DOI : [http://doi.org/10.22438/jeb/42/4\(SI\)/MRN-1538a](http://doi.org/10.22438/jeb/42/4(SI)/MRN-1538a)

Probing cellulolytic yeast from forest ecosystem for the saccharification of Napier fodder biomass

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Received: 24.12.2020

Revised: 24.02.2021

Accepted: 23.03.2021

Abstract

Aim: This study aimed to search for novel cellulolytic isolates with high cellulase titre for the production of fuels and chemicals.

Methodology: The yeast isolate YES5 isolated from the forest soil was screened for cellulase production. The cellulase activity of YES5 was optimized via RSM. The saccharification potential of YES5 using Napier biomass as substrate was evaluated.

Results: The maximum cellulase activity obtained after optimizing pH, temperature, and incubation period was 35.70 U. A reliable statistical model was developed for maximizing the cellulase activity in YES5 *Trichosporon asahii*. The cellulase activity was 23.87U, when carbon source in CMC medium was replaced by Napier biomass. The maximum saccharification potential of 33.15% was observed on 3rd day.

Interpretation: The study of optimizing the media composition of *Trichosporon asahii* cellulase using Napier biomass, a natural source of carbon for maximizing the cellulase production via RSM, is first of its kind.

Key words: Cellulase, Napier biomass, Saccharification, *Trichosporon asahii*

How to cite : Valliammai, M.G., N.O. Gopal and R. Anandham: Probing cellulolytic yeast from forest ecosystem for the saccharification of Napier fodder biomass. *J. Environ. Biol.*, **42**, 1152-1161 (2021).

Introduction

As the world population is increasing at an alarming rate, the energy consumption ratio is also rising. Forests and people are tightly coupled in this country (Baboo *et al.*, 2017). Studies have reported that millions of people reside within or close to forests and harvest forest products (Davidar *et al.*, 2010; Gosain *et al.*, 2015; Bargali and Bargali 2016). The activities occurring in the forests include exploitation through commercial logging, seasonally set forest fires, fuel wood removal, charcoal production, cattle grazing, pruning and land clearing for agricultural activities (Bhuyan *et al.*, 2003; Bargali *et al.*, 2019). These disturbances have changed the forest composition, structure and reduced species diversity as well as resources by restricting size of forest patches (Sagar and Singh 2005; Karki *et al.*, 2017). Continued increase in human population together with livestock populations, the pressure on these forests has increased tremendously and consequently resulting in reduced carrying capacity of these forests (Bargali *et al.*, 2018; Manral *et al.*, 2020). The increasing population and reduced resources have increased the gap between demand and supply of ecosystem services (Bargali *et al.*, 1992a, b; 1993; Gosain *et al.*, 2015).

Nonrenewable resources may not be sufficient to meet out the energy requirement of the society so there is a swift in the utilization of resources from nonrenewable to renewable for the production of value added compounds (Gaurav *et al.*, 2017; Valliammai *et al.*, 2021). The environment concerning factors which have paved the path for the production of microbial cellulases are global warming and greenhouse effects (Vanhala *et al.*, 2016). In the current era, microbes play a key role in biomass conversion (Shariq and Sohail, 2018). The effective roles contributed by microbes in bio refinery concept are pretreatment, hydrolysis, detoxification and fermentation. The performance of microbes varies according to the desired yield (Dien *et al.*, 2003). Application of microbes or microbial enzymes for the pretreatment of lignocellulosic biomass is gaining momentum in the industry. Cellulases are inducible enzymes which can be produced by microbes during their growth on cellulosic material (Lee and Koom, 2001). Structurally fungal cellulases are simpler than bacterial cellulase systems (Artzi *et al.*, 2015).

