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Biomass Utilization of Burma Reed (*Neyraudia reynaudiana*) by an alkaliphilic cellulase free Xylanase Producing *Saccharopolys pora antimicrobica* strain 20-23

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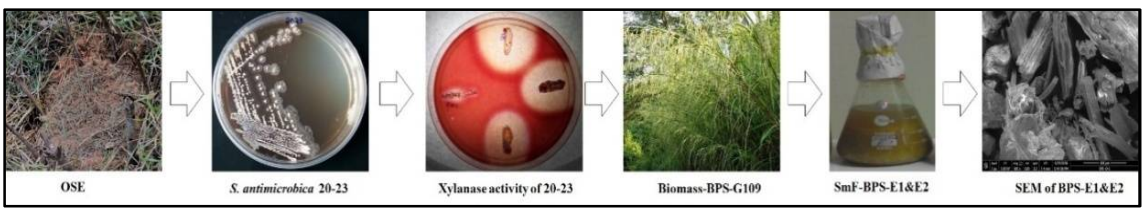
ABSTRACT

On the behest of isolating hemicellulose degrading actinobacteria from sub-tropical forest ecosystem, an on-site enrichment system (OSE) was designed comprising of naturally occurring plant materials which were successful in isolating eight xylanase producers, with 57% overall bacterial recovery. These xylanase producers belonged to Streptomyces, Saccharopolys pora and Nocardiopsis genera of which isolate 20-23 (Saccharopoly spora antimicrobica) exhibited the highest xylanase activity of 39.88±0.42U mL^{-1} at 6th day, pH 8 and 30°C.S. antimicrobica 20-23 (BPS-E1 and E2) potential to utilize BPS-D1 the lignin devoid thermo-alkali pre-treated Burma reed (Neyraudia reynaudiana BPS-G109), for its hemicellulose hydrolysis yielded optimal xylanase activity 47.70 ± 0.31 U mL^{-1} on the 6th day itself. BPS-E1 and E2 submerged fermentation (SmF) was successful in utilizing the 90% of total hemicellulose, resulting in enhanced cellulose recovery (38% to 76%). This result was substantiated by the lack of characteristic hemicellulose peaks by Fourier-transform infrared spectroscopy (FTIR) analysis and subsequent cellulose gain as indicated by increased crystallinity by X-ray powder diffraction (XRD) analysis. To the best of our knowledge, it is for the first time Saccharopolyspora antimicrobica strain 20-23 was exploited for its alkaliphilic cellulase free xylanase productivity was successfully implemented for hemicellulose utilization of Neyraudia reynaudiana for enhanced cellulose recovery.

Graphical Abstract



Keywords: Xylanase, enrichment, *Saccharopolyspora antimicrobica*, OSE.

INTRODUCTION

Lignocellulosic plant biomass mostly builds of holocellulose (cellulose + hemicellulose) represent the most abundant naturally occurring biopolymer in the earth [1]. But, accessing the cellulosic resource that approximates to 60% of the lignocelluloses is hindered due to other polymers like lignin and interlinkages between the hemicelluloses [2]. Numerous pre-treatments were devised to overcome these issues ranging from chemical, physical to hydrolytic enzyme treatments over the years, but their efficiency and reusability vary depending on the biomass sources [3, 4]. Hemicellulases like xylanases that were categorized as accessory enzymes have gained significance recently having identified its importance in acting as an enhancer for biomass conversion. Hemicellulose elements like xylans do hinder the availability of cellulose by entangling them within its cross-linkages in raw biomass that prevents action cellulase or such hydrolytic treatments [5, 6].

The significance of exploring non-edible biomass like perennial grasses is not only for the fact that they encompass rich holocellulose material, fewer lignin contents but also because they thrive on infertile hilly or wastelands [7]. According to [8, 9], Burma reed (*N. reynaudiana*) that are profoundly seen growing in sub-tropical and tropical conditions do provide substantial biomass, with the above-ground plant material reaching heights of 29.0 t/hm^2. All these facts and its abundance especially in North-east India, make the less explored Burma reed an ideal candidate for second generation alternative fuel source [10].

Sub-tropical, tropical forests are a source of vast amounts of biomass and a repertoire of unexplored and diverse microbial communities. They do provide an insight into potent biomass that could be explored, microbes that possess hydrolytic enzymes capable of deconstructing plant debris and materials [11]. The enhanced microbial diversity makes it difficult for isolating specifically plant biomass or lignocellulose degrading microorganisms and hence there is a pertaining need to improve on the existing enrichment and isolation techniques. To garner the unique microbes that are usually shielded by the fast-growing ones, applying on-site enrichments composed of specific biomaterials, can ensure the natural feeders particular to the need are attracted and isolated.

