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Determination of bioethanol potential from banana waste using indigenous yeast (*Saccharomyces cerevisiae.* KX033583)

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Abstract
In present study was aimed to utilize banana wastes residues (Banana peel, Banana pseudo stem and Spoiled banana) for the production of bioethanol by using potential indigenous ethanol genic yeast isolated *Saccharomyces cerevisiae* (KX033583) derived from spoiled banana. A totally 10 yeast strains (*SB*1, *SB*2 - *SB*10) isolated from spoiled banana. The 10 yeast strains with different colony morphology were selected and subjected to identification studies based on morphological and cultural characteristics. Among the 10 isolates screened for some attributes essential for ethanol production, the yeast strains *SB*10 recorded to possess all the attributes like fermentative ability, ethanol tolerance, and osmotolerance, flocculating ability, invertase activity and thermotolerance. The yeast isolates *SB*10 identified to species level by using *rRNA* sequencing studies and confirmed as *Saccharomyces cerevisiae* (KX033583). The *SB*10 employed in pretreated banana wastes materials for bioethanol production. Among the 3 substrates tested spoiled banana wastes showed better yield 23.42 ± 0.18gl^{-1} this was followed by banana peel (17.00 ± 0.07gl^{-1}) and banana pseudo stem (15.61 ± 0.07g/l^{-1}). The parameter optima are important in any type of fermentation. Here an attempt was made to optimize different parameters like inoculums size, pH, and temperature and nitrogen source. The highest yield of ethanol production studied by inoculums level at 5%, pH is 6, temperature at 35ºC and Ammonium sulphate incorporate lead to rise in ethanol production.

Keywords: Banana waste, *Saccharomyces cerevisiae*, fermentation, ethanol production

Introduction
Global warming, urban pollution, oil reserves depletion and high cost of fossil fuel, have been the driving forces for current research on the use of alternative energy sources, particularly those deriving from biomass. Biofuel can be either solid, liquid or gas fuel made from relatively recently dead biological material but the most common sources of biofuels are photosynthetic plants [14]. Sustainable biofuels are essential to ensure a constant, secure supply of energy for individuals and industry.

The conversion of corn and other food-feed crops into ethanol by fermentation is a well-known and established technology. The United States and other countries desperately need a liquid fuel replacement for fossil oil in the future. The use of oil was projected to peak about 2007 and the supply is then projected to be extremely limited in 40-50 years [37]. Alternative liquid fuels from various sources have been sought for many years and since the cost of raw materials which can account up to 50% of the total production cost is one of the most significant factors affecting the economy of alcohol, nowadays efforts are more concentrated on using cheap and abundant raw materials [8]. Several forms of biomass resources exist (starch or sugar crops, weeds, oils plants, agricultural, forestry and municipal wastes) but of all biomass cellulosic resources represent the most abundant global source [36, 21, 22, 3]. Nevertheless, in recent years, much attention has been directed toward bio surfactants owing to their advantages such as low toxicity, high biodegradability, better environmental compatibility, high foaming capability, higher selectivity, specific activity at extreme temperature, pH, salinity etc.,

Ethanol production from biomass can be summarized briefly into following steps: depolymerization of holocellulose polymer into monomeric fermentable substrate, fermentation of depolymerized substrate, and the distillation of the fermentation broth to obtain dehydrate ethanol. The choice of the best technology for lignocelluloses to bioethanol conversion should be decided on the basis of overall economics (lowest cost), environmental (lowest pollutants) and energy (higher efficiencies) that are comprehensive process development and optimization are still required to make the process economically viable [24].

Banana is one of major constitute the principal food resources in the world and occupy the fourth world rank of the most significant foodstuffs after rice, corn and milk [19]. Most of the fruit peels/residues are dried, ground, pelletized, and sold to the feed manufacturers at a low price which is not considered a highly viable proposition [30]. The banana fruit and its associated residual biomass can be converted into glucose which can be used as feedstock to produce ethanol by fermentation and distillation. Since banana peels contain lignin in low quantities [12], it could serve as a good substrate for production of value-added products like ethanol. Banana pseudostem contains good amount of cellulose and starch and can be used as cattle feed [23].

The utilization of mixture (skin and pulp) of rotten fruit was more suitable for bioethanol production as renewable energy which could reduce the cost of the initial process. Banana waste that have been discarded due to the imperfections are normally dumped as a huge masses of wastes, which ultimately cause contamination of water source as well as can affect the environment and health of living microorganisms. Thus, to avoid the environmental problem due to the decomposition of waste, it is usable to make energy from banana waste as biofuel production source [15]. Hence the present study was undertaken to explore the possibility of using different banana waste as a raw material to produce bioethanol. The present study was designed with the collection and pretreatment of the banana waste samples, isolation and selection of ethanol genic yeast from banana waste sample, fermentation of bioethanol from pretreated banana waste by employing potent yeast isolate and optimization of the fermentation process to increase the yield of bioethanol production.

Materials and Methods

Collection of Samples

The banana wastes such as pseudo stem, peel and spoiled banana were used in the present study. The banana wastes (Spoiled banana and Banana peel) were collected from wholesale fruit market, whereas a Banana pseudo stem was obtained directly from a field around Vallampadugai village, Cuddalore District Tamil Nadu, India.