Different kinds of microbes involved in the production of cellulase are aerobic, anaerobic, mesophilic or thermophilic. Among them, *Clostridium*, *Cellulomonas*, *Thermomonospora*, *Trichoderma* and *Aspergillus*. They are the most extensively studied cellulase producers (Sukumaran *et al.*, 2005). *Trichosporon asahii* belongs to genus *Trichosporon*, a genus of anamorphic fungi of family *Trichosporonaceae*. Limited studies are available with regard to cellulolytic potential of *Trichosporon asahii*. The predominant yeast species found in waste biomass conversion to bioproducts reported so far are *Candida tropicalis*, *Candida intermedia*, *Candida parapsilosis*, *Pichia guilliermondii*, *Saccharomyces cerevisiae* and *Trichosporon asahii* (Schwan *et*

al., 2007). Yeasts like *Candida tropicalis* are involved in saccharification of rice straw, corn-cob (Guo *et al.*, 2013). Exploring the nature of cellulase activity of *Trichosporon asahii* in Napier (*Pennisetum purpureum*) fodder biomass conversion into ethanol is first of its kind. Cellulase activity can be observed in diverse group of microbes while utilizing cellulosic materials (Lu *et al.*, 2010). Cellulase activity in microbes can be improved by nutritional and process parameters during fermentation (Rasul *et al.*, 2015). Response surface methodology is an efficient technique utilized for optimization of fermentation processes (Valliammai *et al.*, 2020). Mathematical models are proven tools in the optimisation of process parameters during fermentation (Imamoglu and Sukan, 2013). Napier fodder (*Pennisetum purpureum*) is a fast growing perennial crop widely observed in tropical and subtropical regions of the world. It has a higher yield per unit area and the biomass productivity is also commendable (Woodard and Prine, 1993). High cellulose content in Napier fodder makes it a promising substrate for the biobased economy. In this study, yeast YES5 *Trichosporon asahii* was isolated from the forest soil sample. The cellulase activity of YES5 using Napier biomass as carbon source was optimized via RSM. The study of standardising the cellulase activity of YES5 for the Napier biomass saccharification is a novel approach.

Materials and Methods

Isolation and screening of cellulolytic yeasts from forest ecosystem:

Forest soil samples were collected from the Eastern Ghats region (N 10. 29 363°) (E 77. 70 674°) of Thandikudi, Tamil Nadu, India. Isolation was initiated via serial dilution technique followed by pour plating. Ten gram of soil sample was suspended in 99 ml of water and used for isolation of cellulolytic yeast using carboxymethyl cellulose agar medium and incubated at 28°C for 2-3 days (Hendricks *et al.*, 1995). The appeared colonies were purified and then screened for cellulase activity via congo-red agar medium (Hankin and Anagnostakis, 1977). Among different yeast isolates, YES5 showed maximum cellulase activity and, hence, was selected for further study.

Identification of cellulolytic yeast isolate YES5:

Cellulolytic yeast isolate was cultivated in CMC agar medium, and DNA was extracted (Harju *et al.*, 2004). Nearly-full length 18S rRNA gene was amplified from the genomic DNA of YES5 isolate using a forward primer as NL-1 (5' GCA TAT CAA TAA GCG GAG GAA AAG 3') and reverse primer as NL-4 (5' GGT CCG TGT TTC AAA GAC GG 3') primers. The PCR product was sequenced through ABI prism terminator cycle sequencing ready reaction kit, and electrophoresis of the products were carried out on an Applied Biosystems (Model 3100) automated sequencer (M/s. Yaazh Genomics, Coimbatore, India). Isolate YES5 exhibited 96.96 % similarity to *Trichosporon asahii* MN368077. The 18S rRNA gene sequence was submitted to NCBI with accession number MK640632.

Cellulase assay: *Trichosporon asahii* YES5 was cultivated in CMC broth at 28°C for 2 days, and the culture supernatant was collected by centrifuging at 13000 rpm for 8 min. A 200 µl of culture supernatant was mixed with 200 µl of 1% CMC solution and incubated at 50°C for 30 min. DNS reagent was added and incubated at 100°C for 10 min. The absorbance was read at 575 nm against reagent blank. In this study, one unit of enzyme activity refers to the release of 1 µmol of reducing sugar per min per ml (Wood and Bhat, 1988).

