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SCREENING FOR DIVERSE BIOPOLYMER DEGRADING MICROBES FROM VEGETABLE MARKET WASTE OF KADI

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ABSTRACT:

The fruit and vegetable wastes are rich source of diverse biopolymers *viz,* cellulose, pectin, and xylan. Presence of such biopolymer selectively promotes the growth of microorganisms which produce the cellulase, pectinase and xylanase enzymes to degrade cellulose, pectin, and xylan respectively. These organisms have potential to be used for waste treatment and composting of organic matter rich waste including fruit and vegetable wastes. The present study was conducted for isolation and screening of cellulase, pectinase and xylanase producing microorganism from vegetable market waste of Kadi. The isolation for cellulose, pectin, and xylan degraders was carried out by enrichment followed by screening on solid medium. In the primary screening, 42 isolates were selected on basis of zone hydrolysis ratio. Among these, 34 Mesophilic bacterial isolates, 4 thermo-tolerant bacterial isolates and 4 fungal isolates were there. For all the selected isolates, the zone ratio for cellulose degraders was in range of 1.42 to 7. The zone ratio of pectin degraders was in range of 1.33 to 9.33. The zone ratio for xylan degraders was in range of 1.2 to 9. The total of 9 isolates showed good degradation potential for all the three polymers tested and also seem promising for further studies.

KEYWORDS: *Biopolymer degrading microbes, Screening, Vegetable market waste.*

INTRODUCTION:

0India is second largest producer of fruit and vegetable in the world (Tayade, S. D. *et a*l, 2017**)**.Total fruit and vegetable production in 2017-2018 was 95 mt and 181 mt respectively (GoI, 2017b).But from this production, about 40 to 50 percent of the total output worth Rs. 13,300 crore is wasted every year due to number of problems (Tayade, S. D.*et a*l, 2017**)**. Fruit and vegetable losses indirectly include wasting of critical resources such as land, water, fertilizers, chemicals, energy, and labour (Narashans A. *et al*, 2018). The huge amount of this waste is dumped on land to rot in open air without any treatment. It emits a foul odour, is a source of greenhouse gases, and also creates a big nuisance by attracting birds, rats and pigs, vectors of various diseases (Singh, A. *et al*, 2012). The rotting vegetable and fruit waste thus, is a threat to environmental and makes it a matter of grave concern.

Recycling of fruit and vegetable wastes can be beneficial to the environment by reducing the amount of waste disposed, promoting the fertility of soil and improving the physical and chemical properties of soil (Park, J.I., Yun, Y.S., Park, J.M, 2002). Furthermore, recycling of fruit and vegetable wastes reduce the unpleasant odors of garbage, benefits the sanitation of the environment. Fruit and vegetable waste is less harmful to the environment than industrial waste (Gill, S., Jana, A.M. and Shrivastav, A., 2014).

The recycling of fruit and vegetable market waste by composting is a time consuming process. To reduce the composting period efficient decomposing microorganisms need to be developed. (Gautam, S.P. *et al*, 2010). Microorganisms are the key factor in nutrient transformation. Microorganisms that are involved in composting process excrete several enzymes viz, cellulase, protease, lipase, pectinase, xylanase and other enzymes that contribute in degradation of macro-molecules of organic wastes. Cellulose, pectin, and xylan polymers are the main substrates of lignocellulose-degrading enzymes. They are present in large amounts in the primary cell wall and dietary fibers of major fruits and vegetables. (Toushik, S. H. *et al*, 2017).

Cellulose is the major constituent of plant biomass. It is the most abundant carbohydrate in nature (Koo, Y.M., 2001). Cellulose, represents approximately 1.5×10^{12} tons of the annual biomass produced through photosynthesis (Sukumaran, R.K., R.R. Singhania, and Pandey A., 2005). The cell wall polysaccharides of fruits and vegetables contain approximately 20% to 35% of cellulose in dry weight (Toushik, S. H. *et al*, 2017). All cellulose-degrading organisms possess cellulase systems. Moreover, cellulose degradation is accomplished by the synergistic action among endoglucanases, cellobiohydrolases, and β-glucosidases (Woodward, J., 1991). Cellulose can be also used as a carbon source during composting process.