Processing of samples

The waste materials (Banana pseudo stem and peel) were washed, cut into small pieces using knife and sun-dried for several days. Then the dried substrates were finally powdered using electric grinder and sieved through 56 µm mesh sieve. These samples were used throughout the study. The spoiled banana samples were blended along with water to in a mixer grinder. The resulting juice was filtered through sterile muslin cloth and used for further studies.

Isolation of ethanol genic yeast strains from banana waste samples

The spoiled banana fruits were collected from the local fruits markets around Chidambaram, Tamilnadu. The spoiled fruits along with peels were washed with sterile distilled water, and blended using electric blender. The resulting juice was filtered using sterile muslin cloth and transferred to sterile conical flask. In order to enrich the yeast population, aliquots of samples were transferred to liquid media such as Glucose-Peptone-Yeast extract (*GPY*) broth along with Chloramphenicol and incubated at 28 ± 30ºC for 24 to 48 hrs. After incubation, 0.1ml of the diluted sample was spread plated onto *GPY* agar with a pH of 4.5 and incubated at 28±30˚C for 24 to 48 hrs. Morphologically distinct colonies were selected, purified by streaking and stored in Yeast extract-Malt extract Agar *(YM)* slants for further characterization studies.

Identification of potential yeast isolate by 18s rRNA gene sequencing

The potential yeast strain for alcohol production was selected by testing various attributes essential for ethanol production. For sequencing studies the yeast culture was submitted to Macrogen, Inc. Seoul Korea. The strain *SB*10 was further subjected to molecular characterization mainly by 18S *rRNA* gene sequencing. The template *DNA* was prepared by picking the colonies with sterile toothpick, and the colony was suspended in 1.5 ml centrifuge tube containing 0.5 ml of sterile saline. It was centrifuged at 10,000 rpm for 10, min. after removal of supernatant, the pellet was suspended in 0.5 ml of Insta Gene Matrix (Bio-Rad, *USA*) and incubated at 56ºC for 30 min and then heated 100ºC for 10 min. After heating, supernatant was used for Polymerase Chain Reaction *(PCR)* studies. PCR was performed using 1µl of template *DNA* in 20µl of PCR reaction solution. The primers used were *NS*-1/*NS*-8 primers (NS-1 GTAGTCATATGCTTGTCTC and NS-8 TCGGCAGGTTCACCTACGGA) specific for eukaryotes, and then performed 35 amplification cycles at 94ºC for 45 sec, 49ºC for 60 sec, and 72ºC for 60 sec. *DNA* fragments are amplified about 1,400bp. Sequencing was performed using the same primers employing Big Dye terminator cycle sequencing kit (Applied Bio-Systems, USA). Sequencing products were resolved on an Applied Bio-Systems model 3730 *XL* automated *DNA* sequencing system.

Enzymatic hydrolysis

For the study of enzymatic hydrolysis, all the substrate *viz.*, Banana peel, pseudo stem and spoiled banana was taken in each 50 ml Erlenmeyer flask, pH adjusted and inoculated with fungal consortia. The culture obtained filtrate after 7 days of incubation contained the enzyme source. The crude enzyme extract (10 ml quantity) was added in a flask containing substrates, acetate buffer 0.1M, pH of 4.8, kept on a rotary shaker at a temperature of 50ºC for 72 hrs. The clear supernatant of the hydrolysate from different time intervals *viz.*, 0, 24, and 48 up to 72 hrs was taken for the estimation of reducing sugars.

Distillation of alcohol

About 250 ml of ferment wash was taken into a 500 ml clean distillation flask and mixed with 250 ml distilled water. Distillation flask was then kept on the heating mantle and connects the flask carefully to the condenser, switched on the mantle to boil the ferment wash and after stating of boiling carefully collected the distilled liquid is clearly washed conical flask. This distilled liquid was then used for alcohol estimation.

Effect of inoculum size on ethanol production

The cellulosic hydrolysate (100 ml, pH 5.0) was inoculated with 1%, 2%, 3%, 4% and 5% of inoculum levels of yeast culture (*Saccharomyces cerevisiae*) and kept for fermentation at 35 °C for 7 days and thereafter samples were analyzed for ethanol yield.

Statistical Analysis

The estimates, graphs were plotted with Microsoft Office Excel version 2010. The values reported are the means and standard deviations (Mean ± SD) of three replicates.

