity).

3.9 Application of pectinase in vegetable juice clarification

Pectinase from the 3 selected isolates was further checked for vegetable juice clarification by treating the selected vegetables with CFS from pectinolytic cultures before juice extraction. According to the results obtained, the volume of juice increased after treatment compared to that of the control (table 2). Among the selected vegetables, carrots had the highest volume of juice. Comparatively, the maximum amount of pectinase in K4 culture-treated carrot juice extract was 1.94-fold greater than that in the control. Similarly, beetroot and cucumber treated with pectinase from K2 culture produced the most juice, which was 1.22- and 1.3-fold greater, respectively, than that of the control.

Table 2. Volume of juice obtained from the samples.

Samples	**Carrot**		**Beetroot**		**Cucumber**	
	Volume of juice extracted	Fold increase	Volume of juice extracted	Fold increase	Volume of juice extracted	Fold increase
K2	10 ml	1.9	10.1 ml	1.12	13 ml	1.29
K4	10.3 ml	1.94	11 ml	1.22	13.1 ml	1.3
K10	9.2 ml	1.75	10 ml	1.11	11.1 ml	1.1
Control	5.3 ml		9 ml		10.1 ml	

Tables 2 and 3 display the volume and yield of the juice. Accordingly, pectinase from the K4 culture significantly hydrolysed the treated vegetable and produced the highest yield at 51.5%, 55% and 65.5% with carrot, beetroot and cucumber, respectively. Figure 14 displays the volume of juice obtained from samples K2, K4, and K10.

Table 3. Yields of juice obtained from the samples.

Samples	**Carrot (%)**	**Beetroot (%)**	**Cucumber (%)**
K2	50	50.5	65
K4	51.5	55	65.5
K10	46.5	50	55.5
Control	26.5	45	50.5

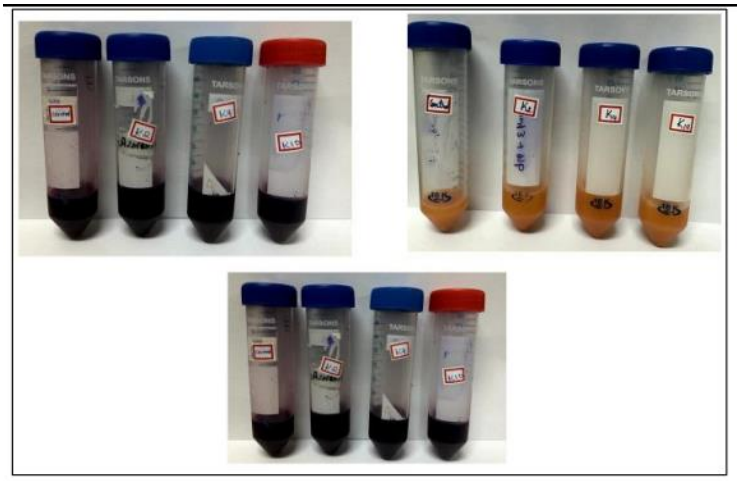

Figure 12. The volume of juice obtained from samples K2, K4, and K10.

3.10. Testing the turbidity of clarified juice

The fluidity of the juice was determined by measuring the turbidity of the juice at 600 nm. K4, which had a relatively high yield of juice and showed maximum clarity in carrot juice is 23% (Table 4 and 5).

Table 4. Turbidity of clarified juice

Samples	**Carrot**	**Beetroot**	**Cucumber**
K2	0.17	1.45	0.19
K4	0.23	1.56	0.10
K10	0.19	1.39	0.13

Table 5. Percentage of juice in samples

Samples	**Carrot (%)**	**Beetroot (%)**	**Cucumber (%)**
K2	17	14	19
K4	23	15	10
K10	19	13	13

4. Conclusion

In conclusion, the evaluation of pectinase from bacteria isolated from vegetable waste for juice clarification shows promising potential for application in the juice industry. The use of pectinase enzymes derived from bacterial sources offers advantages such as cost-effectiveness, availability, and eco-friendly production compared to traditional extraction methods. This study focused on isolating and characterizing pectinase-producing bacteria from vegetable waste, indicating the feasibility of utilizing agricultural by products for enzyme production. The isolated bacterial strains exhibited the ability to produce pectinase enzymes, which can effectively degrade pectin, the major component responsible for cloudiness in vegetable juices. The pectinase enzymes involved in the optimization of various factors, such as pH, temperature, incubation time, substrate, and enzyme concentration, were evaluated. These parameters were systematically investigated to determine the optimal conditions for pectin degradation and juice clarification. The results demonstrated the effectiveness of the pectinase enzymes in reducing cloudiness and improving the clarity and stability of vegetable juices. Overall, the evaluation of pectinase from bacteria isolated from vegetable waste for juice clarification highlights the potential of using microbial enzymes as a sustainable and efficient alternative for juice processing. Future studies are focused on characterizing pectinase-producing bacteria and further evaluating the sensory and nutritional attributes of clarified vegetable juices to ensure their market acceptability.

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