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Bioconversion and saccharification of banana agrowaste by *Amycolaptosis fastidiosa* for fermentable sugar production.

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Abstract

The recent interest in bioconversion of agricultural crop residues rich in cellulose, hemicellulose, protein and minerals into fermentable substrate is best for microbial production of various products. In the present study, three lignocellulosic substrates viz. banana plant leaves, pseudo stem and rhizome were pre-treated with steam, dilute acid, dilute alkali and enzyme obtained from *Amycolaptosis fastidiosa*. The combined effect of different pre-treatment methods with enzymatic bioconversion of lignocellulosic agro waste has been investigated. The physical (steam) and enzyme treatment combination gave maximum saccharification of banana leaves, pseudo stem and rhizome viz. 54%, 75% & 63% respectively. Quantitative analysis of saccharified agro waste for reducing sugar indicated that pseudo stem contains highest reducing sugar as compared to rhizome and leaves. These results suggest that pseudo stem of banana can be utilized as a potential substrate for fermentable sugar production.

Keywords: Bioconversion, saccharification, *Amycolaptosis fastidiosa*, agro waste, fermentable sugar

Introduction

India is one of the richest countries in the agricultural resources. Agricultural wastes are the by-products of various agricultural activities such as crop production, crop harvest, saw milling, agro industrial processing and others. A huge banana lignocellulosic agro waste is generated after harvest of banana fruit. It is estimated to be approximately 30MT/Ha/year.

Plant biomass (lignocellulose) represents the most important renewable feedstock on the planet. These agricultural wastes are the most abundant raw material consisting of cellulose as the major component, which is suited for growth of microorganisms [1].

Sugars in lignocellulose are locked into very stable polymeric structures including cellulose and hemicellulose [2, 3]. Different types of lignocellulosic biomass vary in the composition of cellulose, hemicellulose, and lignin [2]. Chemical pre-treatment processes are commonly required for lignocellulose conversion. Steam pre-treatment with dilute mineral acids is an efficient approach to depolymerize hemicellulose into sugar monomers and to increase the accessibility of cellulase enzymes to degrade cellulose [2, 4-5]. After pre-treatment and cellulase digestion, most of the sugars in agricultural waste will be released into the broth and thus ready to be converted into desired products. The cost of cellulase enzymes is currently still prohibitive to wide application of lignocellulose conversion. Continuing efforts of synthetic biologists from academic and industrial labs are improving cellulase enzymes.

Actinobacteria are a group of gram-positive bacteria, which plays an important role in decomposing plant biomass and mostly exist in the soil. From this group of bacteria, the genus Streptomyces are common in degrading lignin containing biomass. *S.viridosporus* T7A are capable of degrading synthetic lignin, Kraft lignin, aromatic dyes and polyethylene plastic. *Streptomyces flavovirens, Streptomyces badius* ATCC 39117, *Streptomyces cyaneus* CECT 3335, *Streptomyces psammoticus* and *Amycolaptosis sp*. are some of the bacteria from *Streptomyces* genus which showed lignin depolymerization and mineralization activities [6-10]. Many reports are available for delignification of different agro wastes like rice straw, corn cob, sugarcane bagasse but very few reports are there for bioconversion of banana agro waste. Hence this study is designed to obtain efficient cellulase producing organisms for saccharification of banana agro waste and conversion of the agro-waste into fermentable sugar which can be used further for production of value added products.

Methodology

Materials:

Agro-waste Sample: Banana agro-waste samples were collected from the Ardhapur region of Nanded District. The samples comprised of leaves, pseudo stem and rhizome of popularly grown cultivars of banana plant,. Ardhapuri-1,

Chemicals: All the chemicals of analytical grade were procured from Qualigens, S.D. Fine Chemicals and Spectrochem whereas all the culture media were procured from Hi- Media Laboratories Pvt. Ltd.

Microbial Strains: Cellulose utilizing actinomycetes were isolated from the soil sample collected from banana plantation area.

Methods:

1. Isolation of cellulose utilizing Actinomycetes:

A loopful of the enriched broth was streaked on cellulose casein nitrate agar (Kuster and Williams) plate. This media was fortified with the antibacterial agents i.e. penicillin, streptomycin and the antifungal agent griseofulvin before pouring the media into sterile plates. The plates were incubated at 30^0C for 5 to 7 days for the development of colonies. Well isolated colonies were maintained on the slants of respective media for further use.

Screening of isolates for cellulase & hemicellulose activity:

Isolates were screened for cellulase activity by their ability to utilize carboxy methyl cellulose (CMC) in the CMC agar plate [11] and for hemicellulase activity on xylan mineral agar plate.

Identification of isolate:

The most efficient isolate was identified by 16 S rRNA sequencing.