Results

The banana waste samples *viz.*, spoiled banana, peel, and pseudo stem were used as a substrate for ethanol production. The potential indigenous yeast strain *Saccharomyces cerevisiae* isolated from spoiled banana fruit samples was employed for fermentation studies. A total of 10 yeast strains with different colony morphology were isolated from spoiled banana samples. All strains recorded gram positive reaction, their morphology ranged from oval to budding yeast cells and the results for morphological and cultural characteristics of the different yeast strains are tabulated in the Table-1. For convenience, the yeast isolates have been designated as *SB1* to *SB10*. The yeast isolates were subjected to screening of various attributes which are essential for ethanol production. All the 10 isolates were assessed for their fermentative ability to ferment glucose with gas production. In the present study, all the yeast isolates fermented glucose with gas production. The isolates *SB5, SB7, SB9* and *SB10* produced high volume of gas in the Durham's tube than the other isolates. The isolate *SB*10 recorded high ethanol production from 5.0% glucose. Ethanol tolerance of the isolates were checked by incorporating yeast culture onto *YM* broth containing 5%, 7%, 9%, 11%, and 13% alcohol (*v/v*). All the 10 isolates showed good growth at 5% ethanol incorporated broth. As the concentration of ethanol increases the growth rate of the yeast isolates get reduced. The isolate *SB10* alone tolerated 11% ethanol, other isolates showed no growth at this concentration. All the isolates showed no growth in 13% ethanol supplemented broth. The osmotolerance of the yeast isolates was analyzed in the present study. For the test, different concentration of a glucose ranging from 5 to 25% was incorporated into *YM* broth. All the isolates exhibited better growth at 5 and 10% sugar concentrations. At high concentration (25%) of glucose, the strain SB10 recorded good growth which indicated their sugar tolerance. The invertase activity of the isolates is presented in the Figure-3. The invertase activity was calculated as the amount of reducing sugar released per minute. The invertase activity ranged from 3.46 to 56.8 µmole/min. The highest activity was recorded by the yeast isolate *SB10* (56.8 µmole/min), other isolates recorded less activity when compared with *SB10*.

The superior strain selected from the above studies *(SB10)* was sequenced and it was identified as *Saccharomyces cerevisiae* based on the phylogenetic analysis carried out using the Mega 6. The phylogenetic tree shows the coherence of the strain *SB10* with the phylogenetically related neighbor. The 18S *rRNA* gene sequence was further deposited in the Gen Bank and accession number was obtained (KX033583). Fig.5. the strain SB10 was identified as *Saccharomyces cerevisiae* based on the Basic Local Alignment Search Tool Nucleotide *(BLASTN)* search conducted in the National Center for Biotechnology Information *(NCBI).* Further, its phylogenetic position was correctly inferred by selecting phylogenetically nearest sequences, multiple sequence aligning and subjected to neighbor joining method of phylogenetic tree construction using the *MEGA 6* software. In the treeing programme *Candida* was used as out-group. The 18S *rRNA* gene sequence of *SB10* strain closely aligned with the already available and retrieved *Saccharomyces cerevisiae* sequences. The banana waste were inoculated with culture filtrate of different fungal culture increased the reducing sugar content than the control and the results obtained are presented in Table-6. In general, 10ml enzyme source with 72 hrs of incubation time was found to be better in the hydrolysis of banana wastes. Enzymatic hydrolysis released fermentable sugars to the level of 28.51 ± 0.10% from banana peel, 20.48 ± 0.14% from banana pseudo stem and 48.56 ± 2.38% from spoiled banana waste over an incubation of 72 hrs.

The ethanol produced from different pretreated banana wastes materials was estimated by colorimetric method and the results are presented in the Table-7. The potential ethanol genic yeast strain *Saccharomyces cerevisiae* (KX033583) obtained from banana waste was used as a fermentative organism. Among the 3 substrate tested, spoiled banana waste showed better yield *i.e*, 23.42 ± 0.18 gl^{-1}, this was followed by banana peel (17.00 ± 0.07 gl^{-1}) and banana pseudo stem (15.61 ± 0.07 gl^{-1}). The inoculum size of 1%, 2%, 3%, 4% and 5% levels prepared with *Saccharomyces cerevisiae* separately to inoculate the medium containing the hydrolysates of banana peel, banana pseudo stem, and spoiled banana. The inoculum size of 5% found to be more ideal for fermenting the hydrolysates Table 8. *Saccharomyces cerevisiae* at 5% inoculum size recorded ethanol yield of 19.46 ± 0.157 gl^{-1} in banana peel hydrolysate, 16.00 ± 0.179 gl^{-1} banana pseudo stem, and 23.56 ± 0.155 gl^{-1} in spoiled banana hydrolysate. The ethanol yield significantly influenced by different pH levels as indicated in Table-9. *Saccharomyces cerevisiae* preferred pH 6.0 for fermenting banana waste hydrolysates. The spoiled banana hydrolysate yielded ethanol of 24.46 ± 0.154 gl^{-1}, banana peel hydrolysate yielded ethanol of 19.82 ± 0.184 gl^{-1} and banana pseudo stem hydrolysate yielded 16.45 ± 0.145 gl^{-1} of ethanol with *Saccharomyces cerevisiae e*at pH 6. The ethanol values decreased after pH 7.0. The ethanol yield from enzyme hydrolysed waste substrates significantly varied between temperature levels as indicated in Table.10. The ethanol production from enzyme hydrolysates was studied at temperature level of 35ºC gave highest ethanol yield followed by 30˚C. The ethanol yield at 35ºC with *Saccharomyces cerevisiae* fermentation was 19.73 ± 0.066 g/l^{-1} in banana peel, 16.00 ± 0.165 g/l^{-1} in banana pseudo stem, 24.25 ± 0.260 gl^{-1} of ethanol from spoiled banana hydrolysates at 40ºC, a significant reduction in ethanol production was observed in all the substrates used. The effect of addition of nitrogen sources to fermentation media was studied and the results were tabulated Table 11. Among the nitrogen sources used, ammonium sulphate incorporation lead to increase in the ethanol yield from all the substrate used. Other nitrogen supplements have no impact on ethanol production.

