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Biocatalysis of agro-processing waste by marine *Streptomyces fungicidicus* strain RPBS-A4 for cellulase production

Rajanikanth Akurathi[1], Damodharam Thoti[2]*

[1]Department of Biotechnology, Sri Venkateswara University, Tirupati, Andhra Pradesh, India, [2]Department of Environmental Sciences Sri Venkateswara University, Tirupati, Andhra Pradesh, India

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ABSTRACT

The fundamental reason for this investigation is to lessen the production cost of cellulase by advancing the generation medium and utilizing an option carbon source such as agro-processing waste deposit. *Streptomyces fungicidicus* strain RPBS-A4 was subjected to fermentation medium with supplementation of agro-processing waste powder as a solid substrate for cellulase enzyme production. The impact of various process parameters was examined for increasing cellulase production. Cellulase was observed to be produced by *S. fungicidicus* strain RPBS-A4 using rice bran (5–6%), as best substrate among the tested. Solid state bioprocessing increased the production of enzyme activity at temperature - 40°C, pH - 9.0, inoculum size - 0.6/100 ml, incubation period - 96 h, substrate concentration - 0.6 g/100 ml, carbon source - maltose (1%), and nitrogen source - yeast extract (1%). Under these most favorable conditions, the highest activity 13.18 U/ml of cellulase was observed. The *S. fungicidicus* RPBS-A4 has been considered as the best cellulase producer in biocatalysis of agro-processing waste deposits.

1. INTRODUCTION

Products of agro-industrial practices result in the generation of numerous structural plant components such as groundnut shell, sawdust, paddy straw, rice bran, wheat bran and peels which are renewable, chiefly unexploited, and inexpensive. Partial decomposition of these solid wastes produces leachate and affects groundwater and land environment. It also causes a bad odor and increases the chance for pathogens which cause serious diseases to organisms. One of the most effortless methods for dealing with contamination issues is biocatalysis of these agro-processing wastes into cellulase enzyme [1]. The production of this enzyme has been examined in numerous filamentous fungi, despite the fact that a little work has been done on bacterial cellulase and a constrained consideration is being actinomycetes. Vinogradova and Kushnir [2] stated that actinomycetes are one of the important microbial communities responsible for cellulose degradation found abundantly in the plant cell walls. Literature survey revealed that some of the soil actinomycetes – *Streptomyces lividans*, *Streptomyces albaduncus*, *Streptomyces reticuli*, and *Streptomyces* sp. Strain M7a and strain M7b and *Streptomyces* sp. F2621 have just been accounted for creating extracellular endoglucanase altogether in upgraded culture media utilizing cellulose powder as substrate [3]. However, a detailed investigation on the development of this enzyme from *Streptomyces* species has been hindered.

Solid wastes are used as informants for the production of novel enzymes. This is one of the best methods for the management of solid waste in an experienced way. Solid state bioprocessing has more advantages than submerged state bioprocessing due to low capital investment, generalization of the fermentation media, minimized energy requirement, absence of multipart machinery, and enhanced product recovery [4]. Much of the cellulose in nature exists as waste material from agro-processing industry in the form of husk, stalks, stems, and peels. Hence, to utilize these waste products and to develop a cheaper method for the production of cellulase enzyme for enzymatic degradation. The present study describes the production and optimization of cellulase by solid-state biocatalysis of agro-processing waste.

2. MATERIALS AND METHODS

2.1. Microbial Strain

The organism used in this study was *Streptomyces fungicidicus* strain RPBS-A4, which was reported as cellulase producer in our past investigation [5].

