Enhancement of Lycopene Extraction from Tomato peels using Cellulase of *Bacillus cereus*

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Abstract: The present study was aimed to enhance the extraction of lycopene from tomato peel waste with cellulase mediated process. Cellulase was produced by cellulose degrading bacteria through submerged fermentation utilizing tomato peels agro wastes as substrate. Cellulolytic bacteria *Bacillus cereus* KR9 was optimized for pH, temperature, and suitable carbon and nitrogen sources. The data obtained indicate that Rice Straw was found as best substrate with high enzyme production (60.8 Uml^{-1}). The yield obtained with cellulase mediated extraction lycopene from tomato peels was 12. 25 mg/100g of lycopene. Enzyme mediated lycopene was found to more effective in colouring both Bioplastic and Sugarcubes. Hence, *B. cereus* KR9 cellulase assisted lycopene proves to be a potential biocolourant. Further the strain *B. cereus* KR9 can be investigated for scale up processes and other suitable industrial applications.

Key words – Cellulase, *Bacillus cereus*, Rice straw, tomato peel.

I. INTRODUCTION

Lycopene is a natural red tomato pigment that has many beneficial effects to maintains human health. It changes the way of unceasing circumstances of neoplastic diseases, reduces the risk of myocardial infarction, reduces blood pressure and avoid low density lipoprotein (LDL) cholesterol oxidation. High blood lycopene concentrations are also related with lower risks of developing prostate, lung, uterine and breast cancer. Its antioxidant properties proves positive effects on the skeletal system and on neurodegenerative diseases, including Alzheimer's and Parkinson's Przybylska (2020). Due to its strong colouring and non-toxic properties it is used as colouring agent ideal for application in the food industry and used at wide range of yellow-orange-red (Domenguez et al. 2020). Extraction of lycopene from tomato and tomato waste is performed with wide method that utilizes conventional organic solvent (Harini and Sumathy 2016[a]). However, on using the organic solvents for the recovery of products from plant has many limitations such as needs of long extraction times, large organic solvents and reduces quality of product. Thus, the development of an effective and selective method of bioactive compound extraction would be beneficial (Zhang et al. 2017).

Enzyme assisted extraction is one of the most efficient methods of lycopene extraction in which enzyme preparations with polygalacturonase and pectin methylesterase in addition to pectin lyase or cellulase activities are added during tomato processing. This method is used to enhance the recovery of lycopene by degrading the plant cell-wall, thus assisting in the release of the intracellular content (Zuorro and Lavecchia 2010). *Bacillus* species, such as *B. subtilis, B. amyloliquefaciens*, *B. thuringiensis*, *B. cellulomonas* were the best producers of cellulase using submerged fermentation and have been widely used for commercial production of the enzyme for various application. Agrowastes like rice straw, rice husk, and sugarcane waste have replaced the high cost media generally used in submerged fermentation for cellulose production because of their simplicity, low cost and easy availability (Sarrouh et al. 2012).For the large scale production of enzyme it is important to make the process economically viable using bioconversion of agro wastes which could cause pollution to the environment into a more useful product. Till now solvent extracted lycopene were found to be used as colouring agent. Hence, the present study includes the extraction of lycopene from tomato peels with enzyme obtained from cellulose degrading bacteria through submerged fermentation utilizing agrowastes as substrate. The extracted lycopene was used as colouring agent for bioplastic and sugarcubes.

II. MATERIALS AND METHOODS

2.1 MICROORGANISM USED

The strain used in this study is *Bacillus cereus* KR9 which was isolated, identified (Krishma and Radhathirumalaiarasu 2017), stored laboratory of Department of Microbiology, SFR College for women, Sivakasi, Tamilnadu and preserved in nutrient agar slants at 4 °C. Further it was revived using nutrient broth, grown in nutrient agar medium with 2 % (w/v) of CMC (Carboxmethyl cellulose). After 48 hours incubation the growing colonies were stained with 1% congo red, washed with 10% NaCl solution and cellulolytic activity was characterized by observing clear zone the colony.

2.2 CELLULASE PRODUCTION

Cellulase production were carried out using submerged fermentation with medium taken in 250 ml flasks containing 10 g/l of carboxy methyl cellulose (CMC), $MgSO_47H_2O$ - 0.2 g L^{-1}, NaCl -1.0 g L^{-1}, peptone -1.25 g L^{-1}, $FeSO_4$ 0.01 g L^{-1}, KH_2PO_4 0.5 g $L^{-1}$with pH 7. The flasks were autoclaved and inoculated with 1% (v/v) of inoculums and incubated at 37 °C on rotary shaker at 150 rpm for 72 h. After incubation the sample were aseptically withdrawn and centrifuged at 10,000 rpm for 10 min in order to settle the pellet and supernatant was assayed for cellulolytic activity.

