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Degradation of kitchen waste by cellulose degrading bacteria

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Abstract : *Cellulosic biomass is one of the dominating waste materials in nature resulting from human activities. Keeping in view the environmental problems like disposal of large volumes of cellulosic wastes and shortage of fossil fuel in the world, the main aim of the present investigation was to characterize and study the cellulolytic activity of selected bacterial isolates K1, K2 and K3 isolated from kitchen wastes, on natural cellulosic substrates viz. beet root, corn stover and finely grated vegetable peels. Stanier's Basal broth containing each of the substrates was inoculated separately with selected isolates and incubated at 37°C for 7-8 days. A control for each substrate was also kept. Besides this one set of the different cellulosic substrate in basal broth and test culture as well as a control was also kept to get the optical density spectrophotometrically at 420 nm. The cellulosic substrates were weighed after incubation and the difference between the initial weight and the final weight gave the amount of substrates degraded by the isolate. Also OD was taken at 420 nm in spectrophotometer after 7 days incubation by keeping one control. The difference between the OD of control and inoculated broth gave the amount of cellulosic substrates degraded by the isolates. It was observed that K2 was the most efficient isolate for the degradation of cellulosic substrate followed by K3 and K1. Therefore K2 can be considered more efficient for kitchen waste degradation over a period of 8 days. The favourable temperature for growth was 26°- 37°C, optimum pH was 6-8 and the isolates could grow at 1% - 3% of NaCl concentration.*

Key Words: *Cellulolytic potential, cellulosic biomass, sustainable fuel, waste material.*

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Introduction :

Kitchen waste forms an important part of domestic waste. This study focused on cellulose degrading bacteria in food waste. These bacteria play an important role in the biosphere by reducing complex polymer cellulose into various economically important products like monomeric sugars, microbial biomass proteins, compost,

antibiotics etc, to everyday use for man. Cellulose is a polymer of glucose and is the most abundant organic material in nature. The cellulosic biomass, once thought to be an ever increasing unmanageable waste, is now considered as an important renewable source of energy. Despite being an abundant and low cost renewable organic matter in nature, cellulose can be utilized as a source of energy and for the production of useful end products only after its hydrolysis to glucose (Obuekwe and Okungbowa, 1986), which can further be used as a substrate for the other bioprocesses. By the selection of efficient cellulolytic microorganisms and cost-effective operational techniques, the production of such useful end products from the biodegradation of cellulose can be very beneficial. Cellulose may be hydrolyzed using cellulolytic enzymes to produce glucose, which can be used for the production of useful end products (Hao et al., 2006). Cellulases are important industrial enzymes and find applications in several industrial processes (Hanif et al., 2004). Researchers have strong interest in cellulases because of their applications in industries of starch processing, grain alcohol fermentation, malting and brewing, extraction of fruit and vegetable juices, pulp and paper industry, and textile industry (Gao et al., 2008). The purpose of this work was basically to examine the possible utilization of cellulose degrading bacteria in kitchen waste for highest cellulase activity and bacterial growth at optimum working conditions such as pH and temperature.

Materials and Methods :

Chemicals : Chemicals used for the preparation of the media were of the highest purity grade and purchased from the local market. The chemicals used were obtained from Merck and Qualigens.

Media : Media used during the course of the present investigation, unless otherwise mentioned, were sterilized by autoclaving at 15 p.s.i. for 15 min. Nutrient Agar (Peptone 5 g/L, beef extract 3 g/L, sodium chloride 5 g/L, agar 15 g/L) was used for isolation and preservation of cellulose degrader; Stanier's basal medium [$(NH_4)_2SO_4$ 1 g/L, K_2HPO_4 1 g/L, $MgSO_4$ 0.2 g/L, $CaCl_2$ 0.1 g/L, $FeCl_3$ 0.02 g/L]; CMC agar (carboxymethylcellulose 0.5 g/L, $NaNO_3$ 0.1 g/L, K_2HPO_4 0.1 g/L, $MgSO_4$ 0.05 g/L, yeast extract 0.05 g/L, agar 15 g/L) and Cellulose Congo Red Agar with slight modification (K_2HPO_4 0.50 g/L, $MgSO_4$ 0.25 g/L, cellulose powder 1.88 g/L, Congo red 0.20 g/L, Agar 15 g/L, Gelatine 2 g/L); Staniers Basal broth supplemented separately with different natural cellulosic wastes viz. Beet root, corn stover and finely grated vegetable peels for estimating cellulolytic activity of the selected cellulose degrader (Kaur and Arora 2012).

Sample Collection : Samples were collected directly from waste dustbin of domestic kitchen in many replicates and brought into the laboratory for isolation of cellulose degrading bacteria.