2.2. Biocatalysis of Agro-processing Wastes

Cellulase was produced in solid-state fermentation using agro-processing waste powder – mineral salt medium (MSM) as a production medium. The MSM was prepared by the procedure

*Corresponding Author
Damodharam Thoti,
Department of Environmental Sciences
Sri Venkateswara University,
Tirupati - 517 502, Andhra Pradesh, India.
Email: thotidamodharam@yahoo.co.in

described earlier [6]. It contained the following (g/l): $Na_2HPO_4.2H_2O$ - 1.1 g, $NaH_2PO_4.2H_2O$ - 0.61 g, KCl - 0.3 g, $MgSO_4.7H_2O$ - 0.01 g, and pH - 7.0. Different agro-processing waste powders (paddy straw, groundnut shell, rice bran, wheat bran, corn cob, and sawdust) were added individually to the production medium (1% w/v) and pH of this medium was fixed to 7.0 and make sterile in condition at 121°C. This medium was inoculated with spore suspension of present organism and examined at room temperature for 6 days. Samples were withdrawn every 24 h and assayed for enzyme activity.

2.3. Cellulase Assay

Cellulase activity was assayed using the method described earlier by Wood and Bhat [7] with slight modifications. 0.5 ml of crude extract was used as cellulase; 0.5 ml of 1% carboxymethyl cellulose was used as substrate. One unit of cellulase enzyme activity is equal to the µmoles of glucose liberated per ml enzyme used per min under assay conditions.

2.4. Factors Influencing the Cellulase Activity

2.4.1. Effect of incubation period (hours)

To find out fermentation period required for highest production of cellulase, the present isolate was inoculated on MSM added with 1% rice bran, as this medium gives generally quick development and enzyme activity. The fermentations were done at 30°C for 144 h and the concentrates were examined for enzyme activity.

2.4.2. Effect of temperature (°C)

To decide the impact of temperature on enzyme activity, fermentation was done at various incubation temperatures ranging from 25°C to 50°C. The samples were pulled back following 96 h and the concentrates were measured for cellulase activity.

2.4.3. Effect of pH

To decide the ideal starting medium pH for actinomycetes development and enzyme generation, the medium pH was balanced from 5.0 to 11.0 utilizing buffering agents (0.1 M/L) and flasks were incubated at 40°C for 96 h. The samples were pulled back after incubation time and unrefined concentrates were tested for enzyme activity.

2.4.4. Effect of substrate concentration (g)

Enzyme activity was studied by incorporating various concentrations of substrate (g/100 ml)0.2, 0.4, 0.6, and 0.8 to the production medium, and pH adjusted to 9.0. Then, flasks were inoculated with a spore suspension of the above-mentioned organism and incubated at 40°C for 96 h and the enzyme activity was tested.

2.4.5. Effect of inoculum size (ml)

The spore suspension of the above organism was prepared by harvesting slants in 100 ml of starch casein broth under sterile conditions. The inoculum sizes (ml/100 ml)0.2, 0.4, 0.6, and 0.8 were transferred to flasks containing production medium (pH - 9.0, with 0.6 g of substrate) and inoculated flasks were incubated at 40°C for 96 h and the enzyme activity was assayed.

2.4.6. Effect of carbon source

Distinctive outer carbon sources such as fructose, glucose, maltose, and lactose were brought into the production medium at the level of 1% (w/v). Under the previously mentioned conditions, the flasks were inoculated and incubated. Then, the cellulase activity was examined.

2.4.7. Effect of nitrogen source

Various nitrogen sources such as peptone, beef extract, yeast extract, and ammonium sulfate were supplied to the production medium at a level of 1% (w/v) and the control was maintained with the absence of any nitrogen source. All investigations were done at previously mentioned cultural conditions and cellulase activity was tested.

3. RESULTS AND DISCUSSION

Cellulose degrading microorganisms distributed in the marine environment play an important role in mineralization of organic matter and thereby increasing the productivity of the sea. There were only few reports on the generation of cellulase by marine actinomycetes, and our literature survey revealed that this is the first report on marine *S. fungicidicus* producing cellulase.