2.3 EFFECT OF pH AND TEMPERATURE

The optimum pH for cellulase production was determined by culturing the strain individually in the production media with different pH range (4, 5, 6, 7, 8 and 9). The optimum temperature was determined by incubating the culture inoculated fermentation media at various temperatures such a 27 °C, 37 °C and 47 °C. The enzyme samples were withdrawn after 72 h of incubation and analyzed for cellulolytic activity.

2.4 EFFECT OF CARBON AND NITROGEN SOURCES

The effect of different carbon source on cellulase production was analyzed by preparing the production medium containing glucose, sucrose and starch at the concentration of 1% (w/v). Influence of nitrogen sources was observed by supplementing fermentation medium with peptone, yeast extract and casein individually at the concentration of 0.05 % (w/v) under optimized condition.

2.5 PRODUCTION OF CELLULASE USING AGROWASTES

The suitability of agriculture byproducts as substrate for enzyme production were investigated by addition different processed substrates such as rice straw, rice husk and sugar cane bagasse to the production medium. Submerged fermentation was carried out with 10 g L^{-1} of substrate supplemented with $MgSO_47H_2O$ - 0.2 g L^{-1}, NaCl -1.0 g L^{-1}, $FeSO_4$ 0.01 g L^{-1}, KH_2PO_4 0.5 g $L^{-1}$and optimized nitrogen source at optimum fermentation condition. After incubation the samples were aseptically withdrawn at different time interval (24, 48, 72, 96, and 120 h), centrifuged at 10,000 rpm for 10 min and supernatant, and assayed for cellulolytic activity. All the experiments were carried out in duplicates.

2.6 ANALYTICAL METHOD

Cellulase activity was estimated by the procedure of Islam et al. (2014) carboxymethylcellulose (CMC) as substrate. The reaction mixtures contained 1% CMC dissolved in 0.1 M phosphate buffer (pH 7) and 1 ml crude culture filtrate incubated at 50°C for 30 minutes. The reaction was stopped by adding 1.5 ml of 3, 5-dinitrosalicylic DNS reagent followed by heating the tubes at 100 °C for 10 minutes. After cooling the absorbance was measured spectrophotometrically at 540 nm and enzymatic activity was determined using glucose as standard.

2.7 PARTIAL PURIFICATION OF CELLULASE

The culture filtrate of the fermentation medium was saturated with 80% (w/v) ammonium sulphate. The precipitate was concentrated by centrifugation at 10,000 rpm for 10min. Pellet was dissolved in 100 mM phosphate buffer and dialyzed with same buffer (pH 7). The partially purified cellulase enzyme was stored at 4 °C and used in further experiments.

2.8 Extraction of Lycopene from tomato peel

2.8.1 Solvent extraction

The whole tomato fruits were immersed in boiling water for 1-2 min. Then they were cooled under tap water and hand peeled. The peels were dried in air for a few hours and then stored at 4 °C. The extraction of lycopene was performed using double solvent system (Acetone- Ethyl acetate) carried out for 5 h at 40 °C. Lycopene extracted was measured using UV - Visible spectrophotometer at 503 nm and expressed as mg of lycopene per 100 g of dry matter (Pandya et al.2017).

2.8.2 Enzyme mediated extraction

The partially dehydrated tomato peels (2 g) were transferred to conical flasks and in water bath at 37 °C with magnetically stirrer. An aqueous enzyme solution (10 ml) were added and incubated for about 20 h. After incubation, 30 ml of the extracting solution was poured into the flasks and kept under stirring, at the same temperature for solubilization of lycopene. The lycopene content was measured spectrophotometrically at 503 nm.

2.9 Lycopene as coloring agent

Cornstarch (1 g) was mixed with 20 ml of water, 5 ml of vinegar, and 5 ml of glycerin and 1 ml of extracted lycopene solution. The mixture was stirred continuously till it turns to a clear gel-like consistency. This gel-like solution was boiled and poured into the petriplate. The sugar cubes were prepared, spread over with standard color and lycopene solution. The coloring efficiency was observed with against the standard orange color.