Unique and less explored actinobacteria proficient in producing plant degrading enzymes like cellulases and hemicellulases have gathered attention recently. These microorganisms that possess varying adaptable features and competence to produce stable enzymes exceptionally capable of tolerating harsher conditions like thermostability, alkaliphlicity, acidophilicity, etc. find substantial industrial usages [4, 12, 13].Efficient cellulase free xylanases sustainable at alkaline pH are more preferred candidates especially in pulp and paper industries [14]. Even though non-edible biomass like perennial grasses, possess substantial cellulosic entities, accessing it is hindered by the hemicellulosic and lignin components. Hence developing a cheaper and efficient pre-treatment and microbial hydrolysis system that could remove ligno-hemicelluloses and free the cellulosic contents could prove significant in biomass conversion studies.

MATERIALS AND METHODS

Chemicals and perennial grass biomass: ISP7, beech wood xylan, DNS (Dinitrosalicylic acid), crystalline phenol, xylose, congo red, nystatin, and nalidixic acid purchased from HiMedia, Mumbai, Maharashtra, India. All chemicals used were of analytical grade. Perennial grass *Neyraudia reynaudiana* (Kunth) Keng *ex* Hitchc. (Burma reed) under the family, Poaceae was collected and processed according to [10] from Murlen National Park in India.

Enrichment and isolation of actinobacteria: An enrichment experiment was designed for the isolation of actinobacterial strains that possess hemicellulose degrading potential. A compost comprising of forest litter, Burma reed, sawdust and beech wood xylan (30:30:30:10) was mixed into an enriched compost. This was placed within a forest litter rich site in Dampa Tiger Reserve (23°36'13.98"N 92°21'37.70"E) of pit sizes of 12cm x 12 cm x 10cm (w x l x d) for 2 weeks. Post-treatment the enriched compost sample was bagged and subjected to serial dilution and grown on ISP7-X media [ISP7(Tyrosine Agar) media supplemented with 1% oat spelt xylan]. Additionally, the media had 50 mg nystatin and 20 mg nalidixic acid to overcome the interference of fast-acting fungi and bacteria. The actinobacterial isolates emerging based on their morphology were re-streaked to obtain pure culture and stored in ISP7-X media [**15-17**].

Screening, quantification, and optimization of xylanase: All the isolates were subjected to xylanase screening using congo red assay [**18**]. To get the efficient xylanase producer, the strains were grown in ISP1 media supplemented with 2% beech wood xylan and subjected to xylanase quantitative estimation following the instructions of[**19, 20**].1U of xylanase activity was the amount of enzyme capable of releasing 1 µmol of xylose min^{-1} mL^{-1}. All samples were assayed in triplicates and the means documented. The impact of incubation time (12 days) on xylanase production was carried out for all the xylanase active isolates at ambient temperature and neutral pH. The proficient xylanase producer was then subjected to optimization for its pH (5, 6, 7, 8 and 9) and temperature (25, 30, 35, 40, 45, 50 and 55 ˚C) parameters respectively.

Identification of xylanase producers: The isolates that exhibited xylanase activity were identified after DNA isolation and amplification of the 16S rRNA gene as described earlier [**21**]. The DNA purified was then sequenced commercially at Genotypic Technology Pvt. Ltd, India. The sequences were then compared with those in EzBioCloud databases, and the sequences were aligned using Clustal W, and a dendrogram was constructed using MEGA6 to establish the taxonomic position [**22**].

Biomass pre-treatments and its utilization assay: The raw *N. reynaudiana*BPS-G109 grounded to 2.0 to 5.0 mm particle size and pre-processed into the slurry form of 20:2 ratio (biomass: sterile water). This slurry was subjected to alkali pre-treatment using 1% w/v NaOH for 15 min maintained at 120ºC and extended 5 min at 140ºC. After treatment, it was cooled to room temperature, filtered and neutralized. This was further subjected to submerged fermentation (SmF) with alkali pretreated *N. reynaudiana* (BPS-D1) as the major carbon source of 20% w/v in 0.5L BPS-YM media, using the best xylanase isolate under its optimized growth conditions (BPS-E1&E2). SmF supernatant aliquots were collected sequentially for 10 days, and on completion, the remaining biomass was filtered out using sterile nylon cloth for further analysis [**10, 20, 23**].

