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International Journal of Current Research
Vol. 10, Issue, 06, pp.70829-70835, June, 2018

INTERNATIONAL JOURNAL OF CURRENT RESEARCH

ISSN: 0975-833X

RESEARCH ARTICLE

PRODUCTION OF POLY METHYL GALACTURONASE (PMG) BY SIX FRUIT ROT FUNGI AND ITS POSSIBLE USE IN FRUIT JUICE TECHNOLOGY

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ARTICLE INFO

Article History:
Received 29th March, 2018
Received in revised form
06th April, 2018
Accepted 29th May, 2018
Published online 30th June, 2018

Key words:
Pectin, Pectinases, Polymethyl galacturonase, Fruit rot fungi Fruit Juice extraction and clarification

ABSTRACT

Pectinases are group of enzymes that attack pectin and depolymerize it by hydrolysis and transelimination. Polymethyl galacturonase catalyzes the cleavage of $\alpha - 1$, 4-glycosidic linkage of pectin with exo (sequential) and endo (random) activities. The maximum endo PMG in Asthana and Hawker's medium showed by *Rhizoctonia solani* during its 14 days (50.0 RVU). the Endo PMG was studied in six fruits i.e., Apples, Mango, Tomato, Sapota, Grapes and Orange. The maximum endo PMG was recorded in Grapes (62.4 RVU) by *Aspergillus niger* and next best organism is *A. flavus,* while the maximum exo PMG was noticed in *Penicillium citrinum* (1070 µg/ml) during its 7 days of incubation and in vivo studies showed the maximum activity in sapota fruits 995 µg/ml by *Mucor racemosus* and in orange fruits by *A.niger*. These potential fungal strains viz., *Apergillum niger, A. flavus, Penicillium citrinum* and *Mucor racemosus* shall pave the way for exploitation of these fungal enzymes in fruit juice technology.

Citation: Aruri Suryam, Rafiyuddin and Singara Charya, M.A. 2018. "Production of Poly Methyl Galacturonase (PMG) by Six fruit rot fungi and its possible use in fruit Juice Technology", *International Journal of Current Research*, 10, (6), 70829-70835.

INTRODUCTION

Pectin found in primary cell wall and middle lamella of fruits and vegetables (Favela *et al.,* 2006) pectin Contains α,1,4 linked D-galactosyluronic residues. Three pectic polysacchorides, homogalacturonan, Rhamno galacturonan-1 and substituted galacturonan have been isolated from primary plant cell walls (Ridely *et al.,* 2001). Pectinases are group of enzymes that attack pectin and de-polymerase it by de-esterification reaction, which by hydrolyse the ester bonds between carboxyl and methyl groups of pectin (Satyanarayana and Panda, 2003). Pectinases are classified based on their preferred substrate (pectin / pectic acid or Poly galacturonic acid) and on the degradation mechanism (trans elimination or hydrolysis) and the type of cleavage (random - endo or terminal – Exo (Kashyap *et al.*, 2001). Among pectin degrading enzymes, Poly Methyl Galacturonase enzyme are known tobe secreted by pathogenic fungi (Shridha Chaurasia *et al.,* 2015). Poly methyl galacturonase (PMG) catalyzes the hydrolytic cleavage of α,1,4 glycosidic bonds in pectin back bone preferentially highly esterified pectin, forming 6- Methyl D- galacturonate (Jayani *et al.,* 2005). Endo PMG (E.C.3.2.1.41)-hydrolyses of pectin in random fashion and Exo PMG (E.C.3.2.1.40) – hydrolyses of pectin in a sequential fashion from a non reducing end. A wide range of pectin degrading enzymes are involved in pectin modifications, which are to be secretes during fungal infection. These enzymes facilitates the entry and expansion of pathogen in the host issue (Jayani *et al.,* 2005). PMG has wide application in juice and food industries. It is required for extraction and clarification of fruit juices and wines, extraction of oil, flavonoids and pigments from materials, preparation of cellulose fibers for linen, jute and hemp manufactures etc (Hang and Dornenburg, 2000). The commercial preparations of pectinases are produced mainly from fungi, especially *Aspergillus niger* (Favela *et al.,* 2005). The microbial pectinases account for 25% global food enzymes sale (Suryam *et al.,* 2018). Food processing enzymes including pectinases account for 45 % of enzyme usage (Sangeeta *et al.,* 2005). *In vitro* the production of Poly Galacturonase (PG) and Poly Methyl Galacturonase (PMG) largely depends on the cultural conditions and their production may also be induced by different nutrients, which are incorporated in the medium (Fiedurek *et al.,* 1995). Mechanical crushing of pectin reach fruit yields a fruit juice with high viscocity, it is difficult to the extract of this juice by pressing or using other mechanical methods, with the addition of pectinises fruit juice is easily obtained and with higher yields (Tapre and jain, 2014). The production of fruit and vegetables juices is impartant both from the human health and commercial stand points (Harsh *et al.,* 2014). Waste material fron agro industrial processing may be used as the substrate for microbial growth in SSF or SMF (Viviani *et al.,* 2010). The present study is oriented to isolate the fungal organisms capable of producing high qualities of PMG and its application