Effect of carbon source in cellulase activity: The influence of different carbon sources was determined by substituting the carbon sources viz., fructose, galactose, sucrose, lactose, starch, and CMC individually in place of carbon source in the medium. About 5 µl of 24-hr-old culture (7×10^8 CFU ml⁻¹) was inoculated in 100 ml medium supplemented with different carbon sources and incubated at 28°C for 24 hr. The culture supernatant from each source was collected and assayed for cellulase activity (Wood and Bhat, 1988).

Effect of pH and temperature on cellulase activity: Cellulase activity was determined at different incubation period, temperature and initial pH. Five microliter of 24-hr-old culture (7×10^8 CFU ml⁻¹) of YES5 *Trichosporon asahii* was inoculated into CMC broth and incubated at 28°C for different time period (5 days). The day at which maximum cellulase activity observed was considered for further optimizing the pH range (4.5 to 8.5 with an increment of 1.0) and temperature range (25°C to 40°C with an increment of 5°C).

Statistical optimization of cellulase activity using Napier biomass as a carbon source: For maximizing cellulase production, RSM was employed with CCD. The selected parameters and their range are provided in Table 1. A total of 78 experiments were performed with different combinations (Table 2). Cellulase assay was performed as described earlier. The response value (Y) in each trial is the average of duplicates. The design expert trial version 12 was used for the development of design matrix and statistical analysis of the data. Regression analysis was performed to estimate the response function as a second-order polynomial (Wang et al., 2011):

$$Y = \beta_0 + \beta_i X_i + \beta_{ij} X_i X_j + \beta_{ii} X_i^2$$

Where, Y is the predicted response, β_0 is the intercept term, β_i is the linear coefficient, β_{ij} is the quadratic coefficient and

β_{ii} is the interaction coefficient. Analysis of variance (ANOVA) was adopted to know the significance of the model. The quality of polynomial model equation was judged statistically through coefficient of determination (R^2) and adjusted R^2 . Overall significance was evaluated through Fishers test. Three-dimensional plots illustrated the response for each interaction. In order to validate the model predicted by the software, experiments were performed in shake-flasks. Physical parameters were kept at optimum levels. Cellulase assay was performed as described earlier.

Compositional analysis of Napier biomass: The compositional analysis of Napier biomass was determined as per the standard protocols of NREL, 2004 (Sluiter et al., 2012).

Saccharification of Napier biomass: The leftover Napier fodder in the field was collected and dried in a tunnel drier to get a brittle texture. The dried powder was sieved through a 50µ sieve. One gram of powder was added to 100ml of medium containing optimized levels of nitrogen source and mineral salts (1g K₂HPO₄, 0.5g MgSO₄, 0.5g NaCl, 1.0g peptone, 0.25g (NH₄)₂SO₄) and autoclaved at 110°C, 15lbs pressure for 20 min. 5 µl of 24 h old culture (7×10^8 CFU ml⁻¹) *Trichosporon asahii* was inoculated and allowed for saccharification at 30°C and 120 rpm for 5 days. Samples were taken at an interval of 24 hr to monitor sugar release. The experiments were performed in triplicates. The percentage of saccharification was noted as per the standard protocols of NREL, 2004 (Uma et al., 2010).

Results and Discussion

The yeast isolate YES5 was creamy, glossy colonies with a cell morphology of globose shape present singly, multilateral budding with ascospores. The isolate YES5 was identified based on 18S rRNA sequencing as *Trichosporon asahii*. Certain yeast strains like *Pichia stipitis*, *Saccharomyces cerevisiae*, and *Kluyveromyces fragilis* were reported as good ethanol producers from different types of sugars (Mussatto et al., 2012). Though there are many cellulolytic organisms, researchers are still exploring novel microbes for biomass conversion into value-added products. A clear zone of hydrolysis was observed around the colony of isolate YES5 suggesting cellulose hydrolyzing nature of YES5. (Fig. 1).