Compositional, SEM, XRD, and FTIR analysis: The lignocellulosic components were measured for the raw (BPS-G109), thermo-alkali treated (BPS-D1) and SmF (BPS-E1 and E2) samples respectively according to the methodology of the National Renewable Energy Laboratory [**24, 25**] to verify the effectiveness of the alkaline treatment and SmF. The relative dry weight (RDW) was estimated, with the thermo-alkali pretreated sample (BPS-D1) serving as the control. The topological variations occurred on the BPS-G109 sample due to treatments were observed under Scanning electron microscopy (SEM) as described previously by [**10**]. The variations within the chemical bonds were verified using Fourier-transform infrared spectroscopy (FTIR)readings obtained JASCOFT/IR-6800 spectrometer (Jasco, Japan) read at 700 to 3750 cm^{-1} (Scans–Avg.64 at 4cm^{-1} resolution) following [**20**]. X-ray powder diffraction (XRD) was performed in a Rigaku Rotaflex diffractometer model RU200B (Tokyo, Japan) using monochromatic CuKα radiation (1.54 Å) at 45 kV and 36 mA. Scans were obtained from 5 to 60 degrees 2θ (Bragg angle) at a 5° $minute^{-1}$ scanning rate. Samples were milled before analysis and put through a 40–60 mesh sieve. The crystallinity index (CI) for all the samples were calculated according to the procedures previously described [**26, 27**].

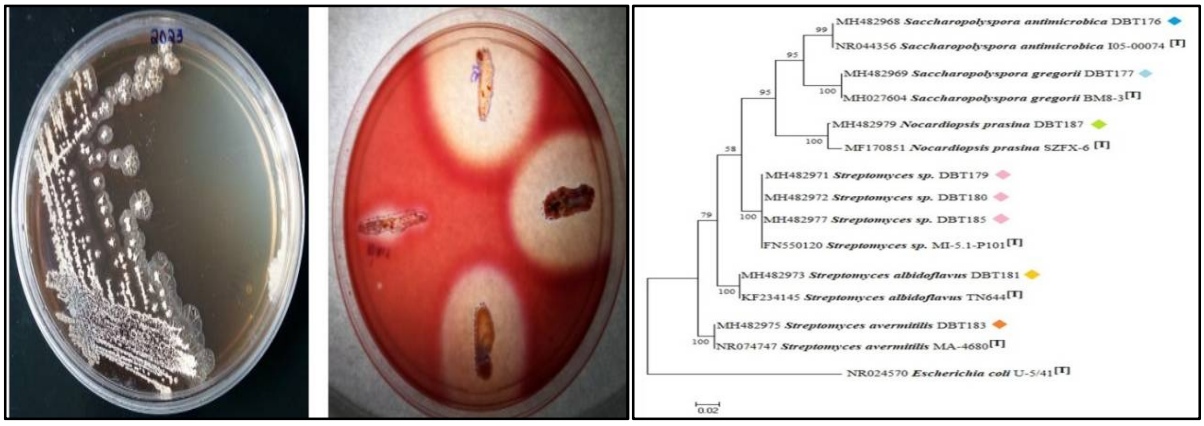
Statistical analysis: The experiments were conducted thrice and statistically evaluated, that was represented as mean±SE. The significance in variations at 5% level of probability was determined by Fisher's LSD multiple range analysis.

RESULTS AND DISCUSSION

Actinobacteria isolation from enrichment and xylanase screening: 14 actinobacteria isolates were recovered based on its morphological and texture natures on the ISP7-X media from the enrichment experiment. Out of this 8 cultures exhibited xylanase activity from congo red assay, with isolate 20-23 showing the highest hydrolysis zone. A 57% hemicellulose degrading actinobacteria was thus recovered from the enrichment method used (Figure 1A, 1B, Table 1).

Table 1. Congo red assay based xylanase screening of OSE isolates

S.No.	Isolate No.	[A] Size of the colony (cm)	[B] Destained Zone (cm)	[B/A] Hydrolysis capacity
1	20-05	0.7	0.6	0.8
2	20-10	0.5	1.5	3.0
3	20-13	0.7	2.0	2.8
4	20-19	0.5	1	2.0
5	20-20	0.5	0.8	1.6
6	20-22	0.5	1.4	2.8
7	**20-23**	**0.5**	**1.8**	**3.6**
8	20-57	0.6	1.2	2.0
9	20-03	-	-	-
10	20-12	-	-	-
11	20-58	-	-	-
12	20-01	-	-	-
13	20-60	-	-	-
14	20-61	-	-	-



1A **1B** **1C**

Figure 1. 1A: Isolate 20-23 (*Saccharopolysporaantimicrobica*)**; 1B:** Congo red screening for xylanase activity of isolates 20-23, 20-22 and 20-10**; 1C:** Phylogenetic tree (Kimura-2 model by Maximum Likelihood Method under 1000 bootstrap replicates) that represents the identity of the selected OSE xylanase producer isolates.

