Enhancement of Rice Straw Composting Using UV Induced Mutants of *Penicillium* Strain

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***Abstract*—**Fungal mutant strains have produced cellulase and xylanase enzymes, and have induced high hydrolysis with enhanced of rice straw. The mutants were obtained by exposing *Penicillium* strain to UV-light treatments. Screening and selection after treatment with UV-light were carried out using cellulolytic and xylanolytic clear zones method to select the hypercellulolytic and hyperxylanolytic mutants. These mutants were evaluated for their cellulase and xylanase enzyme production as well as their abilities for biodegradation of rice straw. The mutant 12 UV/1 produced 306.21% and 209.91% cellulase and xylanase, respectively, as compared with the original wild type strain. This mutant showed high capacity of rice straw degradation. The effectiveness of tested mutant strain and that of wild strain was compared in relation to enhancing the composting process of rice straw and animal manures mixture. The results obtained showed that the compost product of inoculated mixture with mutant strain (12 UV/1) was the best compared to the wild strain and un-inoculated mixture. Analysis of the composted materials showed that the characteristics of the produced compost were close to those of the high quality standard compost. The results obtained in the present work suggest that the combination between rice straw and animal manure could be used for enhancing the composting process of rice straw and particularly when applied with fungal decomposer accelerating the composting process.

***Keywords*—**Rice straw, composting, UV mutants, *Penicillium.*

I. Introduction

RICE crop is one of the most important crops in Egyptian agriculture. Egypt is the largest rice producer in the Near East region with total area of cultivation is approximately 600 thousand hectar. The average yield of rice grain production is 8.2 tons/ha straw of 3 tons/ha [1].

In Egypt, rice straw has been considered as rice agricultural waste disposed by burning that creates environmental problems (air pollution; smog formation). The burning results in particles of < 10 µm size, which cause respiratory problems such as asthma emphysema. The rice straw burning also results in carbon monoxide and some nitrogen dioxides, causing asthma morbidity [2].

In addition the meteorological conditions of very low wind speed and cold temperature causes the phenomena of thermal inversion [3]. Furthermore, the production of CO_2 during burning has been linked to global warming and greenhouse effects. Therefore, the government has set legislation for prohibition of burning.

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The low digestibility and high ash content of rice straw makes it difficult to use as animals feed. The straw is composed mainly from cellulose and hemi-cellulose encrusted by lignin, and a small amount of protein, which makes it poor in C:N ratio. Therefore it is resistant to microbial decomposition compared to straw from other protein-rich grains such as wheat and barley [4]. Thus, rice straw utilization is still a problem needs to be solved in Egypt. In general, certain organic wastes from agricultural and animal production have become major sources of environmental problems throughout the world in both developed and developing countries. Animal manures also become a serious pollution problem resulting from the huge accumulation of such material. These animal wastes are known to be heavily contaminated with pathogenic bacteria and parasites causing a direct health risk [5]. The potential of composting the wastes to turn field it into a farm resource makes it an attractive proposition. Composting offers several benefits such as; enhancing soil fertility, increasing agricultural productivity, improving soil biodiversity and reducing ecological risks. Composting of rice straw with animal manures may provide a readily compostable mixture that gives high quality compost [6]. Several efforts were done for improving biodegradation of the cellulosic materials using microbial strains with high enzymatic activity [7]-[13]. The strains with mutation techniques were used in this study to obtain superior enhance of rice straw hydrolysis capacity.

The present work aims at UV mutations of fungal strain with enhanced biodegradation of rice straw to improve the rice straw composting after mixing with animal manures to obtain high quality compost. The selection of superior mutants on the basis of higher production of cellulolytic and xylanolytic enzymes judged by clear zones method is the key criteria for selection of promising mutants.

II. Materials and Methods

A. Fungal Strains

The original *Penicillium* sp. strain was obtained from Microbial Chemistry Department; National Research center, Dokki, Cairo, Egypt. The mutants of *Penicillium* sp. were developed by UV-mutagenesis of the original strain.

B. Medium

Malt extract medium (Merck) was used for routine culturing and fungal UV mutants. Fermentation medium (FM) was used for determination of the cellulolytic and xylanolytic activities after degradation of rice straw according to [13].

C. Rice Straw

Rice straws were collected from rice fields. Rice straw was air dried and chopped mechanically into the length of 0.5-2.0 cm to facilitate its mixing with other materials. The physicochemical properties of rice straw are presented in Table I.