Table 1: Morphological and Colonial characteristics of the yeast isolates obtained from Spoiled banana samples

S. No	Yeast strains	Colony characteristics				Morphological Characteristics	
		Colony nature	Margin	Colony colour	Optical Property	Cell shape	Gram Staining
1.	SB1	Smooth	Entire	White	Translucent	Oval	Gram positive
2.	SB2	Smooth	Entire	Creamy white	Not Transparent	Ellipsoidal	Gram positive
3.	SB3	Smooth	Entire	Light white	Transparent	Circular	Gram positive
4.	SB4	Rough	Circular	Creamy yellow	Opaque	Budding	Gram positive
5.	SB5	Smooth	Circular	White	Transparent	Budding	Gram positive
6.	SB6	Smooth	Circular	Creamy white	Opaque	Ellipsoidal	Gram positive
7.	SB7	Rough	Entire	Creamy white	Not Transparent	Circular	Gram positive
8.	SB8	Smooth	Entire	Creamy white	Transparent	Budding	Gram positive
9.	SB9	Smooth	Circular	Creamy white	Transparent	Budding	Gram positive
10.	SB10	Smooth	Entire	Creamy white	Not transparent	Budding	Gram positive

Table 2: Fermentative ability of the yeast isolates obtained from banana waste

S. No	Yeast strains	Gas production	Ethanol production (5% glucose)
1.	SB1	+	15
2.	SB2	++	10
3.	SB3	++	16
4.	SB4	+	8
5.	SB5	+++	18
6.	SB6	+	12
7.	SB7	+++	20
8.	SB8	+	16
9.	SB9	+++	13
10.	SB10	+++	22

+++= Good gas production, ++ = Moderate gas production, + = Poor gas production,

Table 3: Thermo tolerance of yeast strains

S. No	Yeast strains	Thermo tolerance				
		30˚C	35˚C	40˚C	45˚C	50˚C
1.	SB1	+++++	+++++	++	_	_
2.	SB2	+++++	+++++	++	_	_
3.	SB3	+++++	+++++	+	_	_
4.	SB4	+++++	+++++	++	_	_
5.	SB5	++++	+++++	++	_	_
6.	SB6	+++++	+++++	++	_	_
7.	SB7	+++++	+++++	+	_	_
8.	SB8	+++++	+++++	+	_	_
9.	SB9	+++++	+++++	_	_	_
10.	SB10	+++++	+++++	+++++	_	_

+++++=Good growth, ++++= Moderate growth, ++=Poor growth, -= No growth

Table 4: Ethanol production from pretreated banana wastes

S. No	Type of Substrates	Ethanol yield (gl^1)
		Enzymatic Hydrolysis
1.	Banana peel	17.00±0.07
2.	Banana pseudo stem	15.6±0.07
3.	Spoiled banana	23.4±0.18

