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ORIGINAL ARTICLE

Fungal Production of Cellulase Using Fruit Waste as Economical Growth Substrates

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ABSTRACT

Background: Vegetables and fruits are playing an important role in human life and diet. For eco-friendly usage the fruit peel wastes can be used as nutrients for microorganisms and animals as they consist of various growth promoting factors. Cellulolytic enzymes convert cellulose into simpler sugars. Due to suitable cellulase titers and rapid growth bacteria and fungi are becoming choice of interest.

Aim: Production of fungal cellulase by using different types of fruit wastes.

Method: This descriptive study was conducted by collecting the Fruit wastes (mango peels, melon peels, orange peels and watermelon rind) from fruit processing shops and transported to Applied and Environmental Microbiology laboratory.Inoculum was prepared by using basal medium and fruit peel powder.Descriptive statistical analysis of data will be performed. One way Analysis of Variance (ANOVA) will be applied for comparing mean values among species using Minitab 16. $P < 0.05$ will be considered significant.

Result: The statistical analysis showed that in solid state fermentation the enzyme summary within 10 days was (181.32±0.00 µg/ml/min) after every 24 hour was (9.40±1.22µg/ml/min) and (0.39±0.05µg/ml/min) after every 1 hour and (0.01±0.00µg/ml/min) at 1 minute. The statistical analysis showed that in sub-merged fermentation the enzyme summary within 10 days was 191.83±4.96 after every 24 hour was (7.12±0.76µg/ml/min) and (0.30±0.03µg/ml/min) after every 1 hour and (0.0.00±0.00 µg/ml/min) at 1 minute.

Conclusion: The present study clearly indicates the potential of Rhizopussp as the best producer of cellulolytic enzymes. Regarding the substrate fruit peels (mango peels, melon peels, orange peels, and watermelon rind) can be used for the efficient production of cellulase.

Keywords: Rhizopus, cellulolytic enzymes, fruit peels, fungal production.

INTRODUCTION

Vegetables and fruits are playing an important role in human life and diet. Therefore, with changing habit of diets and increasing population, the demand for such commodities has increased significantly [1]. The terms of food waste and food loss are generally given to overall waste and losses in the chain of food supply as during the process of production of fruits, its post-harvesting losses, fruit processing, during distribution to fruit markets and in the last during the consumption of fruits by the consumers[2].

At least one third of the overall production of food is wasted and lost annually which is estimated as 1.3 BMT[3]. For eco-friendly usage the fruit peel wastes can be used as nutrients for microorganisms and animals as they consist of various growth promoting factors like minerals and vitamins as well as high amounts of both complex and simple sugars[4,5]. Enzyme analysis exhibits that citrus fruits such as oranges, release high amount of enzymes including cellulase (0.514±0.03U/mL), α-amylase (7.261±0.83U/mL) and protease (0.129U/mL)[6]. In recent years, the developing countries having an economic transition via urbanization and have high demand of energy resources[7]. Cellulose, being the most abundant carbohydrate in nature and its abundance attracts many industries for making products by using raw cellulose[8,9]. With the cellulolytic degrading system, this concern can be resolved by converting cellulose into glucose in a much safer and cheaper process[10]. Lignocellulosic biomass includes agro-industrial, food wastes and forestry wastes which are renewable, inexpensive and abundant energy sources[11].The second major component of lignocelluloses is hemicelluloses. Unlike cellulose, hemicelluloses undergo easy hydrolysis due to branched and amorphous nature[12,13]. Fungal cellulases are preferred over bacterial cellulases due to fungi versatility in attaining different substrates for their cellulolytic activity. Although cellulolytic activity of fungus is greater than bacteria but still it is very rare to find fungi having all the cellulases[14]. Various kinds of microbes are responsible for the production of cellulose such as fungi and bacteria[15].

Fermentation technique has also been used to produce cellulase[16]. A process of growing microorganisms in an environment with low water content or without water content is known as solid state fermentation (SSF)[17].

Another method for developing cellulases from microbes is submerged fermentation (SmF) in which high content of water is used for the production of products[18]. SSF has advantage over SmF such as less inhibitory effects high productivity, less requirement of energy for enzyme production[19]. The production of enzymes of same strain in SSF is greater than SmF, therefore SSF is proffered for the production of enzymes at industrial level [18]. In short SSF is more feasible economically than SmF for the production of enzyme[20].

Massive generation of fruit wastes in Pakistan necessitates its justified utilization. In the present study, different types of fruit wastes will be valorized for the production of fungal cellulase.