3.1. Biocatalysis of Agro-processing Wastes

Bioconversion of agro waste biomass by actinomycetes is a potential sustainable approach to develop new products. In this research, several agro-processing wastes were utilized as substrates for the generation of cellulase. The results [Figure 1] revealed that *S. fungicidicus* strain RPBS-A4 produced maximum quantity of cellulase from rice bran (6.59 U/ml). Microorganisms in the environment degrade disposed agro-processing waste by biocatalysis so utilizing these cheap waste materials for producing high-value enzymes is a suitable waste management plan for cleaning the environment and reducing the production cost of enzymes in industry [8].

3.2. Factors Influencing the Cellulase Productivity

Cellulases have potential applications in food, feed, textile, fuel, and chemical industries. As of late various applications are being proposed and commercially applied for cellulase [9]. For this reason, the present strain had inspected for its capacity to use agro-processing waste deposits as a nutrient for enzyme production under the control of various factors. The physicosynthetic attributes of the fermentation medium assumed an imperative part in cellulase generation by *S. fungicidicus* RPBS-A4.

3.2.1. Effect of incubation period (hours)

The impact of various time interims on cellulase generation by *S. fungicidicus* RPBS-A4 was determined. At first, cellulase production (1.75 U/ml) was noted at 24 h of incubation, it was expanded to 12.29 U/ml at 96 h of incubation and afterward it was diminished to 3.85 U/ml at 144 h [Figure 2]. This demonstrates that incubation time prominently affected cellulase production by the strain and the ideal incubation time for cellulase generation is 96 h. Cellulase activity of *S. fungicidicus* RPBS-A4 (12.29 U/ml) under the optimized conditions is especially greater than the activity of mentioned strains *Streptomyces* sp. BRC1 (6.4 U/ml) and BRC2 (6.6 U/ml) isolated from the soil of decomposed garden [3].

3.2.2. Effect of temperature (°C)

The highest cellulase production (12.32 U/ml) was observed at 40°C [Figure 3]. On increasing the temperature of the cellulase production was decreased to 9.77 U/ml. Temperature assumes an essential part in the development of actinomycetes; under the ideal temperature, the development rate of *S. fungicidicus* RPBS-A4 was expanded, as the development rate expanded cellulase generation was additionally expanded. Chellapandi and Jani [3] declared that the highest quantity of enzyme was produced by *Streptomyces* sp. BRC1 and BRC2 in submerged fermentation at ideal temperature 26°C, i.e. 7.1 U/ml. Maximum cellulase activity of *S. fungicidicus* RPBS-A4 at 40°C was better than the activity of revealed strain. This demonstrates the ideal

temperature for high cellulase production by this strain is 40°C, which is in accordance with the findings of McCarthy [10] who announced that an ideal temperature for enzyme activity is in the scope of 40-55°C for a few *Streptomyces* species.

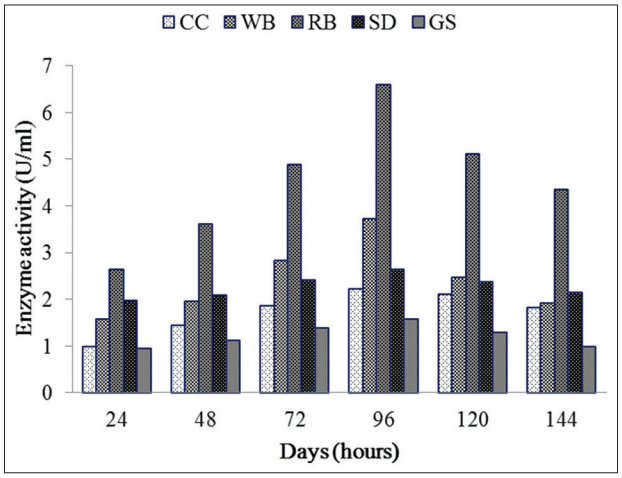

Figure 1: Biocatalysis of agro-processing waste for cellulase production by *Streptomyces fungicidicus* RPBS-A4 (CC: Corncob, WB: Wheat bran, RB: Rice bran, SD: Sawdust, GS: Groundnut shell, and PS: Paddy straw).