Quantification and optimization of the xylanase isolates: Isolate 20-23 under ambient conditions exhibited the highest xylanase activity of 30.23±0.25 U mL^{-1} (6^{th} day) among the selected isolates, followed by isolate 20-22 (23.12±0.14 U mL^{-1}, 6^{th} day) and isolate 20-10 (21.45±0.14 U mL^{-1}, 8^{th} day). Isolate 20-23 exhibited versatile xylanase activity from day 2 of 10.96±0.12 U mL^{-1}) itself till day 10 of 23.38±0.30 U mL^{-1} (Figure 2A, Table 2). The optimization of highest xylanase producer (isolate 20-23) for varying temperatures and later pH revealed an enhanced xylanase activity of 39.88±0.42 U mL^{-1} under alkaline (pH 8) and ambient (30°C) temperature (Figure 2B, 2C, Table 3).

Table 2. Effect of incubation time on xylanase activity of the selected OSE isolates

Xylanase Activity (U mL^{-1})								
Days of Incubation	**20-05**	**(20-10)**	**20-13**	**20-19**	**20-20**	**20-22**	**20-23**	**20-57**
Day 1	1.05 ± 0.02	0.11 ± 0.01	0.14 ± 0.08	0.78±0.02	0.01±0.01	0.37 ± 0.11	0.51 ± 0.14	2.95 ± 0.10
Day 2	2.12 ± 0.04	2.34 ± 0.15	2.06 ± 0.15	2.89± .17	0.11±0.01	10.34±0.15	10.96 ± 0.12	8.67 ± 0.12
Day 4	1.56 ± 0.02	9.87 ± 0.05	10.35 ±0.22	8.77± .19	0.17± .01	18.12±0.18	16.74 ± 0.15	14.65 ±0.17
Day 6	1.11 ± 0.11	17.37±0.11	17.19 ±0.20	15.34± .19	1.14± .12	23.12±0.14	30.23 ± 0.25	10.56 ±0.06
Day 8	1.08 ± 0.06	21.45±0.14	20.80 ±0.28	13.34± 26	1.58± .13	19.97± 17	25.12 ± 0.22	2.65 ± 0.17
Day 10	0.11 ± 0.04	18.94 ± .08	18.23 ±0.19	4.56± .07	2.67± .19	12.18±0.10	23.38 ± 0.30	1.28 ± 0.15
Day 12	0.01 ± 0.01	9.12 ± 0.11	8.67 ± 0.15	1.34± .05	2.15± .10	5.23 ± 0.10	7.95 ± 0.11	0.34 ± 0.09

Table 3. Optimization of temperature and pH parameters on xylanase activity of isolate 20-23 (*Saccharopolysporaantimicrobica*)

Different temperature	**Xylanase activity (U mL^{-1})**	**Different pH**	**Xylanase activity (U mL^{-1})**
25°C	1.26 ± 0.05	pH 5	3.82 ± 0.25
30°C	**30.33 ± 0.48**	pH 6	10.39 ± 0.48
35°C	20.88 ± 0.39	pH 7	30.79 ± 0.45
40°C	15.22 ± 0.36	pH 8	**39.88 ± 0.42**
45°C	4.92 ± 0.18	pH 9	15.55 ± 0.48
50°C	2.39 ± 0.14	--	--
55°C	0.21 ± 0.02	--	--