The carbon sources used in the assay were fructose, galactose, sucrose, lactose, starch, and CMC. The YES5 yeast

Table 1: Factors employed in the RSM experiment for optimum cellulase activity

Factors	+ alpha	- alpha	Low value	High value
Napier biomass	2.174	17.825	5	15
Peptone	2.174	17.825	5	15
NaCl	0.043	0.356	0.1	0.3
MgSO ₄	0.065	0.534	0.15	0.45
(NH ₄) ₂ SO ₄	0.043	0.356	0.1	0.3

Table 2: Optimization of nutrient components for maximum cellulase activity

Factor 1 A:Napier biomass	Factor 2 B:Peptone	Factor 3 C:Dipotassium hydrogen phosphate	Factor 4 D:Magnesium sulphate	Factor 5 E:Ammonium sulphate	Factor 6 F:Sodium chloride	Observed Cellulase activity (U ml ⁻¹)	Predicted Cellulase activity (U ml ⁻¹)
15	15	0.1	0.15	0.3	0.5	19.03	19.66
5	15	0.1	0.15	0.3	1.5	17.79	16.43
10	10	0.2	0.3	0.2	1	23.36	23.87
5	15	0.1	0.15	0.1	0.5	17.69	16.16
15	15	0.3	0.45	0.1	1.5	19.25	19.04
5	15	0.3	0.15	0.3	0.5	17.38	16.67
5	5	0.3	0.45	0.1	0.5	15.82	15.53
15	5	0.3	0.15	0.3	0.5	16.19	16.38
5	15	0.3	0.45	0.3	1.5	15.92	16.02
10	10	0.2	0.3	0.2	0.217458	23.87	23.55
10	10	0.2	0.3	0.2	1.78254	23.45	23.50
15	5	0.3	0.45	0.1	0.5	17.02	17.19
5	15	0.3	0.15	0.1	1.5	15.88	16.09
5	5	0.3	0.15	0.1	0.5	15.04	15.37
5	15	0.1	0.45	0.3	0.5	15.77	16.05
5	5	0.3	0.15	0.3	0.5	15.00	14.91
15	5	0.1	0.15	0.3	1.5	17.82	17.59
15	15	0.3	0.45	0.3	0.5	19.89	19.55
5	15	0.3	0.45	0.3	0.5	15.89	16.65
5	5	0.3	0.45	0.1	1.5	15.02	15.31
15	5	0.3	0.15	0.1	0.5	17.26	16.87
17.8254	10	0.2	0.3	0.2	1	18.27	18.27
5	5	0.1	0.45	0.3	0.5	15.05	14.67
5	15	0.3	0.45	0.1	0.5	17.21	16.56
10	10	0.2	0.0652373	0.2	1	22.18	22.22
5	5	0.1	0.15	0.3	0.5	15.05	15.07
15	5	0.3	0.15	0.3	1.5	16.87	16.88
10	10	0.2	0.3	0.0434915	1	23.45	22.84
15	5	0.1	0.15	0.1	0.5	17.01	17.18
15	15	0.1	0.15	0.1	1.5	18.94	19.35
10	2.17458	0.2	0.3	0.2	1	16.93	17.73
5	5	0.1	0.45	0.1	1.5	15.00	14.97
15	5	0.1	0.45	0.1	1.5	17.01	17.27
15	15	0.1	0.15	0.1	0.5	19.32	19.30
15	15	0.3	0.15	0.3	1.5	19.44	19.39
5	5	0.3	0.15	0.1	1.5	16.59	15.48
15	5	0.3	0.45	0.1	1.5	17.01	17.26
15	5	0.1	0.45	0.3	0.5	16.98	16.66
15	15	0.1	0.15	0.3	1.5	19.24	19.82
15	15	0.3	0.15	0.3	0.5	19.99	19.42
15	5	0.3	0.45	0.3	0.5	16.83	16.62
10	10	0.0434915	0.3	0.2	1	23.22	22.51
15	5	0.1	0.45	0.1	0.5	16.78	17.02
5	5	0.3	0.45	0.3	1.5	15.02	14.88
15	15	0.3	0.45	0.1	0.5	19.06	19.49
15	5	0.3	0.15	0.1	1.5	17.27	17.27
2.17458	10	0.2	0.3	0.2	1	14.58	14.31
5	5	0.1	0.45	0.1	0.5	15.02	14.99
10	10	0.2	0.3	0.2	1	23.43	23.87
5	15	0.3	0.45	0.1	1.5	16.22	15.82
5	15	0.3	0.15	0.3	1.5	16.11	16.36
15	15	0.1	0.45	0.3	0.5	19.05	19.31