Pectin is a carbohydrate found in all fruits and vegetables and is necessary for plant growth. Pectin is commonly degraded by an enzyme called pectinase. Pectinase enzymes breakdown pectin polysaccharides from the plant tissue into simple molecule like galacturonic acid and sugar. These enzymes act on pectin, a class of complex polysaccharides found in the cell wall of higher plants and cementing material for the cellulose network. Fruits and vegetables contain the highest amount of pectin at under-ripe or just ripe conditions, approximately 35% to 40% in dry weight (Luis, F.G, Domingos PFA, Cristina MO, 2010).

Xylan, consist of a heterogeneous group of polysaccharides that includes also important constituents of plant cell walls (Chen, H., X.-L. Li, and Ljungdahl, L.G., 1997). Xylanase is a class of enzymes produced by microorganisms to break down a component of plant cell walls known as hemicellulose. Xylan comprise approximately 15% to 19% in dry weight of cell wall polysaccharides of fruits and vegetables (Luis, F.G, Domingo's, PFA, Cristina M.O, 2010; Nyman and Haska, 2013).

The vegetable and fruit waste have abundant cellulose, pectin and xylan content. These waste are also rich source of large variety of microorganisms which can efficiently degrade these polymers. The aim of present study is isolation and screening of microorganisms with high ability to produce extracellular enzymes like cellulase, pectinase and xylanase for degradation of natural polymers which are inherent in fruit and vegetable wastes. The inoculation of potential enzyme producing microbial strains in organic waste can lead to efficient composting and also decreasing the time for degradation phase of composting process.

MATERIALS AND METHODS:

Collection of sample:

Diverse vegetable and fruit waste samples were collected from the Kadi vegetable market and were immediately transferred in laboratory for isolation of cellulose, pectin, and xylan degrading organisms. Source of vegetable market wastes are tomato, potato, carrot, cabbage, onion, apple, raspberry, cucumber, ivy gourd, etc.

Enrichment studies.

Enrichment for cellulose, pectin and xylan degrading microorganisms was carried out by inoculation of samples in 100 ml liquid basal mineral salt medium supplemented with 1% carboxy methyl cellulose (CMC), 1% pectin, and 1% beechwood xylan respectively with pH 5.5 and 6.8. Incubation was done at 37 ^{o}C and 55 ^{o}C for 1 week (Han, Y.W and Srinivasan V.R., 1968).

Isolation of cellulose, pectin and xylan degrading microorganism.

For isolation, samples from the enrichment broth were spreaded on solid medium containing 1% CMC, 1% pectin, and 1% xylan and incubated at 37 ^{o}C and 55 ^{o}C for 48 to 72 hrs. Colonies surrounded by zone of solubilisation were isolated, purified and characterized.

Primary screening by plate assay method.

Primary screening of cellulose, pectin, and xylan degrading isolates was carried out on solid medium containing 1% CMC, 1% pectin, and 1% beechwood xylan as substrate with pH 5.5 and 6.8. Plates were incubated at 37 ^{o}C and 55 ^{o}C till the development of colonies. After development of growth, plates were flooded with Gram's iodine solution (Kasana, R.C.*et al*, 2008).Zone of hydrolysis was measured in mm. Zone ratio was determined using Khandeparkar's method (Gayal and Khandeparkar, 1979).

D/d =Diameter of zone of hydrolysis / Diameter of growth

RESULTS AND DISCUSSION:

Enrichment studies:

After the 7 days of incubation period, the increase in turbidity and visible degradation of inoculated vegetables indicated enrichment of Mesophilic and Thermo-tolerant cellulose, pectin and xylan degrading microorganisms (Photograph 1 and 2).