3.2.3. Effect of pH

The impact of pH on enzyme productivity by the isolate was determined. Cellulase generation was gone between 5.67 and 13.18 U/ml. The highest cellulase production of 13.18 U/ml was seen at pH 9.0 and lowest cellulase production 5.67 U/ml was seen at pH 11.0 [Figure 4]. This indicates that the ideal pH for high cellulase production by *S. fungicidicus* RPBS-A4 is 9.0.

3.2.4. Effect of substrate concentration (g)

The effect of substrate concentration on cellulase production by the present strain was examined at various substrate concentrations. The highest cellulase production (12.64 U/ml) was seen with 0.6 g/100 ml of a substrate (rice bran). After expanding or diminishing the substrate concentration cellulase production by *S. fungicidicus* RPBS-A4 was diminished to 9.55 U/ml [Figure 5]. This proves that the appropriate concentration is 0.6 g per 100 ml of fermentation medium. Similarly, Immanuel [11] was used coir fiber powder as the substrate for the generation of cellulase by bacteria. They found that maximum quantity of cellulase 0.081, 0.041, and 0.023 U/ml at

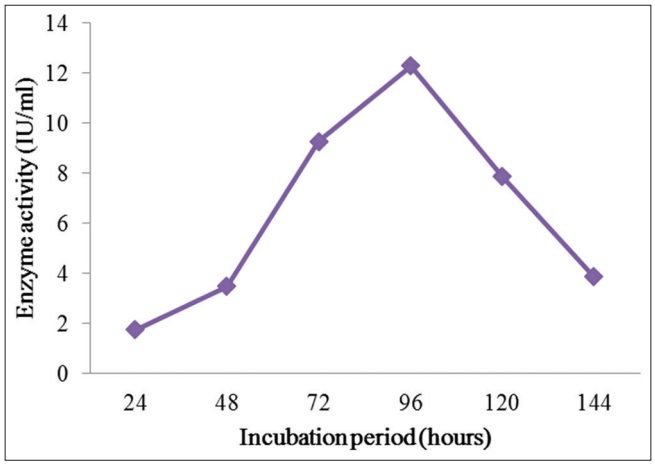

Figure 2: Effect of incubation period (hours) on cellulase production by *Streptomyces fungicidicus* RPBS-A4.

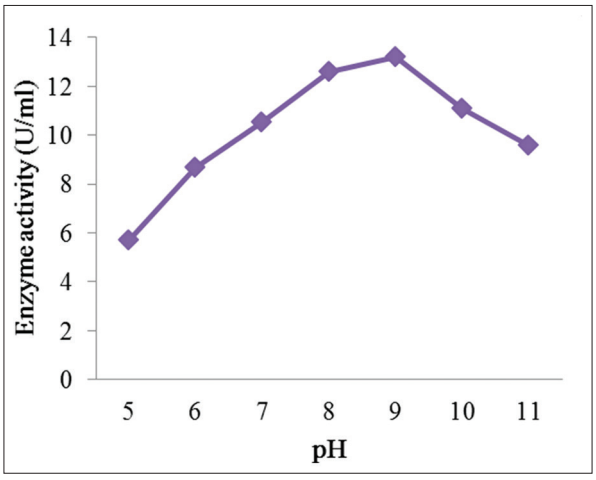

Figure 4: Effect of pH on cellulase production by *Streptomyces fungicidicus* RPBS-A4.