2. Production of Cellulase Enzyme:

The uniform suspension of the selected organism was prepared in sterile saline solution containing (0.05%) Tween 80. The suspension was inoculated in the agro-

waste containing production medium [12] at a concentration of 5% (v/v) for the production of cellulase enzyme. The flasks were incubated on orbital shaking incubator at 30^0C for 48 hours. The contents were centrifuged and supernatant was taken as crude enzyme. The enzyme activity of the cell free extract was measured according to the procedures given by IUPAC [13] by performing CMC ase assay. The assay was performed by incubating 0.5 ml. of enzyme solution with 0.5 ml. of CMC (1%) in acetate buffer (pH 5.0) for 30 minutes at 30^0C. The released sugars were estimated by Dinitro salicylic acid (DNS) reagent [14].

CMC ase activity (IU) = µ mol. reducing sugar liberated $ml^{-1}min.^{-1}$

Protein content of the cell free extract was determined by Lowry's method (1951), and specific activity of the crude enzyme was calculated as,

$$\textbf{\textit{Specific activity}} = \frac{\textit{CMC ase activity (IU)}}{\textit{Mg. of protein}}$$

3. Saccharification of Banana agro-waste:

Banana agro-waste (leaves, pseudo stem and rhizome) was saccharified by physical (steam treatment), chemical (acid and alkali treatment) and enzymatic method individually as well as by combinations of these methods viz. acid and steam, alkali and steam, steam and enzyme, acid and enzyme, alkali and enzyme. [15-17].

The contents of each flask were centrifuged and the resulting supernatant was collected for determination of reducing sugars. Reducing sugar content of the supernatants obtained from all the methods was taken into consideration to calculate percent saccharification.

$$\textbf{\textit{\% Saccharification}} = \frac{\textit{Reducing sugars (mg/ml)}}{\textit{Substrate (mg/ml)}} \; X100$$

4. Analysis of saccharified banana agro-waste:

Saccharified agro-waste was analysed qualitatively for the presence of sugars by thin layer chromatography technique.

Results and Discussions

Morphological studies of 13 actinomycete isolated showed that these comprise the species of *Spirillospira* (3), *Streptomyces (3), Actinomadura (5)* and *Nocardia (2).* This was based upon *Bergey's Mannual of Systematic Bacteriology,* [18].

The specific activity of cellulase revealed that the banana agro-waste degrading microorganisms are varying in their cellulase specific activity. The cellulases obtained from *Actinomadura, Nocardia* and *Streptomyces* have maximum specific activity in the range of 24.59 to 74.07 IU/mg protein (Table-1). Among the actinomycete species, *Streptomyces* cellulase showed 74.07 IU/mg protein specific activity. This isolate identified as *Amycolaptosis fastidiosa* by 16s rRNA sequencing.

Ball and McCarthy [19] studied cellulase activity U/mg of intracellular protein of culture supernatant from actinomycetes against carboxy methyl cellulose. The actinomycete strain *Thermomonospora fusca* MT-100, *Thermomonospora chromogena* MT-808, *Micromonospora* Ll-23, *Streptomyces* EC-22, *Streptomyces* EC-1 and *Microbispora bispora* DSM-4.038 showed the specific activity of 3.0, 1.7, 1.5, 4.6, 2.2, 1.0 and 3.1 U/mg protein respectively whereas *Amycolaptosis fastidiosa* isolated in this study showed the specific activity of 74.07U/mg protein.

The saccharification of lignocellulosic banana agro-waste viz. leaves, pseudo stem and rhizome was attained by physical (steam), chemical (acid and alkali) and enzymatic (cellulase) methods and combination of these methods. Among the physical, chemical and enzymatic method alone, enzymatic method of saccharification showed highest percent saccharification (37%, 39% and 38%) of leaves, pseudo stem and rhizome respectively whereas the acid treatment showed lowest percent saccharification (11%, 12% and 12%) of leaves, pseudo stem and rhizome respectively (Table - 2). The reduced saccharification might be due to production of furfural and hydroxy furfural from xylose and glucose

respectively [20]. They also reported 19% of saccharification with the 1.5% H_2SO_4 treatment of wheat straw. Havnnavar and Geeta, [21] reported 17.74%, 15.63% and 20.81% of saccharification of wheat straw, paddy straw and sugarcane bagasse by the enzymatic treatment by commercial cellulase. These values are less than the banana agro-waste saccharification in the present study indicating maximum activity of the cellulase of *Streptomyces sp.*

Among the combination of physico-chemical and enzymatic methods, steam pre-treatment followed by enzymatic treatment showed highest percent saccharification of leaves, pseudo stem and rhizome viz. 54%, 75% and 63% respectively. Similar results were reported by Baig *et al*., [22]. The steam pre-treatment followed by enzymatic treatment showed maximum percent saccharification because the steam pre-treatment increases accessible surface area and pores, decrease the cellulose crystalinity and degree of polymerization and cause partial hydrolysis of hemicellulose and partial depolymerisation of the lignin of lignocellulosic banana agro-waste.