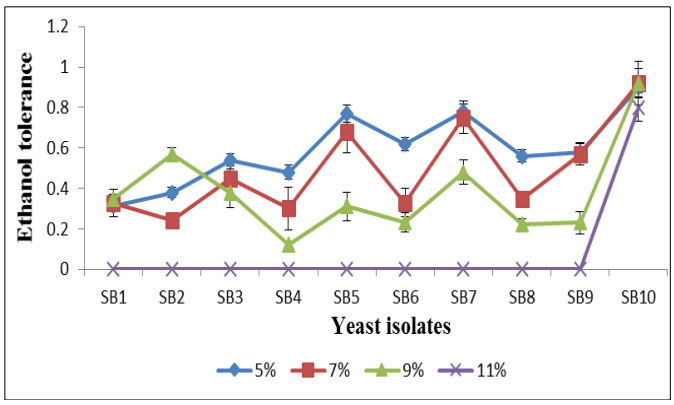

Fig 1: Ethanol tolerance of the yeast strains

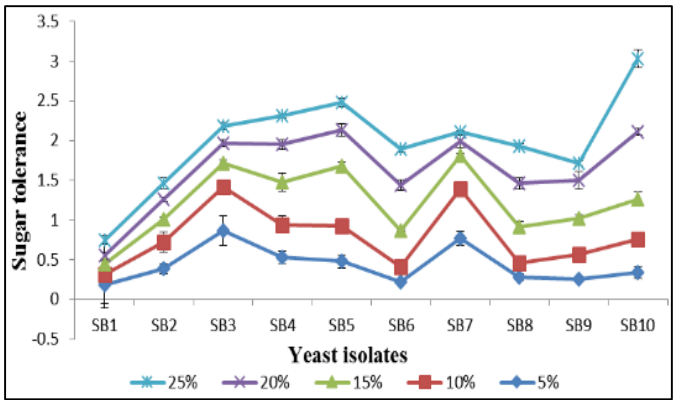

Fig 2: Osmotic tolerance of the yeast strains

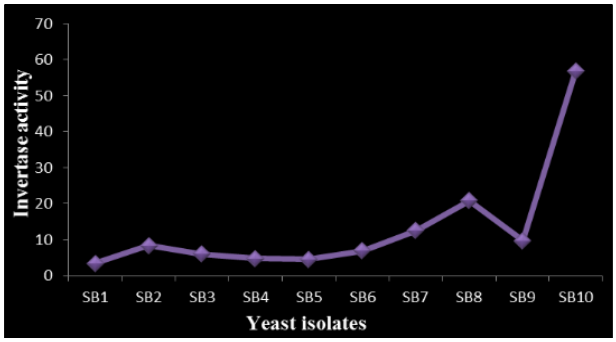

Fig 3: Invertase activity of the yeast isolates

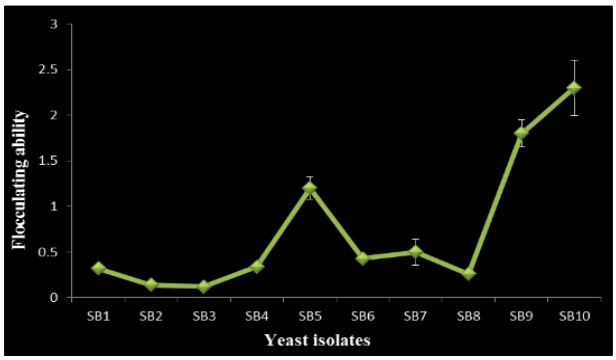

Fig 4: Flocculating ability of the yeast isolates obtained from spoiled banana samples