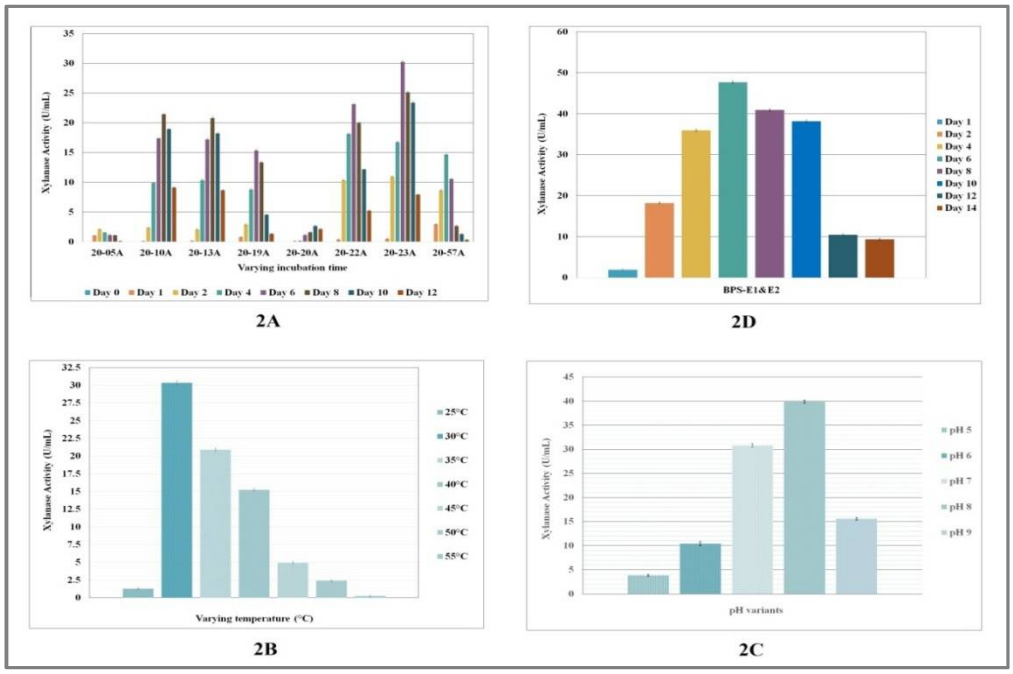


Figure 2. 2A: Effect of incubation time on xylanase activity of the 8 selected OSE isolates; **2B**: Effect of temperature on xylanase activity of *S. antimicrobial* 20-23; **2C:** Effect of pH on xylanase activity of *S. antimicrobial* 20-23; **2D**: Xylanase activity exhibited in SmF conditions of BPS-E1&E2 for varying incubation periods.

Identification of the xylanase isolates: Partial amplification of 16S rRNA gene of the xylanase positive isolates were conducted and the obtained sequence were deposited under the NCBI Gen Bank (Accession number's: Table 4). The Neighbour-joining phylogenetic tree generated helped reveal that the isolates belonged to three genera, out of which *Streptomyces* was the dominant genera, followed by *Saccharopolyspora* and *Nocardiopsis* which were clustered separately (Figure 1C). The transition and transversion ratio was 0.15. Isolate 20-23 that exhibited the highest xylanase production was identified as *Saccharopolyspora antimicrobica* of the accession no. MH482968.

Table 4. Identity and accession numbers of the selected xylanase producers

S. No.	Isolate No.	Accession No.	Identity and strain no.
1	20-23	MH482968	*Saccharopolysporaantimicrobica*DBT176
2	20-22	MH482969	*Saccharopolysporagregorii* DBT177
3	20-13	MH482971	*Streptomyces* sp. DBT179
4	20-10	MH482972	*Streptomyces* sp. DBT180
5	20-57	MH482975	*Streptomyces avermitilis* DBT183
6	20-19	MH482973	*Streptomyces albidoflavus* DBT181
7	20-05	MH482977	*Streptomyces* sp. DBT185
8	20-20	MH482979	*Nocardiopsisprasina* DBT187

Biomass utilization study: The compositional study proved the effectiveness of the alkali pre-treatment (BPS-D1) on the raw biomass (BPS-G109) substantiated with the reduction of the lignin (10% to 5%) as well as hemicellulose contents (30% to 25%) that was further corroborated with enhanced cellulosic content (45 to 60%). This pre-treated biomass subjected to SmF using *S. antimicrobica* (isolate 20-23) under its optimal growth conditions exhibited substantial xylanase activity from day 2 till day 14. The optimal xylanase activity was observed on the 6th day itself of 47.70 ± 0.31 U/ml (Figure 2D, Table 5). The effectiveness of this xylanase production from the BPS-E1&E2 was crosschecked to its hemicellulose content utilization, which revealed a total biomass loss of 40% and reduction in total hemicellulose to 2.32 ± 0.11% from 22.24 ± 0.77%(raw) after 14 days of treatment (Table 6). This utilization efficiency of BPS-E1and E2 and the effect it has on the physiochemical nature of the biomass was verified using the following analysis.

Table 5. Biomass utilization assay of SmF [**BPS-E1 and E2 (**BPS-D1+ *S. antimicrobica* 20-23)] and it's xylanase activity on various incubation periods.