TABLE I
SCREENING FOR FUNGAL HIGH SOME CHEMICAL PROPERTIES OF WASTES USED IN COMPOSTING

Chemical properties	Rice straw (RS)*	Animal manure (AM)*	Mixture 2RS:1AM**
Organic Matter (OM) %	80.22	74.72	78.4
C/N ratio	64.63	31.41	53.55
Total Nitrogen (N)%	0.72	1.38	0.94
Total Phosphorous (P) %	0.57	0.61	0.58
Total potassium (K) %	0.82	0.88	0.84

*These data was obtained from [21],
**calculated data

D. Animal Manure

Animal manure was collected from animal farms. Its physicochemical properties were presented in Table I.

E. Composting Conditioners

Some conditioners such as Rock phosphate, mixture of nutrient elements and urea were added to the compost to accelerate composting process and to improve the nutritional values of the compost.

F. Cellulolytic and Xylanolytic Activities

The preliminary selection was done on the basis of the cellulolytic or xylanolytic clear zones (mm) method on SM according to [14] with some modification; 50 µl of fungal spore suspensions were dropped in wells (8 mm diameter) and incubated seven days at 28°C.

G. Analysis of Straw Degradation

The spore suspensions from seven days old slants were collected with five ml of distilled sterile water containing 0.1% (v/v) Tween 80 and used for inoculation of Flask (250 ml) containing 50 ml of FM. Flasks were incubated with shaking (220 rpm) at 28°C for seven days.

1. Cellulolytic and Xylanolytic Activities Determination

Enzymatic activities were determined in the culture supernatant obtained by centrifugation culture media for 5 min. at 5000 rpm under cooling. A half ml of the clear supernatant was diluted in 4.5 ml of 0.05 M citrate buffer pH 4.8 and part of diluted was used to assay the enzyme activities.

2. Cellulolytic Activity

A half ml of 1% (w/v) CMC in 0.05 M citrate buffer (pH 4.8) and 0.5 ml of diluted enzyme preparation were mixed and incubated at 50°C for 30 min. The reaction was stopped with 3 ml of 1% (w/v) dinitrosalicylic acid (DNS) and heated at 100°C for 10 min.

3. Xylanolytic Activity

Xylanase activity was assayed using a method similar to that used to estimate cellulase activity but 1% (w/v) xylan in 0.05 M citrate buffer (pH 5.4) was used as the enzyme substrate and different concentrations of D-xylose as the standard. A unit of xylanase activity was defined as the amount of enzyme producing 1µmol of D-xylose per min. [15].

H. Percentages of Rice Straw Degradation

The rice straw pieces were collected by filtration and washed several times with water to eliminate any fungal mycelium or spores. The samples were dried at 105°C in oven for ten hours and the dry weights were measured against control sample to obtain the percentages of rice straw degradation.

I. Composting of Rice Straw

1. Preparation of Standard Inoculums

Spore suspension was prepared by adding 10 ml of sterilized 0.1% Tween-80 solution to the 7-day old culture slants of the wild type and the mutant. At the end of incubation, the surface of the culture was scratched with sterilized loop and agitated thoroughly using a shaker to suspend the spores. The number of the spores was measured using a haemocytometer and adjusted to approximately 10^7 spores/ml and was used as inocula throughout the study.

2. Composting Procedure

Chopped rice straw and animal manure were mixed mechanically at a ratio of 2 to 1 (v/v) respectively. The mixture was layered to form pile. Several composting piles were constructed and divided into three parts: the first part was inoculated by wild strain; the second part was inoculated by mutant strain and the third part did not receive any inoculants. Urea and decomposer strains were incorporated into the composting pile just during the building of the heap pile. Moisture content was adjusted to 50-60% and water holding capacity and sprinkled as necessary to maintain the moisture content at the same level during the active composting period. The mixtures were turned at three weeks intervals to maintain porosity and to keep the aerobic process. Rock phosphate and mixed elements were added to the compost just before the third turn (7 weeks from the start of the composting). At the end of composting process, samples were taken to determine properties of the end products.

III. RESULTS AND DISCUSSION

A. UV- Mutagenesis

Suspensions of *Penicillium* sp. spores were exposed to UV-light as mentioned previously in the materials and methods. Data in Table I showed that the survival percentages of fungal spores decreased gradually by increasing UV exposure time. Data in Table II show that the exposure of fungal spores to UV radiation for the 8 minutes was the best for obtaining UV mutants. Extending the exposure period to 12 minutes reduced the mutants by 50%.

