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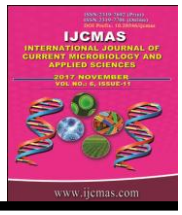

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Optimization of Fermentation Conditions for Pectin Degrading Enzyme Production by Pectinolytic Microbial Consortia Used for Jute Retting

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A B S T R A C T

Three pectinolytic microbial consortia developed for faster retting of jute (*Chorchorus olitorius* L. and *Chorchorus capsularis* L.) were subjected to various pH (4 to 12) and media with variable nitrogen and fixed carbon sources for optimization of fermentation conditions of the consortia for cost-effective pectin degrading enzyme production. All the microbial consortia were active over a wide range of pH from 6 to 10 and recorded maximum polygalacturonase and pectin lyase activities at pH 10. The polygalacturonase and pectin lyase activities of microbial consortium 3 were higher by 2.05 and 3.59 times over microbial consortium 1 and 3.66 and 4.72 times over microbial consortium 2 at pH 10. Among the eight media under study, the yeast extract pectin agar media recorded higher polygalacturonase and pectin lyase production by all the three pectinolytic microbial consortia. Yeast extract was found as the most suitable source of nitrogen over other source of nitrogen and suppliments for polygalacturonase and pectin lyase production. The polygalacturonase and pectin lyase production in yeast extract pectin agar media by microbial consortium 3 were higher by 2.2 and 3.3 times over microbial consortium 1 and 2.8 and 3.94 times over microbial consortium 2. Yeast extract pectin media at alkaline pH (8-10) recorded the maximum polygalacturonase and pectin lyase enzyme production by pectinolytic microbial consortia used for jute retting

Introduction

Jute (*Corchorus olitorius* L. and *C. capsularis* L.), the golden fibre, is an important cash crop of Eastern India, and India earns about 2050 crores rupees annually by exporting diversified jute products. Quality jute fibre is essential for the production of diversified jute products which largely depends on the bio-chemical process of retting. Retting of jute is carried out by the various enzymes like polygalacturonase, pectin lyase (Soriano *et al.*, 2005; Zhang *et al.*, 2000), xylanase etc. secreted by the retting microbes. Uses of pectinolytic bacterial consortium for quick retting of jute have been reported by several researchers (Banik *et al.*, 2007; Majumdar *et al.*, 2009; Das *et al.*, 2012; Das *et al.*, 2015). The microbes of microbial consortium in useful association release pectinolytic enzymes (polygalacturonase, pectin lyase) and xylanase, which in turn degrade the pectin and xylan present as cementing materials. The degradation of these cementing

materials helps in the loosening of the bondage with cellulosic fibre and easy removal of cementing portion, softening of bark tissues to obtain better quality fibre (Das *et al.,* 2015). Higher fibre recovery and quality jute fibre production with reduction in retting duration in improved retting by using microbial consortium compared to conventional retting was reported by Das *et al.,* (2017).

The vitality and potentiality of various pectinolytic microbes to produce enzymes intensively depends upon the selection of appropriate nutrient medium, in particular, carbon and nitrogen sources. Ammonium phosphate was found best N source for growth of *Aspergillus niger* (Joshi *et al.,* 2006), whereas peptone, casein and ammonium sulphate were utilized well by pectinase producing fungi and maximum enzymatic activity was recorded with ammonium sulphate (Kutateladze *et al.,* 2009). Carbon sources like polygalacturonic acid, pectin, lactose enhanced pectinase production (Kashyap and Soni, 2003) and use of 1% pectin recorded maximum PME (pectin methylesterase) (Madhania *et al.,* 2010). Individual pectinolytic bacterial strains were found to exhibit polygalacturonase and pectin lyase activities over a wide range of pH (Kobayashi *et al.,* 2001; Tamburini *et al.,* 2003; Kashyap *et al.,* 2000; Soriano *et al.,* 2005).

Standardization of optimum fermentation conditions like carbon and nitrogen sources and pH of the growth medium is essential for cost effective production of the pectinolytic enzymes by individual or group of pectinolytic microbes. Hence, the present laboratory study was undertaken to optimize the fermentation condition of three pectinolytic microbial consortiums developed for jute retting at various co-operating centres of ICAR-Central Research Institute for Jute and Allied Fibres for cost-effective production of pectin degrading enzymes.

Materials and Methods

Microbial consortium

Three microbial consortia developed for faster retting of jute under the Technology Mission on jute project at ICAR-Central Research Institute for Jute and Allied Fibres (ICAR-CRIJAF), Barrackpore and its collaborating centres. The component microbes of each microbial consortium was isolated and screened out from jute retting water.