MATERIAL AND METHODS

This descriptive study was conducted by collecting the Fruit wastes (mango peels, melon peels, orange peels and watermelon rind) from fruit processing shops and transported to Applied and Environmental Microbiology laboratory, Department of Wildlife and Ecology, UVAS, Ravi Campus, Pattoki. The collected wastes was completely oven dried (60c) and ground well to obtain fine powder of the dried wastes. The pre isolated and characterized fungal

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culture was obtained from Applied and Environmental Microbiology laboratory to use in this study. The fungal culture was employed for the production of cellulose using different fruit wastes independently as growth substrate. All the experiments for this purpose were carried in triplicate under hygienic condition to maintain possible sterility of the environment. The cellulase yield was estimated spectrophotometrically. The produced cellulase was purified on different substrates to enhance the immobilization of the cellulase[21].

Exclusion criteria: Peels of fruits that are toxic and deteriorate

Procedure of fungal growth: For this purpose, the cellulose-selective broth (pH 5.5) inoculated with 1% of the fungal growth was incubated at 25, 37 and 50 oC temperature for 24h. In another set of experiments, cellulose-selective media of different initial pH of 5, 7 and 9 were inoculated with 1% of the fungal culture and incubated for 24 h at its respective temperature optima.

After identifying optimum temperature and pH for cellulase yield, the media was inoculated with 1,5 and 10% (v/v) of the corresponding fungal cultures and incubated at their respective determined enzyme yield optima for 24h. The effect of oxygen requirements for cellulase production was determined by incubating the fungal cultures at 120rpm for aeration and without shaking for non-aeration at their corresponding predetermined temperature, initial pH and inoculum size optima[22].

Statistical Analysis: Descriptive statistical analysis of data will be performed. One way Analysis of Variance (ANOVA) will be applied for comparing mean values among species using Minitab 16. $P < 0.05$ will be considered significant.

RESULTS

The statistical analysis showed that in solid state fermentation the enzyme summary within 10 days was (181.32±0.00µg/ml/min) after every 24 hour was (9.40±1.22µg/ml/min) and (0.39±0.05µg/ml/min) after every 1 hour and (0.01±0.00 µg/ml/min) at 1 minute (Table 1). The statistical analysis showed that in sub-merged fermentation the enzyme summary within 10 days was 191.83±4.96 after every 24 hour was (7.12±0.76µg/ml/min) and (0.30±0.03 µg/ml/min) after every 1 hour and (0.0.00±0.00µg/ml/min) at 1 minute (Table 1). Cellulase production by orange substrate through solid state fermentation is 179.32±5.22 in 10 days, in 24 hours 7.40±1.22, in 1 hour 0.19±0.05 and in 1 minute is 0.01±0.00 (Table 2). Production through submergedfermentation is in 10 days 189.83±4.96, in 24 hours 5.12±0.76, in 1 hour 0.10±0.03 and in 1 minute 0.01±0.00 recorded (Table 2).Cellulase production by using melon is recorded as in 10 days 182.32±5.22, in 24 hours 10.40±1.22, in 1 hour 0.49±0.05 and in 1 minute 0.01±0.00 in solid state fermentation (Table 3). Through submerged fermentation, cellulase production is recorded as in 10 days 192.83±4.96, in 24 hours 8.12±0.76, in 1 hour 0.40±0.03 and in 1 minute 0.00±0.00 (Table 3). Cellulase production through watermelon in solid state fermentation is recorded as in 10 days 183.32±5.22, in 24 hours 11.40±1.22, in 1 hour 0.59±0.05 and in 1 minute is 0.01±0.00 recorded (Table 4).

Table 1: Statistical Analysis of Cellulase Production using mango substrate

Fermentation Process	Treatment	10 days of Cellulase Activity	24 hours of Cellulase Activity	1 hour of Cellulase Activity	1 minute of Cellulase Activity
Enzymes Summary					
Solid State Fermentation		181.32±5.22	9.40±1.22	0.39±0.05	0.01±0.00
Sub-merged fermentation		191.83±4.96	7.12±0.76	0.30±0.03	0.00±0.00
Treatment summary					
	R1	178.91±5.84	8.29±0.92	0.35±0.04	0.01±00
	R2	195.46±5.42	9.12±1.74	0.38±0.07	0.01±00
	R3	185.35±7.24	7.38±0.99	0.31±0.04	0.01±00
Interaction Summary					
Solid State Fermentation	R1	187.74±8.37abb	8.77±0.91aa	0.37±0.04aa	0.01±0.00aa
Solid State Fermentation	R2	193.81±9.09aa	11.35±3.30aa	0.47±0.14aa	0.01±0.00aa
Solid State Fermentation	R3	162.41±7.04c	8.09±1.41aa	0.34±0.06aa	0.01±0.00aa
Sub-merged Fermentation	R1	170.08±7.53bcc	7.81±1.65aa	0.33±0.07aa	0.01±0.00aa
Sub-merged Fermentation	R2	197.12±6.39aa	6.89±0.90aa	0.29±0.04aa	0.00±0.00aa
Sub-merged Fermentation	R3	208.29±7.41aa	6.67±1.41a	0.28±0.06a	0.00±0.00aa
Anova					
Enzyme		0.0998	0.1244	0.1246	0.1294
Treatment		0.1045	0.6255	0.6255	0.6243
Enzyme × Treatment		0.0005	0.5717	0.5716	0.5568