TABLE II
MUTAGENIC EFFECT OF UV-IRRADIATION EXPOSURE TIME ON *PENICILLIUM* SP. STRAIN

UV Exposure time (min)	Fungal Survival		No. of tested colonies	Morphological mutants No.	Mutation %
	No.	%			
Zero	1631	100.00	200	0	0.0
4	717	43.96	100	2	2
8	283	17.35	75	6	8
12	69	4.23	50	2	4

B. Screening for High Cellulolytic and Xylanolytic Activities

Conidia of *Penicillium* sp. strain were subjected to mutagenesis by using UV-light. Out of 150 colonies grown from single spore suspension on agar plates, about 15 mutants showed the greatest cellulolytic and xylanolytic activities as indicated by clear zones around colonies on SM. These mutants were selected for evaluating their efficiency in rice straw degradation.

C. Enhancement of Rice Straw Degradation

Table III presents cellulase and xylanase activity and percentages of rice straw degradation by 15 selected mutants obtained after the exposure of wild type strain of *Penicillium* sp. to UV-light for 4, 8 and 12 min. The obtained results show that all tested mutants produced cellulase higher than the parent strain with the exception of the mutant 12. Moreover, the percent of xylanase activity ranged between increases up to 209.91% in mutant 12 which received 12 minutes UV exposure. This mutant and two other mutants No. 1 or 4 were more efficient in straw degradation as compared with parent strains as shown in Table III.

The ultraviolet irradiation is a successful tool for the induction of genetic variabilities in many industrial microorganisms used by several researchers. Montenecourt and Eveleigh [7] used UV-irradiation doses with *T. reesei* GM6a to isolate series of mutants with hypercellulases productivity. They isolated the mutant QM 9414 which produced 2.4 times more enzyme than the wild type. Moreover, [16] isolated the mutant *T. reesei* MCG 80 which was an excellent producer for extracelluar protein and cellulases after exposure the parental strain C30 to UV-light. On the other hand, [9] isolated a number of mutant strains over producing cellulase, ß-glucosidase and xylanase enzymes from the cellulolytic fungus *Penicillium pinophilium* 87160iii after mutagenesis by UV-irradiation and /or the chemical treatment with N-methyl- N-intro- N-nitrosoguanidine (MNNG). Cellulase production by some of these mutants in shake flask cultures was approximately 4- fold, the wild type. Improvements in ß-glucosidase production reached 8 to 9-fold and enhancements in xylanase production reached 2- fold the wild type strain. The morphology of mycelium of the mutants was quite different from that of the wild type and several of these mutants showed altered kinetics of straw hydrolysis. Moreover, [11] used multi-step physical (UV) and chemical MNNG, sodium azide and colchicine mutagenesis in the mutation of *A. niger* RK3 strain. This resulted in highly cellulolytic mutant, UNSC-442, having an increase of 136, 138 and 96 percents of endoglucanase, exoglucanase and ß-glucosidase activities, respectively.

Fatma [17] isolated several mutants of *T. reesei* after treatment with different mutagens. The results indicated that the treatment with UV-light induced 183 colonies, 48 of them were morphologically different and 6 are auxotrophic mutants. However, some mutants showed higher cellulase activity which reached about 2.5 or 1.9 times of those of the parent strain. They also concluded that UV exposure gave the best induction of cellulase improvement in the original strain under the same environmental conditions. Furthermore, [13] isolated several induced mutants of *Penicillium* sp. producing cellulase and xylanase enzymes with high hydrolysis capacity of rice straw. It is worth to state that the cellulotlytic and xylanolytic genetically improved mutants of *Penicllium* obtained in this study can be efficiently used for biodegradation of cellulosic farm wastes especially rice straw in composting of rice straw instead burning which causes environmental pollution in addition to the loss of nutrients in wastes.

TABLE III
CELLULOLYTIC AND XYLANOLYTIC ACTIVITY AND PERCENT OF RICE STRAW DEGRADATION BY 15 SUPERIOR UV *PENICILLIUM* SP. MUTANT