Isolation of cellulose pectin and xylan degrading microorganism:

The microbial colonies, isolated from enrichment of cellulose, pectin and xylan degraders were selected for primary study on the basis of zone of hydrolysis. The 38 bacterial and 4 fungal isolates were selected for further study. The bacterial isolates showed diversity of culture characteristics in terms of colour, margin, shape, and texture of the colonies .The variety of pigments like pink, yellow, pale yellow, and chalky white, white, were observed (Photograph 3 and 4). From the 38 bacterial isolates obtained, 8 isolates were pigmented and 30 isolates were non- pigmented. Based on Gram staining, the isolates were found mostly to be Gram-positive. The microscopic observation revealed 5 rod-shaped bacilli, 23 Cocci, and 9 oval yeast. The bacterium were arranged in single, in cluster, in chain and 1 *actinomycetes* also found (photograph 5 and 6).

Primary screening by plate assay method.

Cellulose degraders:

The zone ratio for cellulose degraders was in range of 1.42 to 7.On the basis of zone ratio, maximum cellulose degrading potential was observed for isolates MBC 23 and MBC19 (Table 1 and Photograph 7,8).From the total isolates obtained , 2 isolates had very good degradation potential and 11 isolates had good degradation potential. 18 isolates had moderate degradation potential and 11 isolates had poor degradation potential.

Pectin degraders.

The zone ratio for pectin degraders was in range of 1.33 to 9.33 mm. On the basis of zone ratio maximum pectin degrading potential was observed for isolate MBC4 (Table 1 and Photograph 7,8) .From the total isolates obtained, 5 isolates had very good degradation potential and 14 isolates had good degradation potential. 17 isolates had moderate degradation potential and 6 isolates had poor degradation potential.

Xylan degraders.

The zone ratio for Xylan degraders was in range of 1.2 to 9 mm. On basis of zone ratio, maximum xylan degrading potential was observed for isolate MBC28 (Table 1 and Photograph 7, 8). From the total isolates obtained, 8 isolates had very good degradation potential and 13 isolates had good degradation potential. 8 isolates had moderate degradation potential and 13 isolates had poor degradation potential.

CONCLUSION:

Results indicate that cellulose, pectin and xylan degrading organism were present in vegetables market waste. From the isolates obtained, 26% were good cellulose degrader (Graph 1A), 33% were good Pectin degrader (Graph 1B) and 31% were good xylan degraders (Graph 1C). Irrespective of their enrichment source, all the isolates had capacity to degrade cellulose, pectin and xylan i.e. all polymers tested. However their degradation potential for each polymer varied considerably. The total 9 isolates showed good degradation potential for all the polymers tested and also seem promising for further studies.

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Table 1: Enzyme profile of bacterial isolates by plate assay method

	Zone of hydrolysis (mm)								
	Cellulose			Pectin			Xylan		
Isolates code	Colony size	Zone size	Zone ratio	Colony size	Zone size	Zone ratio	Colony size	Zone size	Zone ratio
MBC 1	5	25	5	4	32	8	5	30	6
MBC 2	7	30	4.28	6	29	4.83	15	37	2.46
MBC 3	8	23	2.87	7	30	4.28	6	20	3.33
MBC 4	5	22	4.4	3	28	9.33	5	21	4.2
MBX 5	7	10	1.42	6	10	1.66	6	13	2.16
MBX6	6	10	1.66	4	20	5	4	30	7.5
MBX7	6	30	5	5	30	6	5	33	6.6
MBX8	6	29	4.83	4	28	7	5	30	6
MBX9	5	20	4	5	24	4.8	6	22	3.66
MBP10	7	35	5	5	28	5.6	7	36	5.14
MBP11	6	24	4	6	19	3.16	5	6	1.2
MBP12	5	32	6.4	6	16	2.66	4	33	8.25
MBP13	5	20	4	6	30	5	6	32	5.3
MBC14	7	20	2.85	7	33	4.71	5	11	2.2
MBC15	5	8	1.6	5	24	4.8	7	20	2.85
MBC16	4	22	5.5	6	28	4.66	5	28	5.6
MBC17	6	29	4.83	5	26	5.2	5	22	4.4
MBC18	4	8	2	6	8	1.33	5	20	4
MBC19	5	35	7	6	28	4.66	8	22	2.75
MBC20	8	20	2.5	3	18	6	9	15	1.66
MBC21	5	28	5.6	5	30	6	5	34	6.8
MBC22	4	10	2.5	4	33	8.25	5	36	7.2
MBC23	5	35	7	5	31	6.2	5	35	7
MBC24	5	32	6.4	7	30	4.28	6	11	1.83
MBC25	3	9	3	6	29	4.83	5	33	6.6
MBC26	5	15	3	5	16	3.2	5	32	6.4
MBC27	4	10	2.5	4	29	7.25	5	30	6
MBC28	7	26	3.71	9	31	3.44	4	36	9
MBC29	5	15	3	7	28	4	6	34	5.66
MBC30	5	28	5.6	5	30	6	5	33	6.6
MBC31	6	30	5	5	32	6.4	5	10	2
MBC32	7	33	4.71	5	14	2.8	4	27	6.75
MBC33	5	20	4	4	23	5.75	5	37	7.4
MBC34	6	16	2.66	6	32	5.33	4	30	7.5
MBC35	5	23	4.6	5	23	4.6	5	7	1.4
MBC36	7	26	3.71	5	32	6.4	4	6	1.5
MBC37	4	19	4.75	6	25	4.16	5	35	7
MBC38	5	26	5.2	4	27	6.75	5	8	1.6