Days of incubation	Xylanase Activity (U mL^{-1})
Day 1	1.90 ± 0.22
Day 2	18.20 ± 0.24
Day 4	35.92 ± 0.29
Day 6	47.70 ± 0.31
Day 8	40.92 ± 0.24
Day 10	38.19 ± 0.29
Day 12	10.45 ± 0.23
Day 14	9.35 ± 0.28

Table 6. Compositional analysis of biomass during various stages of treatments

Sample name	Moisture content(%)	Extractives (%)	Cellulose (%)	Hemicellulose (%)	Lignin (%)	Ash (%)	Reference
BPS-G109*	9.75 ± 0.10	14.74 ± 0.12	38.69 ± 0.23	22.24 ± 0.77	11.64 ± 0.49	2.95 ± 0.05	[10]
BPS-D1*	7.47 ± 0.14	12.3± 0.11	58.85 ± 0.13	18.32 ± 0.32	3.92 ± 0.81	0.25 ± 0.02	Present study
BPS-E1&E2*	6.95 ± 0.12	9.12 ± 0.12	77.35 ± 0.34	2.32 ± 0.11	3.90 ± 0.10	0.10 ± 0.02	Present study

***BPS-G109:** – Raw, ***BPS-D1:** - thermos-alkaline pre-treatment, ***BPS-E1 and E2**: - SmF of BPS-D1+ isolate 20-23

SEM, XRD, and FTIR analysis: The SEM analysis revealed clear distinction on the enhancement in the appearances of pores and channels due to the effect of BPS-E1 and E2 treatment on the pre-treated BPS-G109 (Figure 4).

The degree of crystallinity and changes in the crystal structure of cellulose was studied. All the samples showed significant intensity peaks related to their crystallinity at 2θ values of around 15.9° and 22.3°. The BPS-E1 and E2 showed additional peaks at 8.4°, 26.5°, 29.5°, and 54.7° and increased crystallinity of 83.84 % compared to control BPS-C (56.17 %), which indicated the increase in cellulose content by a reduction in hemicellulose and lignin hydrolysis (Figure 3A, 3B).

The chemical structural variations occurred during the BPS-E1 and E2 were documented using the FTIR spectral analysis in comparison to its untreated and alkali treated biomass samples. Small peak intensity variations were observed at 1160 cm^{-1} corresponding to the C-O-C stretching of hemicellulose as well as cellulose. Though in this study it has to be assumed the differences in readings might be due to hemicellulose hydrolysis. But the significantly almost comprehensive reduction in the peak was obtained from the spectral range of 1730 to 1738 (C=O conjugates in xylans stretching). This was in synchronization with the enhanced hemicellulose utilization and hydrolysis by SmF. The alkali pre-treatment effect on lignin reduction was also documented at 1595 cm^{-1}, and 2937 cm^{-1} which corresponds to the lignin-related bond stretches (Figure 3C).

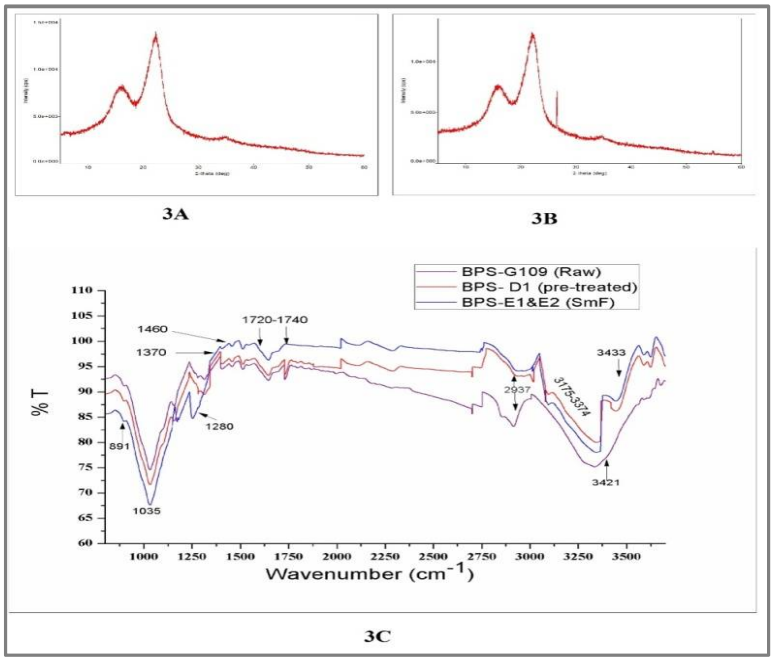


Figure 3. **3A:** XRD of raw control BPS-C; **3B:** XRD of BPS-E1&E2;
3C: FTIR spectra of BPS-G109 (raw), BPS-D1 (pre-treated) and BPS-E1 and E2 (SmF)