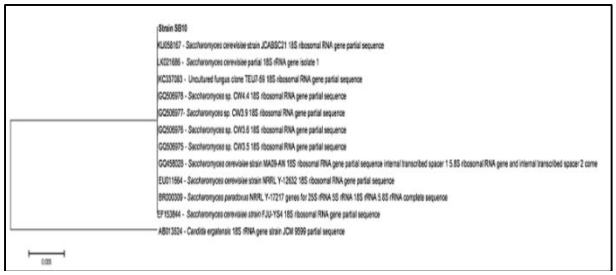

Fig 5: Phylogenetic tree for SB10 yeast strain

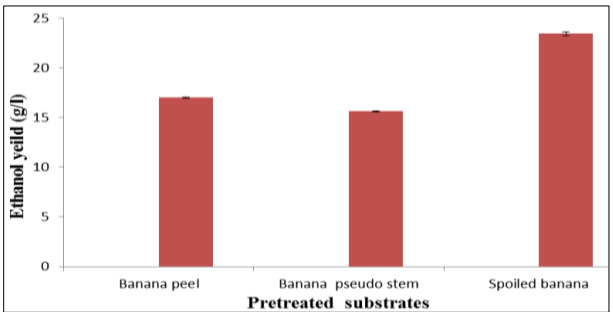

Fig 6: Enzymatic hydrolysis

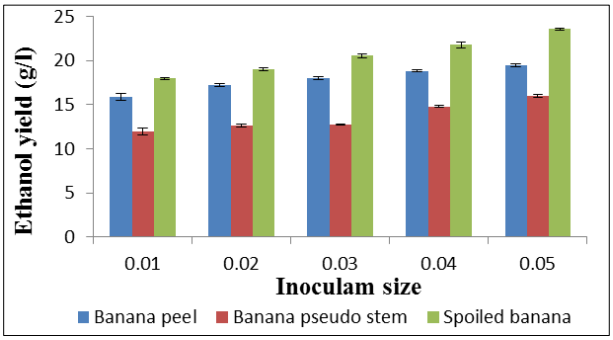

Fig 7: Effect of inoculum size on the ethanol yield from banana waste

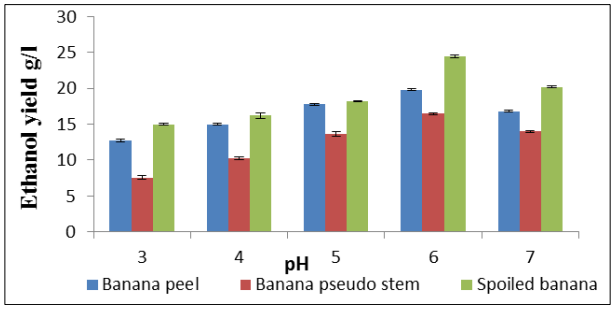

Fig 8: Effect of pH on the ethanol yield from Banana waste

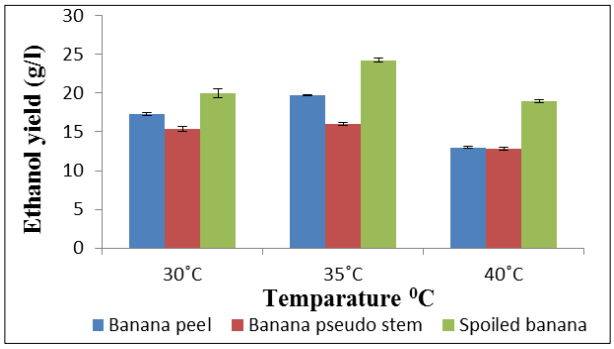

Fig 9: Effect of temperature on the ethanol yield from banana waste

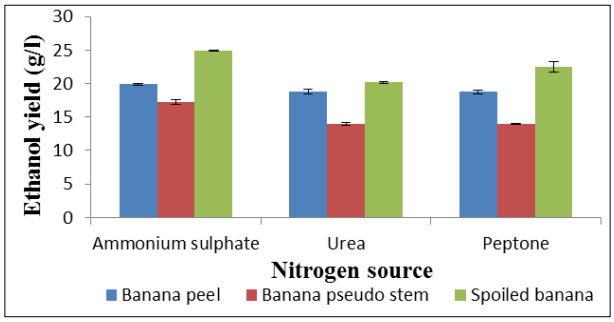

Fig 10: Effect of Nitrogen source on the ethanol yield from banana waste

Discussion

Ethanol is one of the important alcohols, derived from renewable biomass. It is widely used as a partial gasoline replacement in the U.S. Fuel ethanol that is produced from corn has been used in gasohol or oxygenated fuel since the 1980s. These gasoline fuels contain up to 10% ethanol by volume. As a result, the U.S. transportation sector now consumes about 4,540 million liters of ethanol annually, about 1% of the total consumption of gasoline. Recently, U.S. automobile manufacturers have announced plans to produce significant number of flexible-fueled vehicles that can use an ethanol blend- E85 (85% ethanol and 15% gasoline by volume) alone or in combination with gasoline. Using ethanol-blended fuel for automobiles can significantly reduce petroleum use and exhaust greenhouse gas emission [49]. Although different processes for ethanol production from sugar, starch or cellulose are feasible, production costs and energy consumption strongly depend on raw materials [32]. The current research on ethanol production is focused on reducing production costs, using alternative feedstock and increasing energy efficiency by means of energy integration of the plant processes.

The present study was mainly focused on banana waste materials as potential substrate for the production of bioethanol using native yeast isolate. The banana fruit and its organic residues are feedstock that can be used to produce

ethanol through hydrolysis, fermentation and distillation. Through these processes, agricultural waste can be used to produce ethanol and reduce environmental concerns [48]. Increased yield of ethanol production depends on the use of ideal microbial strain, substrate and best process technology [5]. The yeast strain employed for ethanol production should possess some of the essential attributes like sugar, ethanol tolerance, flocculating ability, fermentative capability, invertase activity and thermo tolerance. Many researchers have isolated ethanol-producing yeast strains from various sources. [5] Isolated 8 strains from ripe banana peels, subsequently assessed for some fermentation attributes such as ethanol producing ability, ethanol tolerance, flocculence, thermal and sugar tolerance. Among the 8 yeast isolates, 5 yeast strains were selected as potential strains for ethanol production.

In our present study, about 10 indigenous yeast strains were isolated from spoiled banana fruit. The cultures were identified as yeast based on colony characteristics and microscopic examination. Based on the results obtained from morphological and cultural characteristics the strains were designated as *SB*1 to *SB*10 for convenience. All the yeast isolates were screened for some essential attributes in order to select the potential ethanol genic yeast strain. The result of the fermentative ability of the test isolates revealed 4 isolates *viz.*, *SB*5, *SB*7, *SB*9, and *SB*10 showed better ethanol production with gas production by fermenting glucose. The osmotolerance of the yeast isolates were also analyzed in the present study. High concentration of sugar increases osmotic pressure, only osmotolerance yeast can survive. High osmotic pressure is associated with increased stress on the organism, ultimately results in low productivity of ethanol. Herein, 5 yeast isolates showed tolerance to high sugar concentration (25%). The study conducted by [46] demonstrated that about 31 yeast isolates obtained from rotten banana were able to withstand the 25% sugar concentration.

Generally, ethanol is toxic to microorganism which inhibits the growth of organism. It damages mitochondrial *DNA* in yeast cells and causes inactivation of enzymes [47]. The ethanol tolerance is an important aspect in ethanol production, because the yeast should tolerate high concentration of ethanol. In the present study, the yeast isolates obtained from spoiled banana fruits were analyzed for ethanol tolerance. Out of 10 isolates tested, 5 isolates showed appreciable ethanol tolerance against different concentration of ethanol tested. The yeast strain SB10 tolerated up to 11% ethanol. [45] Reported that the indigenous yeast isolate (*BRM* 17) obtained from banana, showed maximum ethanol tolerance upto 14%. [5] Examined some attributes of yeast isolates for ethanol production, the ethanol tolerance of the isolates ranged from 6-12% (v/v) ethanol [47]. Reported maximum of 12% ethanol tolerance exhibited by yeast strains isolated from fruits. During the process of fermentation, due to metabolic activity of microbes and frictional effects of agitation serve to generate large amount of heat. So, the yeast strain employed for fermentation studies should be thermotolerance one. In the present study, all the yeast isolates showed growth upto 35˚C. At 40˚C, only the isolate SB10 exhibited good growth. No isolates showed growth at the temperature of 45 and 50˚C. In contrast, the results of [46] declared that the yeast strains *BRC*05 and *BRM*17 had able growth at 50˚C.

The strain with high flocculation ability, fermentative capacity, sugar and thermotolerance are the requisite criteria for selecting yeast for industrial production of ethanol [5]. In the present study, 5 yeast strains are flocculent, the flocculation rate ranged from 0.5 to 2.3 ml/10 min. The strain *SB*10 was highly flocculent (2.3ml/10min) among the other strains used in the present study [35] have stated that yeast with high invertase activity is required for growth in medium where the principal carbohydrate is sucrose. Herein, the highest invertase activity of 56.8 µmole/min was contributed by isolate *SB*10 [38] isolated 17 wine yeast from cashew apple juice and were screened for ethanol and sugar tolerance. Among them, two strains of *Saccharomyces cerevisiae* were found to possess higher invertase activities.