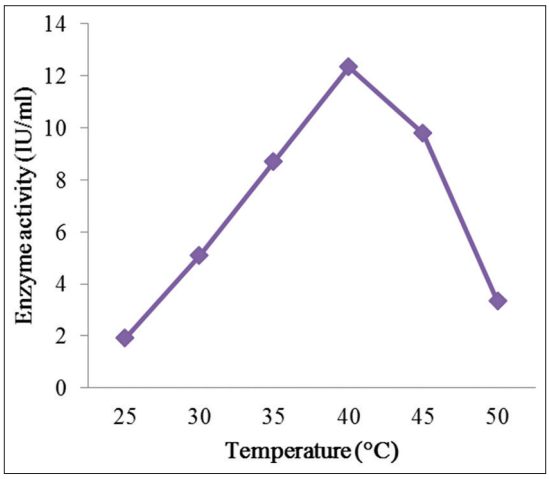

Figure 3: Effect of temperature on cellulase production by *Streptomyces fungicidicus* RPBS-A4.

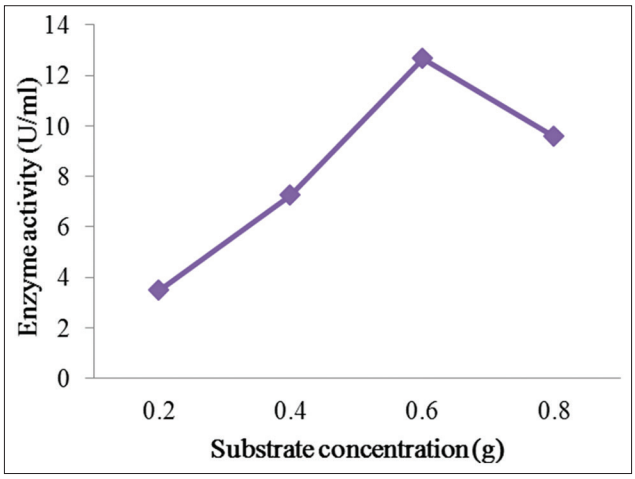

Figure 5: Effect of substrate concentration (g) on cellulase production by *Streptomyces fungicidicus* RPBS-A4.

1.5% concentration of substrate. The highest cellulase production of *S. fungicidicus* RPBS-A4 using rice bran was 150 times more than the above-mentioned strain.

3.2.5. Effect of inoculum size (ml)

The inoculum concentration had a prominent effect on cellulase production. The cellulase showed maximum activity (12.59 U/ml) using 0.6 ml inoculum [Figure 6]. Results obtained in the present investigation proposed that the ideal inoculum concentration is 0.6/100 ml of fermentation medium for the generation of enzyme by *S. fungicidicus* RPBS-A4 in solid state bioprocessing. These outcomes are in accordance with an earlier report of cellulase generation by *Bacillus circulans* in solid state bioprocessing [6].

3.2.6. Effect of carbon source (1%)

The impact of various carbon sources on cellulase generation by the marine isolate was resolved. The highest cellulase production (10.14 U/ml) was observed in the presence of maltose (external carbon source) [Figure 7]. The presence of additional carbon sources, glucose, and fructose was decreased the cellulase generation than the control. This indicates that the carbon source exist in the generation medium had assumed a key part, i.e., the more concentration of additional carbon source decreased the growth rate of *S. fungicidicus* RPBS-A4. Hence, there was no need of an external carbon source for cellulase production by this strain. Similar results were found when 1% glucose treated as an external carbon source in *Bacillus subtilis* [12].

3.2.7. Effect of nitrogen source (1%)

The impact of various nitrogen sources on enzyme generation by *S. fungicidicus* RPBS-A4 was resolved. Results revealed that maximum production of cellulase 12.64 U/ml was observed when yeast extract was served as external nitrogen source [Figure 8]. These findings were very close to *Bacillus cereus* MRK1 producing cellulase in the presence of external nitrogen source as yeast extract [13].