Isolation, purification and maintenance of isolates : Samples from kitchen waste were serially diluted in 10 ml of sterillized normal saline and direct plating of six fold serial dilution in triplicates were done on Nutrient Agar and kept in the incubator at 37°C for 24 hours. Different colonies of bacteria thus obtained were purified by streaking (Dubey and Maheshwari, 2004) and maintained on Nutrient agar slants at 4°C with periodic sub culturing and further studied.

Screening of cellulose degrading microorganisms from the isolates : Screening of

cellulose degrading microorganisms was conducted by using Congo red dye. The isolates were grown on CMC Agar (pH 7.0) and incubated at 37 °C for 5 days to allow the secretion of cellulase enzyme. The agar medium was flooded with an aqueous solution of Congo red (1 % w/v) for 15 min to visualize the hydrolysis zone. The Congo red solution was then poured off and the plates were further treated by flooding with 1 N HCl for 15 min. Diameters of clear zone around colonies on CMC agar were measured indicating cellulase activity of the microorganism. The colony producing the largest clear zone was selected for further studies.

Cultural and morphological characterization of the selected isolates : The isolated colonies were identified on the basis of colour, margin, texture and elevation. Cultures were characterized morphologically and physiologically by Gram's staining.

Effects of various growth parameters on the isolates: The influence of temperature on the growth of the selected bacteria was studied by incubation of the steaked plates at 4°C, 10°C, 26°C, 37°C and 50°C for 24 hours. Similarly The influence of pH was observed by adjusting pH of the media by using pH meter to 2, 4, 6, 8, and 10 and incubated at 37°C for 24 hours. The influence of NaCl was studied using Nutrient agar with different NaCl concentration i.e 1%, 3%, 5%, 7%, 9% (Shaikh et al., 2013).

Estimation of degradation potential of isolates : The degradation potential of the selected isolates were estimated by using different cellulosic substrate. Cellulosic substrate(Corn stover, Beet root and finely grated vegetable peel) were washed, kept in the hot air oven at 60°C for 1-2 days to dry the substrate as well as to kill the natural microflora. It was then crushed and weighed on the electronic balance. 10ml of Stainer's Basal broth with 100 mg of crushed cellulosic substrate were taken in different test tubes. The substrate was mixed in the basal broth and were inoculated with the test isolates. The tubes were then incubated in shaker incubator at 37°C for 7-8 days. One tube as control was kept for each sample which was devoid of the test culture. Besides this one set of the different cellulosic substrate in basal broth and test culture and control was also kept to get the optical density spectrophotometrically at 420 nm. After 7-8 days of incubation, the culture broths were filtered through filter paper. The filter paper with residues were dried in hot air oven at 80°C for 30 mins. The residue was then scrapped and weighed. The difference between the initial weight of the substrate and final weight after treatment gave the amount of cellulosic substrates degraded by the isolates. Also OD was taken at 420 nm in spectrophotometer after 7 days incubation by keeping one control. The difference between the OD of control and inoculated broth gave the amount of cellulosic substrates degraded by the isolates (Prasad et al., 2013).

Results and Discussion:

Isolation and screening of cellulose degrading microorganisms from the isolates : Screening of isolates done by using the Congo red test helped in identifying cellulose degraders. Since the sole carbon source in CMC agar medium was carboxymethylcellulose, the clear zone (Fig.1) in the medium indicated cellulose degradation by the isolates. Out of 11 isolates three producing the largest clear zone were selected and named as K1, K2 and K3. The selected isolates were further studied for their degradation potential.

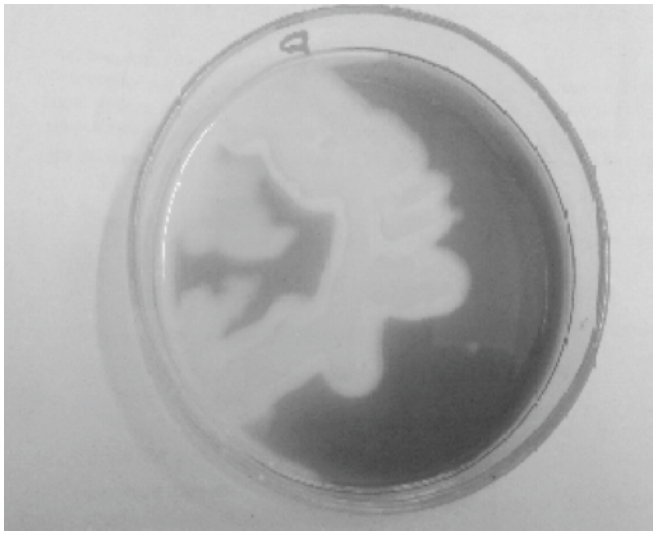

Fig 1. Bacterial isolate showing clear zone of cellulose degradation after Congo red test

Cultural Characterization of the Selected isolates : The selected bacterial colonies isolated on nutrient agar media from the samples (vegetables, corn stover and sugarcane molasses) showed individual characteristics. Isolate K1 and K3 showed the white slimy growth with smooth margin and flat elevation while the isolate K2 showed pale yellow slimy growth with smooth margin and flat elevation. Isolate K2 showed bluish-green colouration of Nutrient agar media.