III. RESULTS AND DISCUSSION

3.1 Optimization of pH and temperature

Cellulolytic activity of bacterial strain *B. cereus* KR9 was confirmed by growing the culture in CMC (Carboxmethyl cellulose) incorporated plate. After 48 hours incubation the on staining with 1% congo red and further washing by 10% NaCl solution showed a clear zone of hydrolysis. The highest activity of cellulase of *B. cereus* KR9 was found at pH 7.0 (32.6±0.2 U/ml) and above that pH there was decrease in cellulolytic activity. At alkaline pH, the cellulolytic activity reduced to 24.5±0.2 U/ml whereas at acidic pH the enzyme activity was extremely low (22.9±0.1 U/ml). However, the cellulase enzyme was active at both acidic and alkaline pH with broad pH range of 4 to 9. Among the range of temperatures tested, highest cellulase production by *Bacillus cereus* KR9 was recorded in temperature 37 °C (34.3±0.3 U/ml). Conversely, minimum cellulase production was found at 27 °C (23.7±0.1 U/ml) (Fig. 1). Similar to the finding of Rasi and Mahalingam (2012) showed the best cellulolytic activity of *Bacillus* sp. at neutral (pH 7.0) and 37˚C. The optimum levels of pH, temperature for cellulase production predicted in pH 7.2, 39 °C, and121 rpm conditions respectively (Deka et al. 2013). Temperature also has a significant effect on enzyme activity by *Proteus vulgaris* where increase in temperature from 30 °C to 35 °C showed an increase in enzyme activity from 4.07 to 9.23 U/ml (Archana et al. 2016).

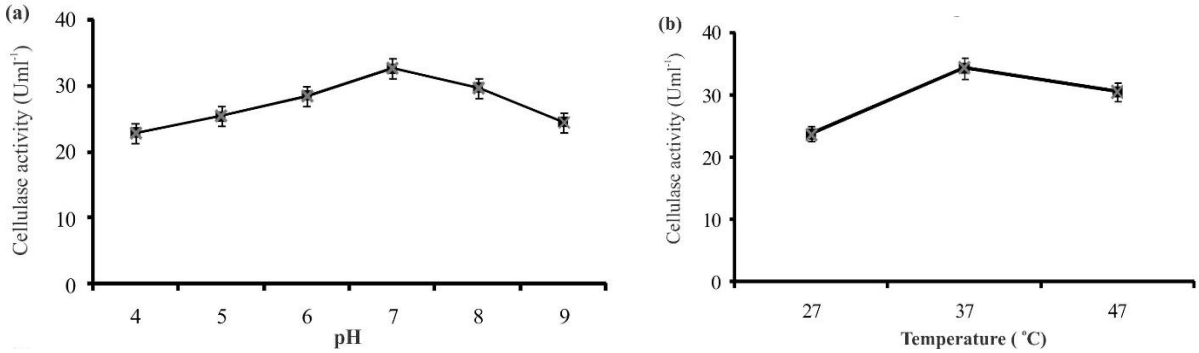

Fig. 1. Effect of pH (a) and Temperature (b) on cellulolytic activity.

3.2 Optimization of Carbon and nitrogen source

The cellulase production was optimized by supplementing various synthetic carbon sources. Among this addition of CMC confirmed the maximum cellulase production (33.1±0.2 U/ml) compared to other carbon sources at 72 h incubation. However, among the nitrogen sources analyzed increased cellulase production was obtained using peptone with highest with enzyme activity of 35.4±0.7 U/ml. Minimum cellulase production was observed while using casein as nitrogen source (24.2±0.5 U/ml) (Fig. 2). This coincides with the earlier work, that records carbon source production by *Bacillus* sp. Y3 the maximum FPase activity (3.74 IU/mL) and CMCase activity (4.49 IU/mL) was found supplemented media with CMC (Lugani et al. 2015). Similarly, the nitrogen source, the highest cellulase production was observed with Yeast extract (0.450 IU/ml), Peptone (0.390 IU/ml), Glycin (0.400IU/ml) and Malt extract (0.810 IU/ml for *Bacillus subtilis* (Reddy et al. 2016).

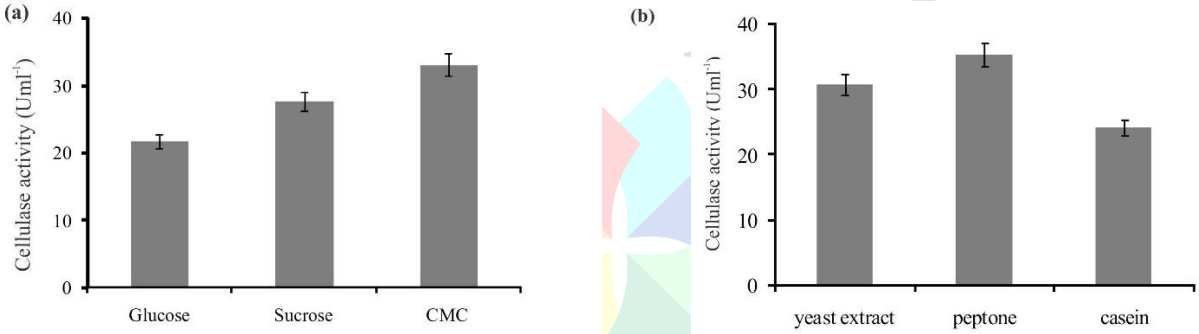

Fig. 2. Effect of Carbon (a) and Nitrogen (b) sources on cellulolytic activity.