Table continue

Table 2: Optimization of nutrient components for maximum cellulase activity

Factor 1 A:Napier biomass	Factor 2 B:Peptone	Factor 3 C:Dipotassium hydrogen	Factor 4 D:Magnesium sulphate	Factor 5 E:Ammonium sulphate	Factor 6 F:Sodium chloride	Observed Cellulase activity (U ml ⁻¹)	Predicted Cellulase activity (U ml ⁻¹)
15	5	0.1	0.45	0.3	1.5	16.92	17.02
15	15	0.3	0.45	0.3	1.5	19.04	19.20
10	17.8254	0.2	0.3	0.2	1	21.34	20.27
10	10	0.2	0.534763	0.2	1	22.12	21.81
5	15	0.3	0.15	0.1	0.5	15.17	16.50
5	5	0.1	0.45	0.3	1.5	15.02	14.75
5	15	0.1	0.45	0.3	1.5	15.08	15.60
15	15	0.1	0.45	0.3	1.5	19.67	19.14
5	5	0.1	0.15	0.1	1.5	15.06	15.62
15	15	0.3	0.15	0.1	1.5	19.02	19.15
15	5	0.1	0.15	0.3	0.5	16.94	16.91
5	5	0.1	0.15	0.3	1.5	15.04	15.48
5	5	0.3	0.45	0.3	0.5	15.08	14.99
15	15	0.1	0.45	0.1	0.5	19.03	19.03
15	5	0.1	0.15	0.1	1.5	18.68	17.77
5	5	0.3	0.15	0.3	1.5	15.09	15.12
5	5	0.1	0.15	0.1	0.5	15.02	15.32
10	10	0.2	0.3	0.356508	1	22.43	22.77
5	15	0.1	0.45	0.1	1.5	15.05	15.18
15	15	0.1	0.45	0.1	1.5	18.85	18.76
15	5	0.3	0.45	0.3	1.5	16.75	16.79
5	15	0.1	0.45	0.1	0.5	15.10	15.73
10	10	0.356508	0.3	0.2	1	22.16	22.60
5	15	0.1	0.15	0.3	0.5	16.01	16.55
15	15	0.3	0.15	0.1	0.5	19.28	19.28
5	15	0.1	0.15	0.1	1.5	15.02	15.93

Table 3: Cellulolytic potential of *Trichosporon asahii* YES5 in synthetic and natural source of carbon

Constant parameters	Variable parameters	Range	Optimum	Maximum activity
Basal medium+ Incubation time+ Temperature+ pH	Synthetic carbon sources		CMC	21.23 U ml ⁻¹
Basal medium+ CMC+ Temperature+ pH	Incubation time	1-5 days	3 rd day	29.90 U ml ⁻¹
Basal medium+ CMC+ Optimum Incubation Time+ pH	Temperature	25-40°C	30°C	35.70 U ml ⁻¹
Basal medium+ CMC+ Optimum Incubation Time+ Optimum temperature	pH	4.5-8.5	6.5	34.49 U ml ⁻¹
Optimum Incubation Time+ Optimum Temperature+	Napier biomass	2.174-17.825 g l ⁻¹	10 g l ⁻¹	
Optimum pH	Peptone	2.174-17.825 g l ⁻¹	10 g l ⁻¹	23.87
	NaCl	0.043-0.356 g l ⁻¹	0.2 g l ⁻¹	U ml ⁻¹
	MgSO ₄	0.065-0.534 g l ⁻¹	0.3 g l ⁻¹	
	(NH ₄) ₂ SO ₄	0.043-0.356 g l ⁻¹	0.2 g l ⁻¹	
	K ₂ HPO ₄	0.2174- 1.7825 g l ⁻¹	0.217 g l ⁻¹	