Among the 10 isolates obtained from spoiled banana samples, the isolate *SB*10 exhibited all the attributes tested in the present study. The strain *SB*10 recorded high ethanol tolerance, flocculating ability, sugar tolerance and thermo tolerance activity with appreciable fermentative capability to produce ethanol. So, the strain SB10 was considered as potential ethanol genic strain and selected for further fermentation studies. The strain *SB*10 was subjected to 18S *rRNA* sequencing studies to identify upto species level. The strain *SB*10 was identified as *Saccharomyces cerevisiae* based on BLAST search conducted in the NCBI Gen bank. The strain has 99.6% similarity with the already available sequences of *Saccharomyces cerevisiae* in the Gen Bank.

The [10] isolated 374 yeasts from a variety of rotten fruits and barks of trees. Out of them, 27yeast strains were able to assimilate xylose and produce ethanol. The phylogenetic analysis of D1/D2 domain sequence of LSU (Large Sub Unit) rRNA gene and phenotypic characteristics the yeast strains were identified as members of the genera *Pichia, Candida, Kluyveromyces, Issatchenkia, Zygosaccharomyces, Clavispora, Debaryomyces, Metschnikowia, Rhodotorula* and *Cryptococcus*. The most suitable feedstocks for ethanol production are high sugar-content crops such as sugarcane, sugar beets, molasses and fruits, because their main components are sugars that can be readily converted into ethanol [7]. Banana fruit and its associated residual biomass are amylaceous and lignocellulosic materials; therefore, they need to be hydrolyzed to be converted into glucose, which is then fermented to produce ethanol [26, 41].

Lignocellulose materials are highly resistant for microbial degradation. To enhance their susceptibility for hydrolysis, number of physical, chemical and microbial/enzymatic methods is advocated. These methods help to liberate cellulose from its protective sheath lignin to increase the surface area of crystalline cellulose by size reduction and swelling. One should consider for the cost-benefit ratio as well as higher recovery of end product. Biological treatment involves the use of whole organisms or enzymes in pretreatment of agricultural waste products. Both fungi and bacteria are used for biotreatment of agricultural waste. Commercial preparations of fungal and bacterial hydrolytic and oxidative enzymes are also widely used instead of these microorganisms. Fungal pretreatment of agricultural residues is a new method for improvement of digestibility [44].

Enzymatic pretreatment of agricultural waste utilize hydrolytic and oxidative enzymes which are mainly derived from fungi and bacteria. Cellulases are usually a mixture of several enzymes. At least three major groups of cellulases are involved in the hydrolysis process: (1) endoglucanase (*EG*, 1,4- D -endo glucanohydrolase) which attacks regions of low crystallinity in the cellulose fiber, creating free chain ends; (2) exoglucanase or cellobiohydrolase (CBH, 1,4-β-D glucan cellobiosehydrolase) which degrades the molecule further by removing cellobiose units from the free chain ends and (3) β-glucosidase which hydrolyzes cellobiose to produce glucose

[42]. To help the enzyme to perform well and degrade the lignocellulose efficiently the fibers in the raw material need to be accessible to the enzymes. The enzymatic hydrolysis was studied by using the 10ml enzyme source obtained from the consortium developed by using three fungal isolates *viz*., *Trichoderma* sp. (PSF-2), *Aspergillus* sp. (SBF-2) and *Mucor* sp. (SBF-1).Enzymatic hydrolysis released fermentable sugars to the level of 28.51±0.10% from banana peel, 20.48±0.14% from banana pseudo stem and 48.56±0.38% from spoiled banana over an incubation of 72 hrs [9]. Carried out Simultaneous Sacchrification and Fermentation (SSF) of banana peels to ethanol by using co-cultures of *A. niger* and *S. cerevisiae* at different temperature and pH. The maximum ethanol yield was 6.54%. They concluded that simultaneous fermentation of starch to ethanol can be conducted efficiently by using co-culture of amylolytic fungus *A. niger* anda non amylolytic sugar fermenter *S. cerevisiae.* The results of the present investigation clearly indicated that the banana wastes could be suitable substrate for ethanol production. Among the 3 substrate used, spoiled banana waste recorded better yield (23.42 ± 0.18 gl^{-1}), this was followed by banana peel (17.00 ± 0.07 gl^{-1}) and banana pseudo stem (15.61 ± 0.07gl^{-1}) by using the indigenous yeast isolate *S. cerevisiae*. The study pointed out the fact that use of indigenous yeast with good fermentation attributes will be more economical to the produce bio-ethanol from easily available agricultural wastes. Innumerable studies have been conducted on ethanol production from banana residues [39]. Utilized banana peels and beet wastes for alcohol production, without any pretreatment by using *Saccharomyces cerevisiae.* Ethanol production in case of banana peels is 1.90% equivalent to dextrose [2]. Carried out simultaneous saccharification and fermentation using *Aspergillus niger* and *S. cerevisiae to* produce alcohol from fruit wastes *viz.,* pineapple peel, banana peel, orange peel and pea peels. Among the fruit wastes used, pineapple peel and banna peel recorded higher ethanol yields (83% v/v) than orange and pea peel [40]. Utilized banana and mango peels to explore their potential application in bioethanol production. The banana fruit peels yielded a maximum reducing sugar content of 36.67% after acid and enzymatic hydrolysis. The hydrolyate obtained from the dilute H_2So_4 pretreated banana fruit peels yielded a maximum of 13.84% ethanol at 42 hrs of incubation [43]. Employed dried and ground peel biomass, ripe waste banana and acid hydrolyzed peel of green and red banana for bioethanol production by using *Saccharomyces cerevisiae.* The maximum yieldof ethanol in ripened red banana and their hydrolyzed peels about 1.3% and 0.27% (v/v) in 10% substrate concentration [27]. Evaluated the possibility of using banana tree pseudo stem as a substrate for alcoholic fermentation. Hydrolysis methods using dilute H_2So_4 and enzymes were evaluated both separately and in combination. Acid hydrolysis, released maximum amount of fermentable sugars and fermentation of the hydrolysates was satisfactory for the maximum yield of ethanol [15]. Compared different chemical and biological pretreatments method to digest banana pseudo stem for bioethanol production.