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in fruit juice extraction and clarification. The selected strains, which are thourougly investigated and critically monitored, are the safe candidates for the application in fruit juice technology.

MATERIALS AND METHODS

(a) Collection of infected fruits and fungal isolation method: The infected fruits of tomato (*Lycopersicum esculentum*), mango (*Mangifera indica*), apple (*Malus pumila*), sapota (*Achras sapota*), orange (*Citrus sinensis*) and grapes (*Vitis vinifera*) were collected carefully in the separate polyethylene bags from the fruit markets of Kumarpally, Hanamkonda, Kazipet, Warangal areas and carried to the laboratory. The infected portions of fruits indicate post-harvest fungal / bacterial diseases. The fruit was surface sterilized with 0.1% mercuric chloride for one minute and washed thoroughly and a small transitional portion of infected and healthy regions was separated and transferred onto the agar slants of Asthana & Hawker's Agar medium (A) (Glucose- 5 g, KNO_3-3.5 g, KH_2PO_4 – 1.75g, $MgSO_4$–0.75g, Agar-Agar 20g) and incubated at room temperature for 3 days.. After incubation period the emerged hyphal tips were picked up and transferred to Asthana and Hawker's Agar (A) slants in aseptic condition and incubated them at room temperature for one week to obtain pure cultures. About 50 fungal species were isolated and identified from different fruits and among these the dominant cultures occurred very frequently were selected for the present study on(*in vivo* and *in vitro*) pectinase production. The important six fungal species used in the present study are viz., *Rhizoctonia solani, Penicillium citrinum, Mucor racemosus, Rhizopus stolonifer, Aspergillus flavus* and *Aspergillus niger*.

(b) Extraction of pectinases from fruits (In *vivo*): Healthy fruits were inoculated with six fruit rot fungi viz. *Rhizoctonia solani, Penicillium citrinuim, Mucor racemosus, Rhizopus stolanifer, Aspergillus flavus* and *A. niger* by giving a small incision on the surface of fruit and sterilized cotton was wrapped on the infected part and after an adhesive tape was fixed in aseptic condition. The fruits were incubated for 4–16 days in case of apples and 2 – 8 days for all other fruits. After incubation period, 20 grams of infected portion of the fruits was separated in aseptic condition and cut the tissue into small pieces of 1-2 centimeters. The cut pieces were transferred into a waring blender and added 100 ml 0.15 M NaCl and macerated for two minutes. This was filtered through two layers of cheese – cloth and transferred the filtrate to centrifuge tubes and centrifuged at 2000 r.p.m. for 30 minutes and supernatant was separated into culture flask and this filtrate was used as an enzyme source. Few drops of toluene was added and enzyme was stored at 4°C in case when enzyme was not immediately used.