isolate showed a positive response towards all the tested carbon sources, however, the impact of CMC as a source of carbon showed much higher cellulase activity compared to other carbon sources. The cellulase activity of YES5 isolate using CMC as a

substrate was 21.22 U. (Fig. 2). Lignocellulosic biomass acts as a source of carbon for the microorganisms during the fermentation process (Battaglia *et al.*, 2011). The most important carbon source in biomass is cellulose and is converted into value-added

products by the enzyme cellulase (Lu *et al.*, 2013; Valliammai *et al.*, 2021). Maximum cellulase activity was observed by optimizing the factors like incubation period, temperature, and pH. (Fig. 3A-C). The cellulase activity was observed throughout the incubation period; however, it was maximum on day 3 (29.90 U). Though cellulase activity was observed 25-45°C, the optimum temperature for obtaining maximum cellulase activity was 30°C (35.70 U). While increasing the temperature, cellulase activity decreased. Among the various pH tested, the optimum pH for cellulase activity was 6.5 (34.498 U). The cellulase activity was minimum at pH of 4.5. The factors influencing cellulase activity were carbon sources, pH, temperature, incubation period. This study highlights that CMC as a source of carbon was ideal for exhibiting maximum cellulase activity when compared with other carbon sources. CMC plays a significant positive impact on cellulase production by cellulolytic microbe (Deka *et al.*, 2011). The optimum pH for the production of cellulase by YES5 was 6.5. Cellulase activity is dependent on temperature and pH (Tai *et al.*, 2004). The pH of the medium has a strong influence on the enzymatic processes and the mobility of components across the cell membrane (Moon and Parulekar, 1991). The most popular design employed for optimization of cellulase production is Response Surface Methodologies (Queen *et al.*, 2002). RSM offers statistical predictions and actual observation (Singh and Kaur, 2012). For optimising the medium composition for cellulase activity, a natural source of carbon (powdered Napier biomass) was substituted in the place of synthetic carbon source. The combination which the maximum cellulase activity was observed using a natural source of carbon was observed (Table 2). The second-order polynomial equation for cellulase activity is as follows:

$$Y = +23.87 + 1.26 A + 0.8132 B + 0.0277 C - 0.1295 D - 0.0195 E - 0.0135 F + 0.3186 AB - 0.0902 AC + 0.0392 AD -$$

Fig. 1: *Trichosporon asahii* YES5 showing cellulolytic activity. The zone around YES5 colony indicates cellulose hydrolyzing nature.

0.0083AE+0.0705AF+0.0727BC-0.0255 BD+0.1589BE-0.1317BF+0.1208CD-0.0548 CE-0.0461CF-0.0192DE-0.0817DF+0.0258 EF -3.10A² -1.99 B² -0.5386 C² -0.7591 D² -0.4366 E² -0.1426 F²; where Y is the cellulase activity (U/ml) and A, B, C, D, E and F are the test variables. The ANOVA results convey that the model was highly significant and it was obvious with F-value as 48.06 and < 0.0001 p-value. The model showed insignificant lack of fit (19.14). The F, P and lack of fit values indicate that the conducted experiments were in good fit. The predicted R² 0.9046 was par with the Adjusted R² 0.9429. The R² value was 0.9629, so the model could explain 96 % of total variation. Pure error was 0.0025, which indicated the good reproducibility of the experiments. 3D plots were obtained, which conveyed the best combination of parameters where the cellulase activity was optimum (Fig. 4).

Fig. 2: Effect of carbon sources on cellulase activity of yeast isolate *Trichosporon asahii* YES5. The yeast isolate YES5 was cultured in different mono, di and polysaccharide sources and cellulase assay was done in triplicate. The values are depicted along with standard deviation.

Fig. 3: The effect of process parameters on cellulase assay of *Trichosporon asahii* YES5: (A) Incubation period, (B) Temperature and (C) pH. The assay values were done in triplicate and presented along with standard deviation.