Steam and enzyme treated banana agro-wastes were analysed for sugars qualitatively as well as quantitatively. The reducing sugars obtained from the hydrolysed banana agro-waste were 540, 750 and 630 mg/g of leaves, pseudo stem and rhizome respectively (Fig.-1) Sugars present in the hydrolysed agro-waste were identified qualitatively (TLC) as maltose, xylose, glucose, arabinose and galactose (Table - 3). Oshoma and Ikenebomeh, [23] also reported that rice bran converted to fermentable sugars by heat treatment

Similarly, Kovacs *et al*., [24] reported presence of glucose, mannose and xylose in steam and enzymatically hydrolyzed substrates of spruce, wheat straw and sugarcane bagasse. Gomez *et al*., [25] also detected release of xylose, mannose, glucose and arabinose in acid pre-treated *Brachypodium distachyon* stems.

Table 1. Cellulase activities of Banana agro-waste Decomposing Microorganisms.

Sr. No.	Cellulase from microbial isolate	CMC ase activity IU/ml	Specific activity IU/mg protein
1	AI-3	0.76	54.28
2	AI-4	0.759	54.21
3	AI-5	0.787	56.21
4	AI-10	0.787	24.59
5	AI-13	1.037	74.07

**AI – Actinomycete*

IsolateResults from each isolates are the mean value of three replicates.

Table 2. Saccharification of Banana agro-waste by physico - chemical and enzymatic methods.*Concentration of Substrate: 10 mg/ml.*

Sr. No.	Saccharification Method	% Saccharification		
		Leaves	Pseudo stem	Rhizome
1	**Steam Treatment**	**20%**	**36%**	**31%**
2	**Acid Treatment**	**11%**	**12%**	**12%**
3	**Alkali Treatment**	**31%**	**37%**	**41%**
4	**Enzyme Treatment**	**37%**	**39%**	**38%**
5	**Steam+ Acid treatment**	**22%**	**30%**	**31%**
6	**Steam + Alkali treatment**	**35%**	**38%**	**30%**
7	**Steam + Enzyme treatment**	**54%**	**75%**	**63%**
8	**Acid + Enzyme treatment**	**13%**	**20%**	**30%**
9	**Alkali + Enzyme treatment**	**22%**	**20%**	**38%**

Table 3. Qualitative analysis of saccharified Banana agro-waste for sugars.

Sr. No.	Product	Saccharified Component		
		Leaves	Pseudostem	Rhizome
1	Sugars	Maltose Xylose Glucose	Glucose Arabinose Galactose Xylose	Glucose Arbinose Maltose Xylose

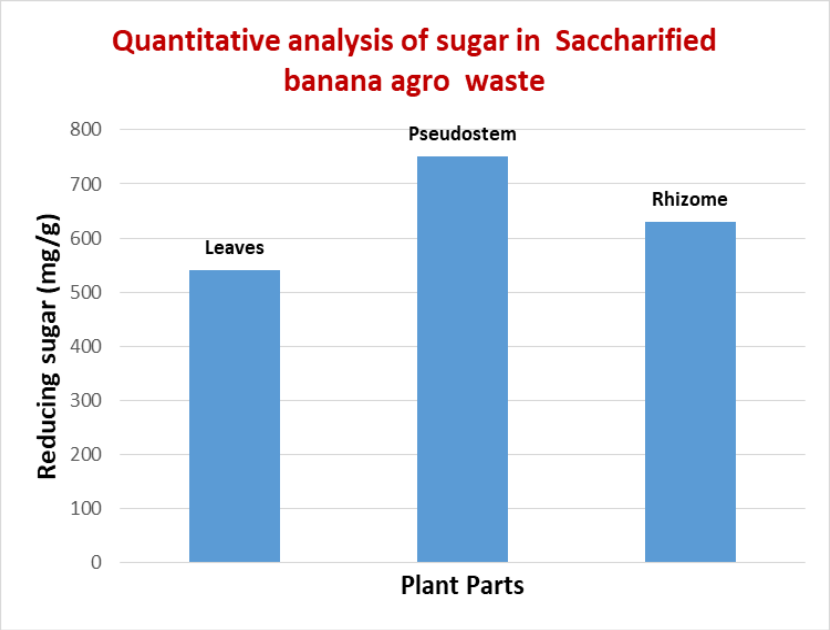

Fig. 1. Quantitative analysis of sugar in saccharified Banana agro-waste

Conclusion

The cellulase produced from *Amycolatopsis fastidiosa* showing highest activity compared with known references and thus can be efficiently used for saccharification of banana agro waste viz. leaves, pseudo stem and rhizomes.

The saccharifications were carried by using different methods. The physical (steam) and enzymatic treatment combination is best as it gives maximum saccharification of banana leaves, pseudo stem and rhizome viz. 54%, 75% and 63% respectively indicating this combination as most efficient method of saccharification.

The saccharified component obtained from banana leaves, pseudo stem and rhizome was analysed qualitatively and quantitatively for liberated sugars.

The sugars were identified by thin layer chromatography as glucose, xylose, maltose, galactose and arabinose suggesting the mixture of fermentable sugars and the bioconversion of the lignocellulose of banana agro waste.

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