***Penicillium* sp mutants**		**Cellulase**		**Xylanase**		**Degradation of rice straw (%)**
Mutant No.	**UV dose in minutes**	**U/ml**	**% from WT.**	**U/ml**	**% from W.T**	
Original wild type strain		2.110	100.00	7.500	100.00	46.58
1	UV4	2.548	120.76	7.654	102.05	54.38
2	UV4	2.294	108.72	6.797	90.63	48.73
3	UV4	4.170	197.63	7.604	101.39	53.53
4	UV4	2.867	135.88	5.730	76.40	52.40
5	UV4	2.539	120.33	9.671	128.95	53.61
6	UV8	3.826	181.33	5.021	66.94	54.22
7	UV8	4.110	194.79	9.807	130.76	54.11
8	UV8	3.285	155.69	6.174	82.32	52.34
9	UV8	3.631	172.09	6.081	81.08	54.36
10	UV8	4.259	201.85	5.887	78.49	53.71
11	UV12	6.461	306.21	15.743	209.91	63.30
12	UV12	4.517	214.08	9.938	132.51	54.71
13	UV12	5.123	242.80	8.049	107.32	58.12
14	UV12	1.923	91.14	6.865	91.53	45.52
15	UV12	3.245	153.79	11.450	152.67	54.20

D. Quality of Compost Produced Using Mutant Penicillium Strain

The results presented in Table IV show the analyses of the compost after 120 days of composting. The results demonstrate that the properties of compost inoculated with either mutant strain or wild strain type were better than those of un-inoculated control. The analysis of compost inoculated with mutant strain was close to that of inoculated with wild strain except for total nitrogen and total potassium which was higher in case of mutant strain. The properties of composted rice straw were considerably improved relative to those of the initial mixture (raw materials). For instance, initial C/N ratio was 56.3 in different compost heaps and after the 120 day of composting, the final C/N ratio was reduced to 11.24, 14.19 and 17.51 for surface heaps inoculated with mutant strain, wild strain and un-inoculated control respectively. The main macronutrients (NPK) contents in all composts heaps achieved the upper level for high quality compost. Results demonstrate that mixing of rice straw with animal manure seems to enhance microbial decomposition of straw due not only to the abundance of microorganisms but also to the high contents of its macronutrients. Marked reduction of volume size (about 50 %) was observed after 120 days from the beginning of composting processes (data not shown). The experimental results show that the final product of all treatments was free from pathogenic nematodes and weed seeds (data not shown).

TABLE IV
ANALYSIS OF COMPOSTS PRODUCED FROM RICE STRAW AMENDED WITH ANIMAL MANURE AND INOCULATED WITH EFFICIENT MUTANT *PENICILLIUM* STRAIN AS COMPARED WITH CONTROLS

Analysis of	Inoculated with wild strain	Inoculated with mutant strain	Un-inoculated	Standard Egyptian Compost
pH (1:10)	7.84	7.80	8.70	7-8
E.C. (1:10) ds/ml	4.38	3.23	1.47	<10
Organic matter (%)	30.86	32.18	47.11	>17
Organic carbon (%)	17.89	18.66	27.33	>10
C/N ratio	14.19	11.24	17.51	15:1
Total Nitrogen (%)	1.26	1.66	1.56	0.5-1
Phosphorus total (P_2O_5) (%)	0.69	0.73	8.70	<0.5
Potassium total (K_2O) (%)	0.83	1.38	1.47	<0.5
Asch (%)	69.14	67.82	52.89	60-80

The decomposition process of rice straw is usually slow because of its wide C: N ratio, in addition to the high lignin content (18%) physically encrusting its cellulose components [4]. Results of the present study demonstrate that rice straw decomposition was greatly enhanced by using animal manure and fungal mutant decomposer active in straw biodegradation. These results were in agreement with other studies [18], [19] which indicate that the decomposition process of rice straw is potentially enhanced using animal manure and inoculation by efficient decomposing microbial strain. The high NPK content of the composted product results in slow release of macronutrients (N and P). This produced compost is considered a good bio-fertilizer for plant growth that may reduce the use of chemical fertilizers. Previous studies showed that commercially available bio-fertilizers can enhance crop yield by increasing NPK and improving soil fertility [20]. Such bio-fertilizers are cheaper source of plant nutrient supply without pollution. The role of animal manure and decomposer strain in rice straw degradation was quite evident in the present study. The high macronutrient content of the obtained product and its good physical properties make the product as bio-fertilizer and soil conditioner excellent particularly for newly reclaimed sandy soils.

IV. CONCLUSION

In conclusion, the UV- mutant *of Penicllium* strain was efficient in biodegradation of rice straw. When the mixture of rice straw and animal manure is treated with UV- improved mutants of *Penicllium sp*., the good compost can potentially be produced in the large scale for agricultural purposes instead of rice straw burning, which causes environmental pollution in addition to the nutrients loss of these economic wastes.

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