(c) Extraction of Pectinases from Pathogen (*In vitro*): Pectin or supplemented Asthana and Hawker's medium (pectin 5g, KNO_3 3.5 g, KH_2PO_4 – 1.75 g, $MgSO_4$ – 0.75 g) was prepared and 100 ml of broth was transferred into 250 ml conical flasks. Aspetically inoculated the flasks with 2 ml of spore suspension or 7 mm mycelial disc from the growing margin of 5 days old culture of respective fungi. The inoculated flasks were incubated at 27°C for 7, 14 and 21 days. After the incubation period the contents were filtered through Whatman No. 1 filter paper and mycelial mat was separated. The filtrate was centrifuged at 2000 rpm for 30 minutes and supernatant was taken as enzyme source. Few drops of toluene was added to the enzyme, when enzyme assay was delayed.

(d) Assay of Exo-Polymethyl Galacturonase (Exo-PMG): Exo-PMG was estimated by DNS method (Miller, 1959) 3.5 ml of 0.5% pectin solution was taken into a test-tube and added with one ml of citrate buffer followed by 0.5 ml enzyme (fruit extract / broth culture) and 3-4 drops of toluene and incubated at 30°C for six hours. After incubation period, 0.2 ml of aliquot was withdrawn from the above reaction mixture and added with three ml of DNS reagent. The contents were thoroughly mixed and kept for 15 minutes in a hot water bath. Two ml of 20% sodium potassium tartarate solution was added to the test-tube while it was hot and cooled the tube under running tap water. The developed brown colour was read at 575 nm by using spectrophotometer. A blank was prepared with the same procedure by using a heat killed enzyme. Reducing sugars liberated were calculated with the help of standard curve drawn for glucose. Poly Methyl Galacturonase (PMG) activity was expressed in terms of mg of reducing groups (as µg/ml) liberated in 6 hours.

(e) Assay of Endo Polymethyl Galacturonase (Endo-PMG): Wood (1955) viscometric method was followed to estimate the Exo-PMG. Pectin (0.5%) was prepared by dissolving 0.5 g of pectin in 100 ml citrate buffer (pH 5.5) and heated at 50°C – 60°C and the contents were blended for 3 minutes and filtered through two layers cheese cloth. The pH was adjusted to 5.2, by the addition of 1N HCl or 1N NaOH using pH meter. The reaction mixture for the estimation of Endo-PMG was with pectin (0.5%) substrate, citrate buffer (pH 5.5) and enzyme source in 4:1:2 ratio. The contents were mixed in a 100 ml beaker and immediately transferred into an Oswald viscometer. The efflux time of the contents were determined with the help of a stop clock at the initial time. The contents were incubated for three hours in the viscometer at room temperature and reduction in the efflux time of the contents in the viscometer was calculated after every 10 minutes. The percentage of reduction in viscocity was calculated by applying the following formula of the substrate by applying the following formula:

$$V = \frac{ET_o - ET_t}{ET_o - ET_w} \times 100$$

Where, V = percent loss of viscosity.
ET_o =flow time of water in seconds at zero time
ET_t=flow time of reaction mixture at 't' intervals
ET_w=flow time of distilled water.

The Relative Enzyme Activity (REA) of endo PMG was calculated by dividing 1000 with time required for 50% loss of viscosity (t_{50}) and expressed the activity in Relative Viscosity Units (RVU).

$$REA = \frac{1000}{t_{50}}$$

RESULTS

In vitro: The production of endo-PMG by six fruit rot fungi during 21 days of incubation was assayed in between 7, 14 and 21 days and presented in table-1. From the table it was evident that the maximum endo-PMG was noticed in *Rhizoctonia solani* during its 14 days of incubation (50.0 RVU). The next best organisms were *Aspergillus niger* (41.6 RVU) and *A. flavus* (37.0 RVU) after 21 days. Among the six fungi

Rhizopus stolanifer was responsible for least enzyme secretion (19.0 RVU) and that was stable up to 21 days of incubation. In the 21 days of incubation the growth rate decreased and subsequently the enzyme production also declined During 7 days of incubation the fungi were initiated the production of endo-PMG and ranged between 12.7 to 40.0 RVU. *Mucor racemosus* is only one organism which showed its maximum endo-PMG production in 7 days of incubation (33.3 RVU). In view of these results it was noticed that for maximum endo-PMG production the ideal incubation time in Asthana & Hawker's medium (supplemented with pectin) was 14 days.