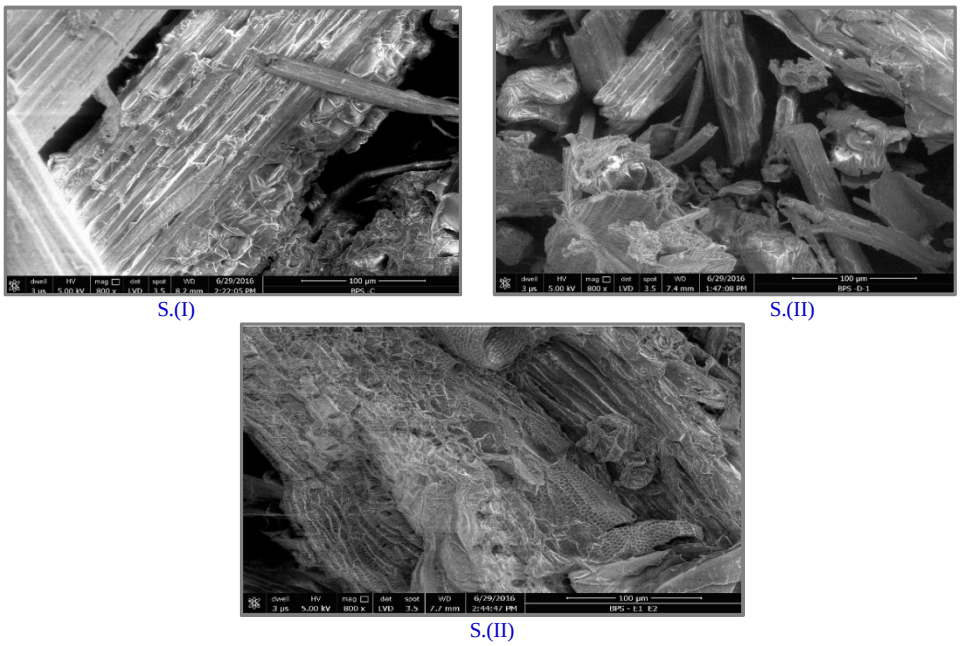


Figure 4. SEM images of **S.(I)** raw BPS-G109,**S.(II)** BPS D1 and **S.(III)** BPS-E1 and E2.

Various enrichment methods have been developed for obtaining enhanced lignocellulolytic inoculum post-sampling from the site of study [**28-30**]. Though on-site enrichment technique has not been explored extensively specifically for isolation of lignocellulose degrading microorganisms. [**16**], envisaged cellulosic traps of filter paper bits kept within steel tea strainers and placed within compost material (food and horse waste along with wood chips) for 1 week of incubation. They thus used such traps as an enrichment material and were successful in isolating thermophilic *Clostridium* bacteria capable of exhibiting high cellulase and low xylanase activities. [**11, 31**] used similar traps of lignin amended and cotton baits for verifying the effects of such enrichment techniques for enhancements in lignocellulose degrading bacteria. But in both these studies, the work was restricted to pyrosequencing or metagenomics analysis, for predicting biomass degrading enzymes or laccases genes presence or capabilities. With more than 50% of the actinobacteria recovered from the on-site enrichment exhibiting xylanase production (57%) capabilities and the lack of such on-site enrichment isolation technique specifically targeting hemicellulose degrading microorganisms, the present study envisages a stepping stone into this paradigm.

Actinobacterial xylanases are restricted to a few genera of *Thermobida*, *Streptomyces*, *Nocardiopsis*, *Actinomadura*, etc. [**4, 32**]. Xylanase from *Saccharopolyspora* has been limited to mostly *Saccharopolyspora pathumthaniensis* which has produced the enzyme in thermostable and slightly acidic conditions [**33**]. *S. antimicrobica* capability of xylan degradation was reported during its initial biochemical characterization by [**34**]. It is in this current work only, for the first time xylanase from *S. antimicrobica*20-23 has been quantified and applied for biomass utilization. Xylanase activity as high as 105 U mL^{-1} was reported by [**35**], from *Streptomyces cuspidosporus* under solid state fermentation of wheat bran (10% w/v) but under acidic (pH 5.5) conditions.