Microbial consortium 1 (MC 1) consists of 4 pectinolytic bacteria of *Bacillus sp.*L6, *Bacillus pumilus* EK 17, *B. pumilus* Geo-03-422 and *B. pumilus* IK-MB12-518 F (Das *et al.,* 2012), microbial consortium 2 (MC2) consists of three different strains of *Bacillus pumilus* and one strain of *Bacillus subtilis* (Srivastava *et al.,* 2012) and microbial consortium 3 (MC 3) consists of *Bacillus pumilus* IMAU80221, *B. pumilus* GVC11 and *B. pumilus* SYBC-W (Majumdar *et al.,* 2009; Das *et al.,* 2015). The microbes of MC1 & MC2 have cellulolytic activities whereas; the microbes of MC3 do not have any cellulolytic activities. The pure cultures of microbial consortiums were used for the study.

pH optimization

For pH optimization, nine different buffers of pH values ranging from 4 to 12 were used respectively as potassium hydrogen phthalate-NaOH buffer (pH 4.0), Potassium hydrogen phthalate-NaOH buffer (pH 5.0), Potassium di-hydrogen phosphate (KH_2PO_4)-NaOH buffer (pH 6.0), Tris-HCl buffer (pH 7.0), Tris-HCl buffer (pH 8.0), Tris- HCl buffer (pH 9.0), Borax-NaOH buffer (pH 10.0), Sodium-bicarbonate ($NaHCO_3$)-NaOH buffer (pH 11.0), Potassium chloride (KCl)-NaOH

buffer (pH 12.0). Pure cultures of each of the component microorganisms of the microbial consortium were streaked on pectin agar plate and incubated for 48 hrs, and then a single colony of the isolates were inoculated in 10 ml pectin broth and incubated at 34^0C at 180 rpm for 24 hrs to synchronize the growth of individual culture. After 24 hrs of incubation, each consortium was inoculated @ 1% in 100 ml pectin broth (1% pectin + 1% yeast extract + 0.5% NaCl) and incubated at 180 rpm for 48 hrs and the supernatant obtained after centrifugation at 10000 rpm for 10 minutes were used for polygalacturonase and pectin lyase assay. Polygalacturonase (PG) enzyme assay was done by DNS method of Miller, (1959), later modified by Phutela *et al.*, (2005). One unit (IU) of polygalacturonase activity corresponds to the release of 1µmol of galacturonic acid/min/ml of supernatant culture keeping D-galacturonic acid as the calibration standard. Pectin lyase (PNL) enzyme assay was done by following the modified TBA method of Nedjma *et al.*, (2001). One unit (U) of pectin lyase activity defined as the amount of enzyme that caused a change in absorbance of 0.01 under the assay condition. On the basis of maximum PG and PNL activities, the pH value was optimized for each consortium.

Media optimization

After determination of optimum pH, each consortium was tested at its individual optimum pH value for production of highest amount of polygalacturonase and pectin lyase enzyme activities using 8 different media having fixed carbon source and variable nitrogen source or supplements. On the basis of maximum PG and PNL enzyme activities, best suitable media was standardized for three different consortiums. Seven media used were respectively as Media 1- (1% pectin + 1% yeast extract + 0.5% NaCl), Media 2- (0.5% glucose + 1% pectin + 1% yeast extract + 0.5% NaCl), Media 3- (0.5% $CaCl_2$ + 1% pectin + 1% yeast extract + 0.5% NaCl), Media 4- (1% pectin + 0.5% ammonium sulphate + 0.5% NaCl), Media 5- (1% pectin + 0.5% ammonium oxalate + 0.5% NaCl), Media 6- (1.5% pectin + 0.5% ammonium nitrate + 0.5% NaCl), Media 7- (1% pectin + 0.5% peptone + 0.5% NaCl) and Media 8- (1% pectin + 0.5% beef extract + 0.5% NaCl). In media 2 and 3, glucose and $CaCl_2$ were added as supplements to the yeast extract pectin medium. In medium 4, 5 and 6, ammonium sulphate, ammonium oxalate and ammonium nitrate were respectively used as the sole source of nitrogen instead of yeast extract. In medium 7 and 8, yeast extract as nitrogen source was replaced respectively by peptone and beef extract. Each consortium was inoculated @ 1% in 100 ml broth of eight media under study and incubated at 180 rpm for 48 hrs and the supernatant obtained after centrifugation at 10000 rpm for 10 minutes were used for polygalacturonase and pectin lyase assay as described earlier.