Table 1. Endo Pectin Methyl Galacturonase (PMG) activity of six fruit rot fungi on Ashthana & Hawker's medium supplemented with pectin after 7, 14 and 21 days of incubation

Fungi	Relative enzyme activity*		
	7	4	21
Rhizoctonia solani	40.0	50.0	19.0
Penicillium citrinum	20.0	29.0	23.8
Mucor racemosus	33.3	22.2	22.2
Rhizopus stolanifer	12.7	19.0	19.0
Aspergillus flavus	22.2	29.0	37.0
A. niger	20.0	28.0	41.6

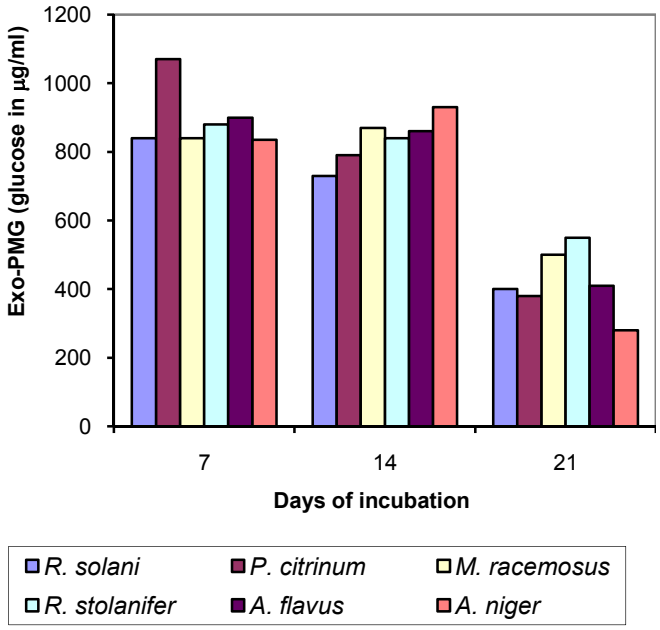

Fig. 1. Exo Poly Methyl Galaturonase (Exo-PMG) activity of six fruit rot fungi on Ashthana and Hawkers medium supplemented with pectin after 7, 14 and 21 days in incubation

In vivo: The endo-PMG production *in vivo* was studied in six fruits and the obtained results were incorporated in table -2. The table clearly revealed that the production rates were increased initially up to 4/6 days and subsequently the quantities were decreased. The apple fruits were incubated after the inoculation by six fungi and after 4, 8, 12 and 16 days of incubation the fruit pulp was assayed for endo-PMG activity. The maximum activity was recorded during 12 days of incubation (23.4 RVU) by *Mucor racemosus. Penicillium citrinum* was the next best fungi in producing high amounts of endo-PMG (22.8 RVU), while *Aspergilus niger* (21.5 RVU) and *Rhizoctonia solani* (21.3 RVU) were moderate in their endo PMG activity. In mango fruit the maximum endo-PMG was reported in 4 days of incubation (49.3 RVU) in *Rhizoctonia solani*. Moderate activity was observed in *Mucor-racemosus* (41.7 RVU) and *Rhizopus stolanifer* (41.7 RVU) after 6 days. Where the endo PMG activity and growth rate increased upto six days and declined after 8 days. The endo-PMG production in tomato fruit during 2,4,6,8 days of incubation under pathogensis was assayed and recorded. Among the six fungi, only *Aspergillus niger* activity was increased up to 8 days and remaining fungi showed their maximum endo PMG activity after six days. The endo PMG maximum was recorded in 4 days of incubation (20.0 RVU) in *Rhizoctonia solani.* In sapota fruit the highest range of endo-PMG activity was recorded in 8 days of incubation period and highest (47.6 RVU) was in *Penicillium citrinum* and *Mucor-racemosus*. In the grape fruit, the maximum endo PMG activity was noticed during six days of incubation (62.4 RVU) by *A. niger.* A moderate activity was showed by *Rhizoctonia solani* (56.0 RVU) and *A. flavus* (55.5 RVU) after eight days, while *Mucor racemosus* showed lowest endo PMG activity after six days (32.5 RVU). In the orange fruit the endo PMG activity was maximum in eight days of incubation by *Mucor racemosus* (11.1 RVU) and gradually endo PMG activity increased upto 8 days by *Penicillium citrinum* and *Mucor racemosus*. A moderate endo PMG was occurred in *A. niger* (10.3 RVU) and *Rhizoctonia solani* (9.2 RVU) after six days and activity declined in both organisms after 8 days.