Hemicellulases that are more specific to the breakdown of hemicellulose entities have been explored recently, with the emphasis on particular substrate acting or hydrolytic enzymes like cellulase free xylanase [**36, 37**]. These type of xylanases which are additionally alkali tolerant finds industrial usages in the paper industry (bio-bleaching or pre-treatment of kraft pulp). Though thermo, as well as alkali tolerant xylanase, have been reported by *Thermobida fusca* which was over expressed in *Pichia pastoris* with the substantial activity of 165 U/mL [**38**], its applications on raw substrates and hydrolysis durability under such conditions are yet to be explored. The alkaline xylanase from BPS-M1 and M2 (47.7 ± 0.31 U mL^{-1})produced in this current study is comparable to that of alkaline xylanase recently reported by [**39**] *Bacillus licheniformis* Alk-1 that showed optimal xylanase activity at pH 9 of 26.4U mL^{-1} using beechwood xylan and 22.6 U/mL using wheat bran as major carbon sources respectively. Even though there are a few reports of alkaline xylanases, *S.antimicrobica* that are naturally devoid of cellulose reduction capabilities are hence ideal candidates for such enhanced cellulase free xylanase's.

Traditionally in case of pulpy biomasses; submerged fermentation (SmF) is a more preferred system than that of SSF, and hence bacterial cultures are better equipped than its fungal counterparts. From our previous studies, it's understood that perennial grasses that are renowned non-edible biomass, that encompasses 20~40% hemicellulose and lignin contents, are more efficiently hydrolyzed when it's treated in its pulpy condition [**10, 20**]. The thermos-alkaline pre-treatment (BPS-D1) that focused on retaining hemicellulose, enhancing the cellulose and reduction in lignin contents, was successful in reducing the lignin contents to just 33%, while lignin was as high as 90% in [**10**], were the biomass under study was similar. Though in [**10**] the majority of hemicellulose was intact, here slight reductions were observed, but it was better than that of [**20, 23**] that used alkaline treatments. Similarly, the cellulosic component enhancement of 20% in BPS-D2, which was 40% more in BPS-E1 and E2 hydrolysis than the raw sample was slightly better than the pre-treatment conducted by [**10**] and similar to that of [**23**] which proved that the reductions in hemicellulose and lignin were successful. This enhancement in cellulose was substantiated using the XRD analysis, were the higher crystallinity values implying solubilization of hemicelluloses and lignin together with less ordered cellulose the main reason for the increase in cellulose crystallinity [**40, 41**]. Even though the

loss in biomass of 40% by BPS-E1 and E2 was lesser (50 to 77%) in comparison to previously reported by [**10, 20, 42**] were biomass degradation of perennial grasses and post-harvest residues, the SEM analysis indicated the surface topology effect exhibited by the treatment in the current study. The expansion on the channels of the biomass that resulted in increased gaps in SEM imaging of BPS-E1 and E2 compared to its raw sample was similarly reported by [**39, 43**].

FTIR spectra readings are a useful tool in identifying the scale of changes among the chemical functional groups and are widely used in biomass conversion studies of wood and pulp materials [**10, 44**]. Thermo-alkaline pre-treatment of BPS-D1 that was responsible for the lignin reduction which was as characteristically shown by its lack of peaks at 1249 cm^{-1} and 1595 cm^{-1} in comparison to its raw (BPS-G109) and comparable to that of findings [**23**] on *Thysanolaena maxima*. The peak reductions in that of hemicellulose in both BPS-D1 and BPS-E1 and E2 especially at 1160, 1370 and 1730–1738 cm^{-1}were similarly observed in studies of [**39**] by the action of alkaline xylanase on eucalyptus kraft pulp. It was substantially differed from triculture system application on *N. reynaudiana* by [**10**]. This difference in peaks from triculture study indicated the enhanced capability of hemicellulose utilization in BPS-E1 and E2 system.

APPLICATION

The OSE technique model will find extensive usage in effective isolation of specific lignocellulose degrading microbes. Additionally, the alkaline tolerant xylanase from *S. antimicrobia* 20-23, will find tremendous applicability in biomass pre-treatment and pulp processing.

CONCLUSION

The on-site enrichment (OSE) technique by the usage of cheaper and common hemicellulose materials devised for isolating actinobacterial strains with xylanase activities was successful in isolating 8 potent strains. This study shed light into the potential of *N. reynaudiana* as a major cellulosic source which was previously hindered by its hemicellulose and lignin contents. The fact that *S.antimicrobia* 20-23 has successfully produced xylanase of superior activity for a prolonged period within alkaline conditions of pH 8, utilizing the hemicellulose entities of BPS-G109 makes this strain very significant and efficient.

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