Statistical analysis

The data were analysed as a single factor one way analyses of variance (ANOVA) in a completely randomized block design using SPSS 10.0 for windows (SPSS Inc., USA). Means were separated by using Duncan's multiple range test (DMRT) at 5% probability level of statistical significance.

Results and Discussion

Effect of pH on PG and PNL activities

The effect of wide range of pH (4 to 12) was tested on the polygalacturonase (PG) and pectin lyase (PNL) activities of three microbial retting consortiums (Table 1). The microbial consortium 1 (MC1) recorded a peak of PG activity at pH 6.0 and thereafter

more than two fold increase in PG activity at pH 8.0 (10.7 IU/ml), which was at par with the recorded activities at pH 9.0 (10.4 IU/ml) and 10.0 (10.8 IU/ml) which then decreased significantly with increase in pH. The microbial consortium 2 (MC2) recorded a gradual increase in PG activity with increase in pH up to 10.0 and thereafter reduced with increase in pH. The maximum PG activity (7.5 IU/ml) by MC2 was recorded at pH 10.0 followed by activities of 5.43 and 5.3 IU/ml respectively at pH 9.0 and 11.0.

The microbial consortium 3 (MC3) recorded a significantly higher PG value at pH 6.0 (9.35 IU/ml) and thereafter two significant peaks of PG activities at pH 9.0 (11.75 IU/ml) and 10.0 (20.2 IU/ml), thereafter reduced significantly at pH 11.0 (10.1 IU/ml). Although, the three microbial retting consortiums recorded maximum PG activity at pH 10.0, but the PG activity of MC3 was higher by 2.05 and 3.66 times over MC1 and MC2 at their respective pH. The retting consortiums were very active over a wide range of pH from 6.0 to 10.0 with higher PG activities in the alkaline range.

The pectin lyase activity was very low at pH 4.0 and 5.0 by MC1 and MC2, then sudden increase in PNL activity at pH 6.0 onwards and the maximum PNL activity was recorded at pH 10.0 by MC1 (50.2 U/ml) and MC2 (38.2 U/ml). Unlike the MC1 and MC2, the MC3 recorded very high PNL activities starting from pH 4.0 and there was significant increase in PNL activity with increase in pH up to 10.0 and the maximum PNL activity (180.5 U/ml) was recorded at pH 10.0 which was higher by 3.59 and 4.72 times over MC1 and MC2 at the same pH. The PNL activity of MC3 was higher by several times at each pH value under study over MC1 and MC2. From the PG and PNL activities over a wide range of pH values, it is clear that MC3 was better among the three retting consortium under study as it recorded higher PG and PNL activities over MC1 and MC2.

Individual pectinolytic bacterial isolates showed higher PG and PNL activities in between pH 8.0 and 8.5 as reported by Kashyap *et al.,* (2000); Tamburini *et al.,* (2003); Das *et al.,* (2011). On the other hand, Soriano *et al.,* (2005) reported higher PNL activities by *Paenibacillus sp*, BP-23 & BP-7 at pH 10.0. In the present study, PG and PNL activities of three retting consortium consisting of more than two pectinolytic bacterial isolates were studied in a wide range of pH from 4 to 12.0.

Table.1 Effect of pH on polygalacturonase and pectin lyase activities of microbial consortia

pH values	Polygalacturonase activity (IU/ml)			Pectin lyase activity (U/ml)		
	MC1	MC2	MC3	MC1	MC2	MC3
4.0	0.70^{f}	1.16^{g}	4.00^{f}	2.00^{f}	1.00^{h}	8.20^{h}
5.0	2.52^{e}	2.22^{f}	7.59^{e}	3.70^{f}	1.60^{h}	16.50^{g}
6.0	4.56^{c}	2.55^{e}	9.35^{cd}	10.00^{e}	7.80^{g}	25.20^{f}
7.0	2.93^{e}	3.21^{d}	7.40^{e}	11.50^{e}	12.50^{f}	40.20^{e}
8.0	10.70^{a}	3.95^{c}	8.66^{d}	48.50^{ab}	20.20^{d}	69.50^{d}
9.0	10.40^{a}	5.43^{b}	11.75^{b}	46.40^{b}	26.80^{c}	120.60^{b}
10.0	10.80^{a}	7.50^{a}	20.20^{a}	50.20^{a}	38.20^{a}	180.50^{a}
11.0	6.26^{b}	5.30^{b}	10.10^{c}	27.50^{c}	32.00^{b}	120.20^{b}
12.0	3.82^{d}	2.61^{e}	9.80^{c}	16.50^{d}	15.20^{e}	95.50^{c}