In vitro: The production of exo-PMG by six fruit rot fungi during 21 days of incubation was assayed in 7, 14 and 21 days and presented in Figure - 1. From the figure it was evident that the maximum exo-PMG was noticed in *Penicllium citrinum* during its 7 days of incubation (1070 µµg /ml). But, in general, the 14 days incubation was viewed to be ideal for optimum production and subsequently in 21 days the production rate was decreased. The next best organisms were *A. niger* (930 µµg/ml) and after 14 days and *A. flavus* after 7 days (900 µg/ml). In the 21 days of incubation the growth rate decreased and subsequently the enzyme production was also declined, which ranged from 280 to 550 µ µg /ml by six fungi. During 7 days of incubation the fungi were initiated their growth and acclimatized for the condition and started production of exo-PMG which ranged between 835 to 1070 µ µg/ml. *Penicillium citrinum* and *Aspergillus flavus* were showed their maximum exo-PMG production in 7 days of incubation (1070 and 900 µg /ml). In view of these results it was noticed that for maximum exo-PMG production the ideal incubation period is between 7-14 days in Ashthana and Hawker's medium supplemented with pectin.

In vivo: The exo-PMG production *in vivo* was studied in six fruits and the obtained results were incorporated in text figure-2. The figure clearly indicated that the production rates were increased up to 16 days and subsequently the quantities were decreased. The apple fruit was incubated after the inoculation by six fruit rot fungi and after 4, 8, 12 and 16 days of incubation, the fruit pulp was assayed for exo-PMG activity. The maximum activity was recorded during 12 days incubation, (960 µg /ml) by *Aspergillus flavus.* In mango fruit the maximum exo- PMG was reported in six days (103 µµg/ml) of inoculation by *R. solani* and *Rhizopus stolanifer.* The moderate exo-PMG activity was noticed in *A. flavus* after 2 days (100µg/ml) and *A. niger* (95µg/ml) after 4 days The maximum activity was observed within two days underpathogenesis by *P. citrinum, M. racemosus, R. stolanifer* and *A. flavus*.

Table 2. Endo-poly methyl Galactaronase (Endo PMG) activity of six fruits rot fungi on six fruits after 4,8,12,16 and 2,4,6,8 days of incubation

Fungi	Relative Enzyme Activity																							
	Apples				Mango				Tomato				Sapota				Grapes				Orange			
	4	8	12	16	2	4	6	8	2	4	6	8	2	4	6	8	2	4	6	8	2	4	6	8
Rhizoctonia solani	5.8	16.7	21.3	14.6	2.11	49.3	16.7	13.9	9.3	20.0	16.7	14.8	37.9	46.7	42.7	41.7	12.8	23.4	39.3	5.60	5.6	8.1	9.2	5
Penicillium citrinum	6.8	22.2	22.8	18.9	3.1	36.0	29.0	16.7	7.3	7.4	11.0	6.7	16.7	38.9	42.5	47.6	8.5	16.7	35.8	28.6	2.9	5.6	7.3	8.1
Mucor racemosus	6.4	15.5	23.4	20.8	5.0	39.4	41.7	27.8	8.3	9.9	11.0	3.5	18.9	22.3	38.9	47.6	5.5	11.1	32.5	26.7	2.9	5.6	5.6	11.1
Rhizopus stolanifer	7.7	12.3	18.5	18.2	2.6	29.6	41.7	19.3	2.8	9.5	14.9	11.1	12.7	19.4	33.3	42.4	6.5	9.1	26.8	41.7	4.1	5.0	6.0	2.9
Aspergillus flavus	7.0	16.7	18.2	18.1	2.2	22.2	37.0	19.7	11.1	16.7	8.1	3.7	11.7	19.4	36.0	46.5	11.1	21.2	37.5	55.5	5.6	7.3	8.00	5.2
A. niger	8.0	18.5	21.5	18.2	2.0	20.6	22.7	22.7	5.5	7.4	11.1	13.3	17.4	20.3	34.7	42.4	23.5	57.1	62.4	58.5	8.0	9.2	10.3	6.0