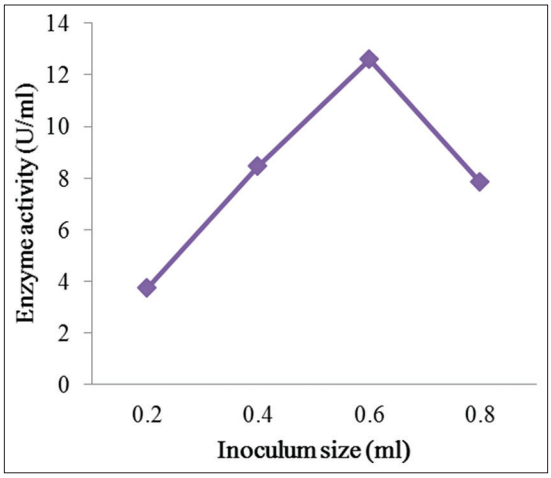

Figure 6: Effect of inoculum size (ml) on cellulase production by *Streptomyces fungicidicus* RPBS-A4.

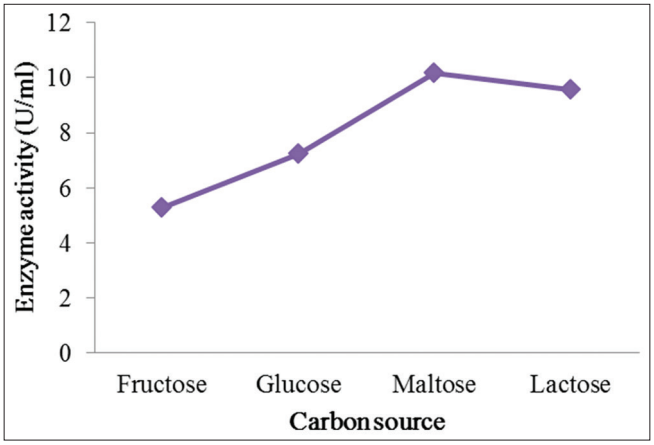

Figure 7: Effect of carbon source (1%) on cellulase production by *Streptomyces fungicidicus* RPBS-A4.

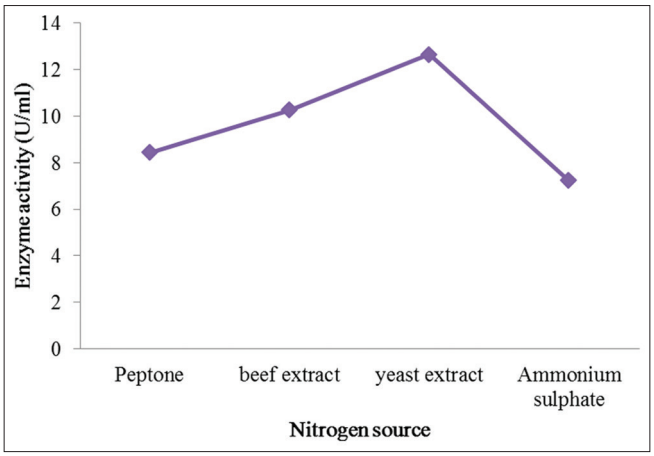

Figure 8: Effect of nitrogen sources on cellulase production by *Streptomyces fungicidicus* RPBS-A.

4. CONCLUSION

In the present study, efforts were made to increase the enzyme production by modifying the nutritional conditions and pH of the fermentation medium. The results revealed that cellulase activity of 13.18 U/ml was seen under appropriate cultural conditions by *S. fungicidicus* strain RPBS-A4 are better than the prior revealed strains. Notwithstanding these, some extra properties such as external carbon source (maltose) and lower substrate fixation, less fermentation period for cellulase production showing the capability of the isolate to be utilized at profitable level in industries. An outline of the outcomes acquired demonstrates that solid-state fermentation of agro-processing waste deposits was appropriate to deliver minimal effort, high esteem item, i.e. cellulase by *S. fungicidicus* RPBS-A4. The ecological contamination caused by the disposal of cellulosic wastes, which are continuously added to the environment through the process of photosynthesis will also reduce by utilizing agro-processing waste as a sole carbon hotspot for the generation of cellulase.

5. ACKNOWLEDGMENT

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