Means with common letters at the superscript in a column are not statistically different at 5% probability level by DMRT

Table.2 Effect of different media on polygalacturonase and pectin lyase activities of microbial consortia

Media	Polygalacturonase activity (IU/ml)			Pectin lyase activity (U/ml)		
	MC1	MC2	MC3	MC1	MC2	MC3
Media 1	8.50[a]	6.50[a]	18.50[a]	60.50[a]	50.80[a]	200.00[a]
Media 2	6.90[b]	3.28[b]	1.03[d]	46.20[b]	38.20[b]	40.20[d]
Media 3	1.43[f]	1.12[e]	0.85[d]	15.40[f]	21.40[e]	34.20[e]
Media 4	4.05[c]	1.84[d]	3.20[c]	38.50[c]	26.20[d]	45.50[c]
Media 5	0.90[g]	0.93[e]	4.62[b]	10.20[g]	16.60[f]	56.20[b]
Media 6	3.77[c]	2.33[c]	0.54[de]	36.80[c]	30.50[c]	30.50[f]
Media 7	2.54[e]	0.24[f]	0.45[e]	22.60[e]	11.20[g]	31.20[f]
Media 8	3.42[d]	0.96[e]	4.33[b]	30.20[d]	20.50[e]	58.80[b]

Means with common letters at the superscript in a column are not statistically different at 5% probability level by DMRT

All the three retting consortium were found to active for PG & PNL activities over a wide range of pH from 6 to 10.0 with maximum values at pH 10.0, while in case of individual microbes, the maximum PG and PNL activities were recorded at pH between 8 and 8.5 as reported by earlier researchers. As there is no report of PG and PNL activities of microbial consortium at different pH, the result could not be compared.

Effect of media on PG and PNL activities

The maximum polygalacturonase (PG) activity was observed by three microbial consortiums in yeast extract pectin (YEP) medium (Table 2). The next higher values of PG activity was recorded in medium 2 (glucose + YEP) by MC1 and MC2, whereas, MC3 recorded higher PG activity in ammonium oxalate, beef extract and ammonium sulphate after YEP medium. Addition of glucose and $CaCl_2$ as supplements to YEP medium decreased the PG activity by MC3. The microbes of MC3 are non-cellulolytic in nature, so addition of cellulose might have affected their growth and reduced the PG activity. Among the three consortiums, MC3 recorded very high PG activity (18.5 IU/ml) in YEP medium compared to other two microbial consortiums and it was higher by 2.2 and 2.8 times over respective PG activity of MC1 and MC2. The results are in agreement with others where they have reported the combination of yeast extract with pectin to be the best medium for polygalacturonase production (Kashyap *et al.*, 2000; Muslim *et al.*, 2015). The MC1 and MC2 also recorded higher values of PG activity in ammonium sulphate and ammonium nitrate. Beef extract was found as a good source of nitrogen for PG production by MC3, but among inorganic sources of N, ammonium sulphate was a good source of nitrogen as also reported by Muslim *et al.*, (2015).

The pectin lyase activity of three microbial consortiums was also affected by various source of nitrogen in the medium (Table 2). The highest pectin lyase activity was recorded by the entire microbial consortium with yeast extract; MC3 recorded pectin lyase activity of 200 U/ml which was higher by 3.3 and 3.94 times respectively over MC1 and MC2. The microbial consortium MC1 and MC2 recorded next higher pectin lyase activity with yeast extract and glucose as supplement followed by ammonium sulphate in case of MC1 and ammonium nitrate in case of MC2.

On the other hand, MC3 recorded next higher values of pectin lyase activity (80.8 U/ml) with beef extract and ammonium oxalate (56.2 U/ml) as N source. Yeast extract was found the best nitrogen source for PG and PNL production probably due to its higher content of minerals, vitamins, coenzyme and nitrogen components (McMillan and Johnson, 2005) for the three microbial consortia.

It can be concluded from the study that by using yeast extract pectin media at alkaine pH (8-10), maximum polygalacturonase and pectin lyase enzyme production by pectinolytic microbial consortia can be achieved. Among the three microbial consortia under study, the polygalacturonase and pectin lyase production in yeast extract pectin agar media by microbial consortium 3 were significantly higher over microbial consortium 1 and 2.

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