**Activity express in Relative Viscometric Units*

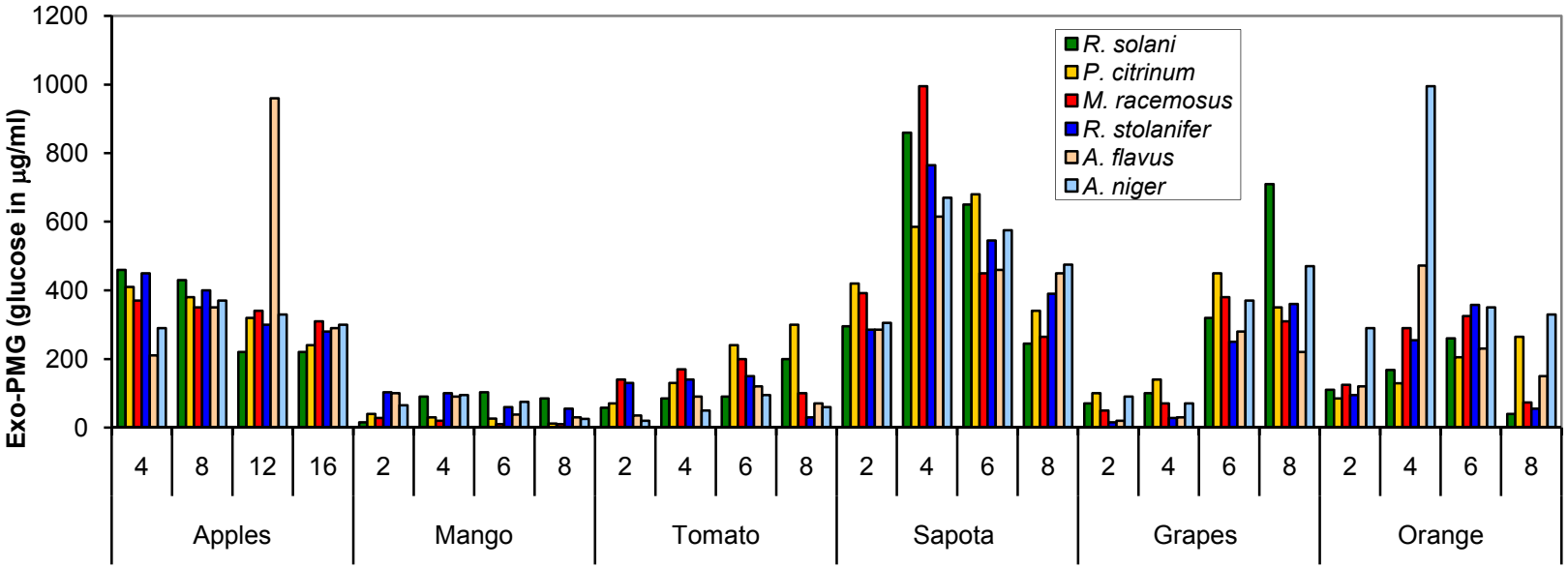

Fig. 2. Exo-polymethyl galacturonase (Exo-PMG) activity of six-fruit rot fungi on six fruits after 4, 8, 12, 16 and 2, 4, 6, 8 days of incubation

The quick spoilage of fruit and vigorous infection are the main reasons for this type of fast decline in the rate of exo-PMG activity. In tomato fruit the highest exo PMG activity was obtained after eight days of incubation period in *R. solani* and *P. citrinum*, while remaining four organisms showed after six days of incubation and enzyme activity was declined up to 8 days. The highest exo-PMG was recorded in *P. citrinum* (300µg /ml) after 8 days of incubation. In sapota fruit the highest range of exo-PMG activity was recorded in four days of incubation period and highest (995 µg/ml) in *Mucor racemosus*. Next best producing fungi were *Rhizoctonia solani* (860 µg/ml) and *Rhizopus stolanifer* (765 µg /ml) after four days of incubation.