Table 2: Enzyme profile of fungal isolates by plate assay

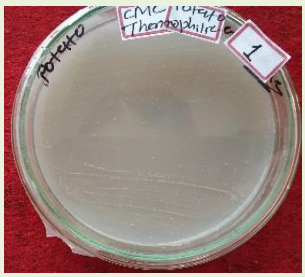
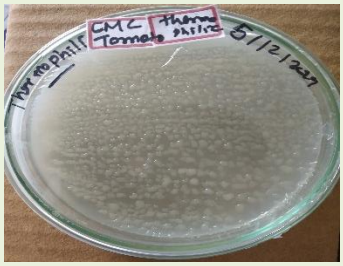
	Zone of hydrolysis (mm)								
	Cellulose			Pectin			Xylan		
Isolates code	Colony size	Zone size	zone ratio	Colony size	Zone size	Zone ratio	Colony size	Zone size	zone ratio
MBCF1	10	38	3.8	15	40	2.6	22	7	3.14
MBCF2	8	18	2.25	8	25	3.1	17	8	2.12
MBCF3	5	15	3	7	30	4.28	7	34	4.85
MBPF4	7	36	5.1	15	28	1.86	7	32	4.57

[Photograph 1: Enrichment of Mesophilic Cellulose, Pectin and Xylan degrading microorganisms]

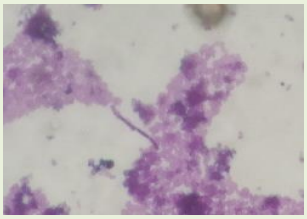
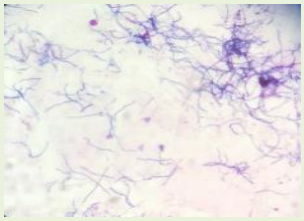
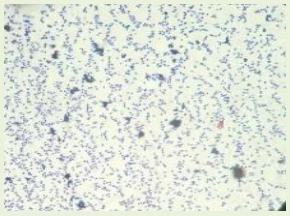
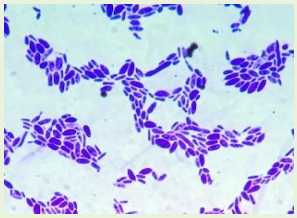
[Photograph 2: Enrichment of Thermo-tolerant Cellulose, Pectin and Xylan degrading microorganisms]

MBC26 **MBP13** **MBX6**

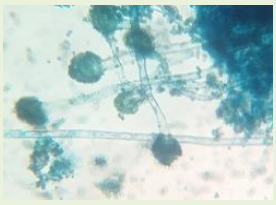
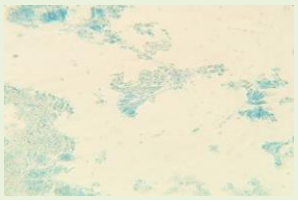
[Photograph 3: Mesophilic Cellulose, Pectin and Xylan degrading isolates.