Morphological characterization of the Selected Isolates : The selected cellulose degrading isolate K1 was gram positive short rods, K2 was gram positive rods while isolate K3 was gram positive slightly long rods as observed under 100X magnification.

Effect of various growth parameters on the Isolates : Growth parameters of the isolates with respect to temperature, pH and saline tolerance on nutrient agar media are listed in Table 1.

Table 1. Effect of temperature, pH and NaCl concentration on bacterial isolates (K1, K2 and K3).

Temperature	Growth		
	K1	K2	K3
4°	Poor	Poor	Poor
10°	Moderate	Moderate	Moderate
26°	Maximum	Maximum	Maximum
37°	Maximum	Maximum	Maximum
50°	Poor	Nil	Nil
pH		**Growth**	
	K1	**K2**	**K3**
2	Poor	Poor	Poor
4	Poor	Poor	Poor
6	Maximum	Maximum	Maximum
8	Maximum	Maximum	Maximum
10	Moderate	Poor	Poor
NaCl concentration		**Growth**	
	K1	**K2**	**K3**
1 g	Moderate	Moderate	Moderate
3 g	Moderate	Moderate	Poor
5 g	Nil	Nil	Nil
7 g	Nil	Nil	Nil
9 g	Nil	Nil	Nil

The favourable temperature for growth was 26° – 37° C, optimum pH was 6-8 and the isolates could grow at 1% - 3% of NaCl concentration.

Estimation of degradation potential of selected isolates: The difference between the OD of control and inoculated broth gave the amount of cellulosic substrates degraded by the isolates (Prasad et al., 2013). The final weight was found to be quiet less than the initial weight and the value of optical density of inoculated broth taken was also less than the control. Hence, the degradation of cellulosic substrate by the selected bacterial isolates is shown in **Tables 2 and 3**.

Table 2. Estimation of degradation potential of selected bacterial isolates by taking weight of residues.

Isolate	Cellulosic substrate	Weight of sample (mg)		
		Initial weight	Final weight	Difference (amount degraded)
K1	Beet root	100	30.8	69.2
	Corn stover	100	24.7	75.3
	Vegetable peel	100	25.8	74.2
K2	Beet root	100	09.3	91.7
	Corn stover	100	23.4	76.6
	Vegetable peel	100	3.6	96.4
K3	Beet root	100	10.8	89.2
	Corn stover	100	28.4	71.6
	Vegetable peel	100	26.7	73.3

Maximum degradation potential was shown by isolate K2 for vegetable peel followed by beet root. Isolate K3 also showed a good degradation potential for beet root. Among the three K1 was a less efficient strain as far as degradation potential is considered.

Table 3. Estimation of degradation potential of selected bacterial isolates by taking the optical density at 420 nm.

Isolate	Cellulosic substrate	OD at 420 nm		
		Initial OD	Final OD	Difference
K1	Beet root	0.507	0.412	0.095
	Corn stover	0.482	0.192	0.290
	Vegetable peel	0.420	0.136	0.284
K2	Beet root	0.507	0.159	0.348
	Corn stover	0.482	0.172	0.310
	Vegetable peel	0.420	0.060	0.360
K3	Beet root	0.507	0.170	0.337
	Corn stover	0.482	0.236	0.246
	Vegetable peel	0.420	0.140	0.280

The difference in the initial and the final OD gave the amount of the cellulosic substrate degraded by the selected isolate. Table 3 shows that K2 showed maximum degradation potential . Similar results were obtained in both the dry weight and the absorbance method.

Conclusion :

The present study revealed cellulolytic activities of isolated strains (K1, K2 & K3) as they could degrade not only the cellulose rich culture media but also the naturally occuring cellulosic materials selected for our investigation. It was observed that K2 was the most efficient isolate for the degradation of cellulosic substrate followed by K3 and K1. Therefore, K2 show can be considered more efficient for kitchen waste degradation over a period of 8 days. The favourable temperature for growth was 26°-37°C, optimum pH was 6-8 and the isolates could grow at 1%-3% of NaCl concentration. By optimising the pH and the temperature, its potential can be utilized to biodegrade the low cost enormous stock of cellulose in nature and the end product may be useful in the preparation of a number of chemicals including bioethanol. Since, the cellulosic waste are biodegradable, it does not cause any environmental pollution and also helps in waste management.

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