In remaining five fungi highest activities were after four days and there was much decline in the production rates of exo-PMG during 8 days of incubation and their range was between 245 to 475 µ µg /ml. In the grape fruit the maximum exo-PMG activity was noticed during 8 days of incubation in *R. solani* (710 µg /ml). Moderate activities were showed by *A. niger* (470 µg/ml) after 8 days and *P. citrinum* (450 µ µg /ml) after six days of incubation periods. In the orange fruit, the maximum exo-PMG activity (995 µg /ml) was noticed after four days of incubation and activity was much declined upto 8 days. *A. flavus* (472 µg /ml) and *M. racemosus* (358 µg /ml) showed their moderate exo-PMG activity after four and eight days of incubation respectively.

DISCUSSION

Gimgihong *et al*. (1991) identified the pectinolytic enzymes production and activity by *Botryosphaeria dothidea* and stated that exo PG and Exo PMG in apple medium showed maximum activity up to 6.4 to 7.2 units at six days of culture respectively. Their maximum activity in Pectin-Poly pectate mineral salts medium was 5.9 and 5.3 units at eight days of culture lower than in an apple medium respectively. Endo PG and Endo PMG in pectin poly pectate mineral salt medium were showed maximum activity (4.4 and 16.2 units) at six and eight days of culture respectively. But in our studies the maximum exo-PMG activity was noticed in Asthana and Hawker's medium supplemented with pectin by *Penicillium citrinum* after seven days of incubation period and in apple fruit the exo-PMG activity was maximum (960 µg/ml) after 12 days by *A. flavus*. While endo PMG was maximum after 14 days in Asthana & Hawker's medium (50.0 RVU) by *Rhizocotnia solani* and in apple extract the endo PMG was noticed in *Mucor racemosus* (23.4 RVU) after 12 days of incubation. Levin and Forchiassin (1998) noticed the pectinolytic enzymes by the white rot fungus *Trametes trogii* on a laboratory scale and observed that high pectinase activities in a media with more alkaline initial pH values (6.2), in the range of 23 to 28^{o}C. Good results were obtained in growth as well as in enzyme production and the addition of Tween 80 promoted growth and gave the highest yield of poly methyl galacturonase and pectinlyase (0.32 and 36.2 EU/ml). But in our investigation pectin promoted the growth and gave the highest yield of PMG in Asthana and Hawker's broth supplemented with pectin.