The fungal strains *Aspergillus ellipticus* and *Aspergillus fumigatus* were used under co-culture fermentation on banana pseudo stem to degrade holocellulose and facilitate maximum release of reducing sugar. Fermentation of cellulosic hydrolysate (4.1 g) gave maximum ethanol (17.18 g l^{-1}) with yield (84%) after 72 hrs [15]. exploited rotten banana as a substrate for bioethanol production employing *Saccharomyces cerevisiae.* The fermented banana waste produced 4.1 to 7.1% bioethanol. Fermented banana treated with mixture of enzymes was the best method for higher production of bioethanol. The results also concluded that mixture of skin and pulp of rotten fruit was more suitable for bioethanol production which could reduce the cost of the initial process. Our results are comparable with studies of [17] herein; the maximum ethanol production was observed when spoiled banana used as a substrate. Parameter optima are important in any type of fermentation. Here an attempt was made to optimize different parameters like inoculum size; pH, temperature and supplement of nitrogen source were analyzed to improve the ethanol yield. Lower inoculum size reduces cost of production in ethanol fermentation. The effect of inoculum size on ethanol production was studied by [27] using response surface methodology and it was found that raised ethanol yields were obtained with high inoculum size [25]. stated that the speed of the fermentation depends on the yeast concentration, the shorter the fermentation period required to achieve maximum production. The studies of [34] demonstrated that inoculum size of 4 to 12% of *Saccharomyces cerevisiae* gave a remarkable increase in the ethanol production from starch. The present findings revealed the inoculum size of 5% *Saccharomyces cerevisiae* recorded rise in the ethanol yield from all the substrate used. According to [43] with the change in the concentration of yeast, the time required for the completion of fermentation decreased dramatically. Using a 12%, 9%, 6%, 3% yeast inoculum, maximum ethanol production was completely achieved in 2, 3, 5, 7 days respectively. Temperature greatly affects the enzymatic activity and membrane turbidity of yeast cells. Most of the studies recorded the optimum temperature for ethanol production is 30-40˚C. In our study, the maximum ethanol production was observed at 35˚C when the temperature increased further the reduction in the ethanol production was observed. Higher temperature may shorten the log phase of yeast cells, subsequent denaturation of enzymes and ribosome, accumulation of toxic results in decrease of yield [28, 31]. Reported that maximum ethanol production was observed at temperature 33˚C. Simultaneous Saccharification and Fermentation of banana peels to ethanol by co-culture of *Aspergillus niger* and *Saccharomyces cerevisiae was* investigated at different temperature (20˚C to 50˚C). The optimum temperature for the fermentation of banana peels was found to be 30˚C [18]. pH of the fermentation medium have direct (or) indirect influence on ethanol production. The optimum pH for *S. cerevisiae* was found to be 6.0. These results are comparable with studies of [11]. According to them, the optimum pH was found to be 6.0 for *S. cerevisiae* in the fermentation of banana peel to ethanol. In contrast [13], recorded the optimum pH for *Saccharomyces cerevisiae* BY 4742 was in the range of 4.0 to 5.0. A wide range of optimum pH (4.0- 8.0) was reported for *Saccharomyces cerevisiae* BY 4742 isolated from Jerusalem artichoke using insulin and Jerusalem artichoke tuber as substrate at 35˚C [13]. The supplementation of exogenous nitrogen sources to the fermentation media enhanced ethanol production in *S. cerevisiae* [11, 13]. Herein, among the different nitrogen sources used, ammonium sulfate incorporation showed significant increase in ethanol production. But [11] reported that the addition of yeast extract, ammonium sulphate, urea and their combination to molasses did not improve ethanol productivity.

Conclusion

Based on the results of our present study, we concluded that

the banana peel wastes offers new way for bio-ethanol production. The choice of newer substrate for the production of ethanol is being a non-seasonal fruit available throughout the year. The waste from the plant can be efficiently utilized based on overall economics and energy. Production of bio-ethanol from agricultural waste residues using indigenous yeast isolates is very economical, especially when the fermentation conditions are optimized.

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