		
MBC1	**MBC2**	**MBPF4**

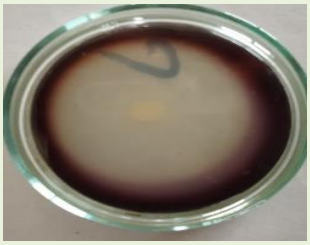
[Photograph 4: Thermo-tolerant Cellulose, Pectin and Xylan degrading isolates]

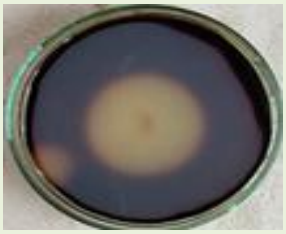
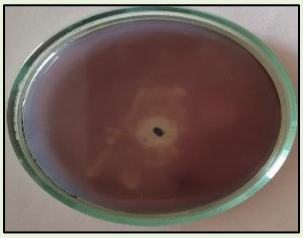
			
MBC4	**MBX5**	**MBC26**	**MBP11**

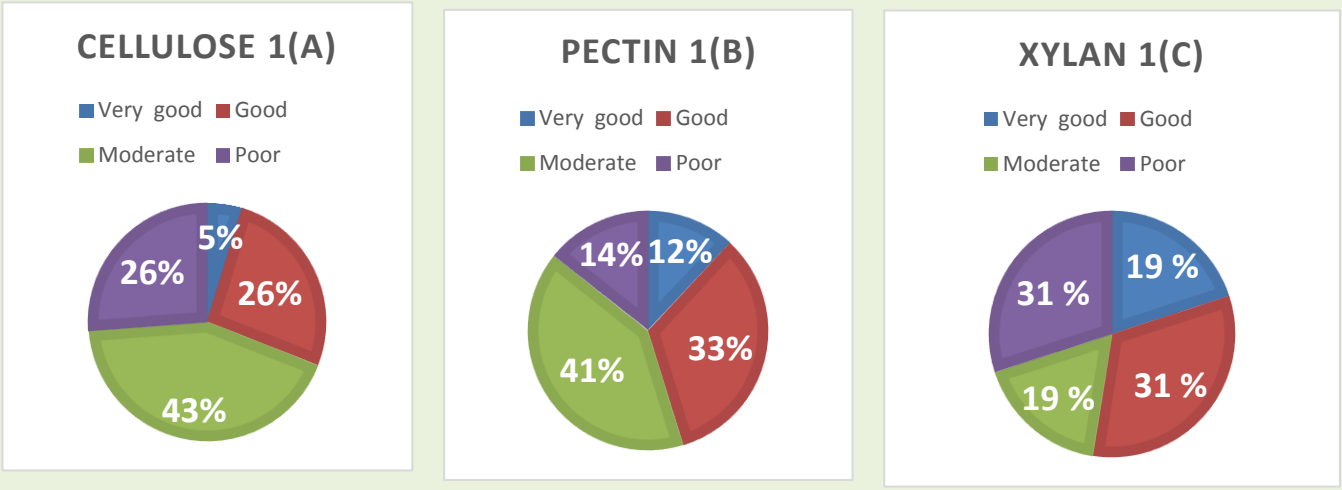
Photograph 5: Microscopic view of bacterial isolates

			
MBCF1	**MBCF2**	**MBCF3**	**MBPF4**

Photograph 6: Microscopic view of fungal isolates

			
MBX8	**MBC32**	**MBC32**	**MBC4**
Photograph 7: Zone of polymer hydrolysis by bacterial isolates			

			
MBCF1	**MBCF2**	**MBCF3**	**MBPF4**
Photograph 8: Zone of polymer hydrolysis by Fungal isolates			



Graph 1: Degradation Potential of cellulose, pectin and xylan degrading microorganisms