Table 2: Statistical Analysis of Cellulase Production using orange substrate

Fermentation Process	Treatment	10 days of Cellulase Activity	24 hours of Cellulase Activity	1 hour of Cellulase Activity	1 minute of Cellulase Activity
Enzymes Summary					
Solid State Fermentation		179.32±5.22	7.40±1.22	0.19±0.05	0.01±0.00
Sub-merged fermentation		189.83±4.96	5.12±0.76	0.10±0.03	0.00±0.00
Treatment Summary					
	R1	176.91±5.84	6.29±0.92	0.15±0.04	0.01±00
	R2	193.46±5.42	7.12±1.74	0.18±0.07	0.01±00
	R3	183.35±7.24	5.38±0.99	0.11±0.04	0.01±00
Interaction Summary					
Solid State Fermentation	R1	185.74±8.37abb	6.77±0.91aa	0.17±0.04aa	0.01±0.00aa
Solid State Fermentation	R2	191.81±9.09aa	9.35±3.30aa	0.27±0.14aa	0.01±0.00aa
Solid State Fermentation	R3	160.41±7.04c	6.09±1.41aa	0.14±0.06aa	0.01±0.00aa
Sub-merged Fermentation	R1	168.08±7.53bcc	5.81±1.65aa	0.33±0.07aa	0.01±0.00aa
Sub-merged Fermentation	R2	195.12±6.39aa	4.89±0.90aa	0.09±0.04aa	0.00±0.00aa
Sub-merged Fermentation	R3	206.29±7.41aa	4.67±1.41a	0.08±0.06a	0.00±0.00aa
Anova					
Enzyme		0.0798	0.1044	0.1046	0.1094
Treatment		0.1043	0.6253	0.6253	0.6241
Enzyme×Treatment		0.0003	0.5715	0.5714	0.5566

Table 3: Statistical Analysis of Cellulase Production using Melon substrate

Fermentation Process	Treatment	10 days of Cellulase Activity	24 hours of Cellulase Activity	1 hour of Cellulase Activity	1 minute of Cellulase Activity
Enzymes Summary					
Solid State Fermentation		182.32±5.22	10.40±1.22	0.49±0.05	0.01±0.00
Sub-merged fermentation		192.83±4.96	8.12±0.76	0.40±0.03	0.00±0.00
Treatment Summary					
	R1	179.91±5.84	9.29±0.92	0.36±0.04	0.01±00
	R2	196.46±5.42	10.12±1.74	0.39±0.07	0.01±00
	R3	186.35±7.24	8.38±0.99	0.32±0.04	0.01±00
Interaction summary					
Solid State Fermentation	R1	188.74±8.37abb	9.77±0.91aa	0.37±0.04aa	0.01±0.00aa
Solid State Fermentation	R2	194.81±9.09aa	12.35±3.30aa	0.48±0.14aa	0.01±0.00aa
Solid State Fermentation	R3	163.41±7.04c	9.09±1.41aa	0.35±0.06aa	0.01±0.00aa
Sub-merged Fermentation	R1	171.08±7.53bcc	8.81±1.65aa	0.34±0.07aa	0.01±0.00aa
Sub-merged Fermentation	R2	198.12±6.39aa	7.89±0.90aa	0.30±0.04aa	0.00±0.00aa
Sub-merged Fermentation	R3	209.29±7.41aa	6.68±1.41a	0.29±0.06a	0.00±0.00aa
Anova					
Enzyme		0.0999	0.1245	0.1247	0.1295
Treatment		0.1046	0.6256	0.6256	0.6244
Enzyme × Treatment		0.0006	0.5718	0.5717	0.5569