The maximum cellulase activity predicted by the model was observed in the treatment holding the combination of 10g of Napier fodder biomass, 0.2 g K_2HPO_4 , 0.3g $MgSO_4$, 0.217g NaCl, 10g peptone, 0.20g of $(NH_4)_2SO_4$ in 1l of distilled water. The decrease in enzymatic activity with increasing incubation time might be due to depletion of nutrients and production of other inhibitors during the fermentation medium (Ikram-ul-Haq *et al.*, 2005). The optimized parameters were validated practically by shake-flask experiments. The observed cellulase activity for optimized media parameters was 23.87 U ml^{-1} , which is at par with the predicted cellulase activity of 23.55 U ml^{-1} . The results positively correlate that the Napier fodder biomass waste could be utilized by yeast YES5, as a good source of carbon for exhibiting maximum cellulase activity. Cellulase activity using a synthetic source of carbon was 35.70 U ml^{-1} at optimal parameters. The cellulase activity using a natural source of carbon (waste Napier biomass) was 23.87 U ml^{-1} at optimal parameters (Table 3). Statistical designs are efficient tool for enhancing cellulase production. This study focused on the optimization of medium components for obtaining maximum cellulase activity in *Trichosporon asahii*, while utilizing Napier biomass waste as a carbon source. The optimum cellulase activity of YES3 (*Candida tropicalis*) isolate, observed after optimizing factors such as pH, temperature and incubation period was 35.53 U (Valliammai *et al.*, 2020). RSM provided the individual and interactive effects of components in the medium during fermentation. Peptone, ammonium chloride, yeast extract were significant components for

cellulase production using *Bacillus tequilensis* (Sharma *et al.*, 2015).

Napier biomass was subjected to physico-chemical characterization. The cellulose content was maximum in Napier fodder biomass (51.34 %). The lignin and hemicellulose content of Napier biomass were 19.10 % and 16.70 %, respectively. YES5 was inoculated for the saccharification of Napier biomass waste, and its percentage of saccharification was noted at regular intervals. The samples were withdrawn at regular intervals for estimating the saccharification percentage. The maximum saccharification percentage (33.15%) was observed on 3rd day. Initially, the percentage of saccharification was low followed by a gradual increase, and later a declining trend was noticed (Fig. 5). Yeasts like *Pichia stipitis* was utilized in the saccharification study of sugarcane bagasse (Lau *et al.*, 2010). The saccharification potential of *Candida tropicalis* isolate YES3 in Napier grass biomass was maximum on 3rd day (31.93%) (Valliammai *et al.*, 2020).

In this study, a novel cellulolytic yeast YES5 isolated from the forest soil was molecularly characterized as *Trichosporon asahii* (Mk640632). A highly reliable RSM model was developed for maximizing the YES5 cellulase activity. This study contributes an active participant YES5 to the lignocellulosic degrading family which could be a boon for the biobased industries and for the production of green chemicals like ethanol.

Fig. 4: RSM 3 D plots show the effect of interactive and individual effects of parameters in cellulase activity of *Trichosporon asahii* YES5. (A) Effect of Napier biomass and peptone, (B) Effect of Napier biomass and dipotassium hydrogen phosphate, (C) Effect of Napier biomass and magnesium sulphate, (D) Effect of Napier biomass and Ammonium sulphate and (E) Effect of Napier biomass and sodium chloride.

Fig. 5: Saccharification of Napier fodder biomass by *Trichosporon asahii* YES5. YES5 (*Trichosporon asahii*) was inoculated into Napier biomass and saccharification potential was observed for 5 days.

Acknowledgement

The first Author, Meyyappan Geetha Valliammai, being an INSPIRE fellow [IF170519], is grateful to DST, INSPIRE Fellowship for the financial support.

Add-on Information

Authors' contribution: M.G. Valliammai: Performed scientific experiments and drafted the manuscript; N.O. Gopal: Planned the scientific work. He guided Meyyappan Geetha Valliammai in performing the experiments; R. Anandham: Suggested ideas for performing the scientific experiment.

Research content: The research content of manuscript is original and has not been published elsewhere.

Ethical approval: Not Applicable

Conflict of interest: The authors declare that there is no conflict of interest.

Data from other sources: Not Applicable

Consent to publish: All authors agree to publish the paper in *Journal of Environmental Biology*.

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