3.3 Submerged fermentation using Agro waste

The results show that in submerged fermentation, with rice straw as substrate exhibit maximum cellulase production by. *cereus* KR9 after 72 h of incubation period (60.8±0.8 U/ml). Next to that supplementation of sugar cane bagasse had cellulase production of 53.4±0.6 U/ml. Minimum amount of cellulase production was observed with rice husk (43.7±0.7 U/ml) supplemented medium (Fig. 3a). Soaka and Sulistiani (2019) used corncob media for observing cellulase enzyme activities of *B. subtilis* A8 that have optimum of incubation time 3 days.

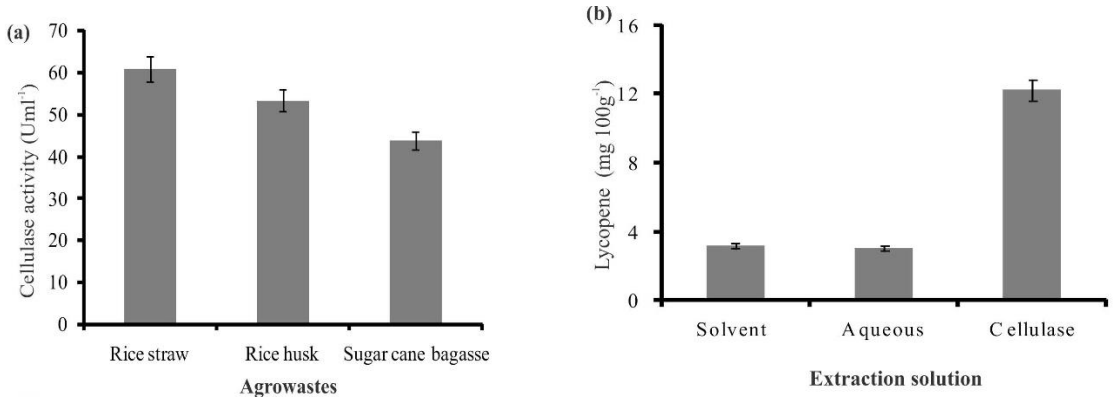

Fig. 3. Production of cellulase using agro-waste as substrate (a) and Extraction of Lycopene from tomato peels.

3.4 Comparison of solvent and enzyme mediated extraction of Lycopene

The crude enzyme extract obtained after submerged fermentation of rice straw added medium was precipitated using 80 % $(NH_4)_2SO_4$ saturation followed by partial purification by dialysis. The results of experiments performed for extraction of lycopene from tomato peels showed maximum lycopene recovery with a yield of 12. 25±0.7 mg compared with the solvent

extraction process performed using Acetone – Ethyl acetate dissolvent system (8.75±0.6 mg) and a least with aqueous extract had 3.07±0.6 mg content of lycopene per 100 g of dry weight of tomato peels (Fig. 3b). Further enzyme mediated extracted lycopene was found to be effectively used for coloring both bioplastic and sugar cubes. In the same manner Munde et al. (2017) found maximum recovery of lycopene from tomato peels using optimized tri-solvent extraction was achieved by pre-treatment with 2% pectinase concentration, pH 5.5, 4-h incubation at 45 °C, 120-min incubation and 200 rpm. Harini and Sumathy (2016[b]) extracted lycopene from papaya by acetone-petroleum ether method and made to spread on sugar cubes showed colouring after 24h observed. The extracted lycopene from tomato and watermelon was added while preparing the bioplastic as the colouring agent (Nair and Lilwani 2016).

IV. CONCLUSION

From the present study it can be concluded that the bacterial strain *Bacillus cereus* KR9 had optimum cellulase production at neutral culture conditions. The screening of a number of agro industrial materials with submerged fermentation is an economical process and is very simple to apply. Rice Straw has been superior to other crude substrate for the synthesis of cellulase. Lycopene recovered with cellulase mediated extraction process was found to be effective and the extracted lycopene showed good color efficiency. Further the cellulase of *Bacillus cereus* KR9 can be employed using scale up processes for many industries.

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