Table 4: Statistical Analysis of Cellulase Production using Watermelon substrate

Fermentation Process	Treatment	10 days of Cellulase Activity	24 hours of Cellulase Activity	1 hour of Cellulase Activity	1 minute of Cellulase Activity
Enzymes Summary					
Solid State Fermentation		183.32±5.22	11.40±1.22	0.59±0.05	0.01±0.00
Sub-merged fermentation		193.83±4.96	9.12±0.76	0.50±0.03	0.00±0.00
Treatment Summary					
	R1	180.91±5.84	10.29±0.92	0.37±0.04	0.01±00
	R2	197.46±5.42	11.12±1.74	0.40±0.07	0.01±00
	R3	187.35±7.24	9.38±0.99	0.33±0.04	0.01±00
Interaction summary					
Solid State Fermentation	R1	189.74±8.37abb	10.77±0.91aa	0.39±0.04aa	0.01±0.00aa
Solid State Fermentation	R2	195.81±9.09aa	13.35±3.30aa	0.49±0.14aa	0.01±0.00aa
Solid State Fermentation	R3	164.41±7.04c	10.09±1.41aa	0.36±0.06aa	0.01±0.00aa
Sub-merged Fermentation	R1	172.08±7.53bcc	7.83±1.65aa	0.35±0.07aa	0.01±0.00aa
Sub-merged Fermentation	R2	199.12±6.39aa	8.89±0.90aa	0.31±0.04aa	0.00±0.00aa
Sub-merged Fermentation	R3	210.29±7.41aa	8.69±1.41a	0.30±0.06a	0.00±0.00aa
Anova					
Enzyme		0.1000	0.1246	0.1248	0.1296
Treatment		0.1047	0.6257	0.6257	0.6245
Enzyme × Treatment		0.0007	0.5719	0.5718	0.5570

DISCUSSION

Vegetables and fruits are playing an important role in the life of human. So, with the changing habit of diets and increasing population, the demand for such commodities has increased significantly[1]. Large quantities of fruit wastes are generated in Pakistan from industries of food processing and agricultural sector. Fruit wastes generated from such sites are apple waste, banana waste, pear waste, pomegranate waste, grapefruit waste and citrus waste [23]. For eco-friendly usage the fruit peel wastes can be used as nutrients for microorganisms and animal as they consist of various growth promoting factors like minerals and vitamins as well as high amounts of both simple and complex sugars[4,5]. Enzyme analysis exhibits that citrus fruits such as oranges, release high amount of enzymes including cellulase (0.514±0.03U/mL), α-amylase (7.261 ± 0.83 U/mL) and protease (0.129 U/mL)[6].

The highest production of cellulase was recorded in submerged fermentation at 10 days of incubation. Determination of pH was also done. In solid state fermentation, the result of treatment R1, R2 and R3 5.18±0.09aa, 5.23±0.14aa and 4.80±0.12b and in submerged fermentation R1 4.80±0.12b, R2 5.11±0.10abab and R3 5.25±0.19aa was observed reported that water Hyacinth can be concluded that 29 diverse isolate of fungal was identified for the production of cellulase[19]. The present study on orange peels, the cellulolytic potential was assessed in a trial of 10 days. The cellulolytic activity started after 24 hours of incubation period and increased with time up to 10 days of incubation period.

In solid state fermentation the enzyme summary of 10 days was (179.32±5.22µg/ml/min) after every 24 hours was (7.40±1.22) and after every 1 hour and similarly at 1 minute. By sub-merged fermentation the enzyme summary within 10 days was (189.83±4.96µg/ml/min) after every 24 hours was (5.12±0.76µg/ml/min) and (0.10±0.03µg/ml/min) after every 1 hour and (0.00±0.00µg/ml/min) at 1 minute was recorded.The highest production of cellulase was recorded in submerged fermentation at 10 days of incubation. Determination of pH was also done. In solid state fermentation, the result of treatment R1, R2 and R3 189.74±8.37abb, 195.81±9.09aa and 164.41±7.04c and in submerged fermentation R1 172.08±7.53bcc, R2 199.12±6.39aa and R3 210.29±7.41aa was observed reported that water Hyacinth can be concluded that 29 diverse isolate of fungal was identified for the production of cellulase[19].Furthermore endoglucanases was produced by the most these isolates of fungal and also determine the most of the isolates of fungus belong to the Acomycetes conducted an experiment for the production of cellulase by using as Prickly Palm Cactus husk growth substrate under solid-state fermentation using Rhizopussp . and Aspergillusniger and In the experiment the optimum conditions was optimized and reported that cellulase and endoglucanase was efficiently produced by Rhyzopussp . and Aspergillusniger. Pandit and Maheshwari Pleurotus Sajor-Caju under solid state fermentation. The result indicated that optimum pH was 5.0 and temperature 25℃. At the end of experiment reported that PleurotusSajor-Caju is more efficient for the active production of cellulase[24].

CONCLUSIONS

The present study clearly indicates the potential of Rhizopussp as the best producer of cellulolytic enzymes. Regarding the substrate fruit peels (mango peels, melon peels, orange peels, watermelon rind) can be used for the efficient production of cellulase.**Conflict of interest:** Nil

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