Jekandra Singh *et al*. (2006) was also studied the PMG production by *Rhizoctonia solani* and Grebechova *et al*. (2007) observed the pectolytic enzymes by submerged fermentation from *A. niger* and *A. foetidus* and stated that the channe growth medium is the best for endo-PMG followed by the Tultobello medium. In fermentation assays *A. niger* in apple pectin produced the highest quantities of endo PMG enzyme followed by carrot and citrus pectins. Production of PMG was increased with increasing in temperature up to 30^{0}c. It means 30 c was most suitable for the production of PMG (ShridhaChaurasia *et al.,* 2015*).* The cellulose repressed the production of endo PG and endo PMG, which enhance the viscocity of poly pectate and pectin respectively in Buffered solution pH 5.0 at 30 c within an hour (Singh *et al.,* 2001*)*. The exo PMG from A.nainiana was most active at pH 7.0 and 60^{0}c, while exo PMG optimal activity from *T.harzianum* strain T6 was obtained at pH 4.3 and 40^{0}c (celestino and filho 2005). The treatment of fruit juice with pectinase samples from Aerophialophora nainiana and T. *harzianum* strain T6 resulted in a decrease of viscocity. Pectinase from T. *harzianum* T6 strain appeared to be more effective in the reducting of turbidity from Apple juice (celestino and Filho, 2005). *Aspergillus niger* is used for industrial production of pectolytic enzymes. This fungus produces PG, PMG and PL (Naidu and Panda, 1998). The fungal strains *A.niger* is highly potential and useful for the production of pectinase (Durairajan and Shivashankari, 2014). *Aspergillus* genus especially *A.niger* as frequently responsible for post harvest decay of fresh fruits such as citrus, grapes, tomatoes (Ajayi *et al.,* 2014). But according to our results other fruits like Apples, Mango, Sapota are also decayed by *Aspergillus* genus. With increasing incubation time, the activity of three enzymes, i.e. cellulase, polygalacturonase (PG) and PMG has been enhanced in *Fusarium oxysporum* (Shukla and Dwivedi, 2012). The enhance enzyme yield, the mixture of two substrates such as sugar beet pulp and wheat bran, having different ratios of carbon, nitrogen and moisture levels was used for the highest PG and PMG production under solid state conditions were determined an wheat bran (Taskin and Eltem, 2008). PMG activity of six *Fusarium* species were evaluated using viscometric assay. In the culture filtrate of 14 days old culture enzyme activity was found to be high, Enzyme production was correlated with the growth of mycelium (Choudary *et al.,* 2013). *Alternaria alternata* (Fr) kiessler was a potential pathogen of onion, was isolated from diseased onion leaves from Nashik district and used for the production of PMG was observed to be least, while the production of PG was produced more by pathogen (Nikumbh and Saler, 2012). Maximum secretion of PMG was observed in *Alternaria* isolatno.1 as compared to four other isolates of *A. Alternata*. Considerable amount of secretion of this enzyme was observed in *A. triticina* isolate No.7 in comparison to other isolates (Petkar, 2013). Pashova *et al*. (1999) studied the physiological aspects of immobilized *Aspergillus niger* cells producing PMGase and stated that conidia of *A. niger* (26 spores) were immobilized in 3% Ca- alginate beads and some intrinsic kinetic characteristics including growth PMG production and specific oxygen uptake rates were determined. A negative correlation between the specific growth rate and specific PMG production indicated a non-growth associated enzyme formation. Inmmobilization did not change the model of PMG synthesis even in repeated replacement cultures and leads under certain conditions to an enhanced of PMG production. The production PMG by immobilized culture was higher then that produced by free cell culture. Performance of pectolytic enzymes was also studied by Naidu and Panda (1999) and stated that the volume ratio of reactants for maximum hydrolysis was determined under the assay conditions. The optimum amount of substrate and enzyme were found to be 0.2 cm^{3} and 0.074 cm^{3} and the pH and temperature optima were found to be 5.3 and 30^{o}C for PMGase. With use of agricultural wastes generates gallons of wastes during preparation of different juices. Its dumping nature causes pollution, this problem can be solved by exploiting these agro wastes for pectinase production by using potential micro organisms (Preeti *et al.,* 2015). Pectinases are ecofriendly in nature in degrading / decomposting the material in the surroundings (Garge *et al.,* 2016). Commertial pectinase with comparison with laboratory produced pectinise was also more effective than the commercial produced enzyme (Ajai *et al.,* 2014). *A. flavus* and *A. niger* showed their potentiality in the pectinase production, these two stains is very effective and replaces the application of costly commercial enzymes in fruit juice and related technologies (Suryam and Charya, 2017). The activity of Pectolytic enzymes i.e. pectin Methyl Esterase, endo and exo PMG and exo and endo pectin trans eliminase produced by *Fusarium oxysporum* was higher. Maximum inhibition of pectin methyl esterase, exo and endo PMG and exo and endo pectin transe eliminase was showed by culture filtrate of *Trichoderma viridae+Pseudomonas fluorescens* (Rajeshwari and Kapoor, 2017).

Conclusion

It was clearly noticed that, the enzyme Exo and endo PMG extraction from these four fungal strains i.e., *Apergillum niger, A. flavus, Penicillium citrinum* and *Mucor racemosus* strains is very effective and replace the application of costly commercial enzymes in clarification and extraction of fruit juice.

Acknowledgements: The authors are very thankful to Head of the Department, Microbiology, Kakatiya University, Warangal Urban, Telangana State for providing laboratory facilities in the Department and one of the author Aruri Suryam is very thankful to University Grants Commission for the award of Rajiv Gandhi National Fellowship (RGNF) during his research.

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