Bioprospecting of Indigenous Microbial Species for Bioethanol Production from the Fruits of Some Selected Exotic Ornamental Plants

ABSTRACT

Bioethanol, descried as a promising renewable energy source, can be produced through the microbial fermentation of various feedstock such as fruits. In this study, the ability of some selected indigenous microbial isolates, namely *Bacillus subtilis*, *Zymomonas mobilis* and *Saccharomyces cerevisiae* to produce bioethanol from the fruits of *Ixora coccinea*, *Duranta repens* and *Syzygium guineense* was determined. The organisms were isolated from soil ad palm wie, ad were identified based on cultural and morphological, as well as molecular characteristics. The fruit samples were obtained from New Campus, Bayero University Kano, Nigeria. The samples were washed, blended, exposed to ultrasonic pretreatment and sterilized using autoclave. Concentrations of reducing sugars in the pretreated fruit samples were determined using DNSA-UV-visible spectrophotometry. Samples were accordingly inoculated with the fermenting organisms in single and in combination. After seven days of fermentation on shaking incubator, the concentrations of bioethanol produced were determined using the UV-visible spectrophotometry. Highest bioethanol concentration was found in *I. coccinea* with significant difference compared to that from *D. repens* and *S. guineense*. The yield in bioethanol production was higher in fermentation media containing the combination of fermenting organisms compared to ones with individual cells. It was recommended that the fruits of these exotic plants should be considered for commercial production of bioethanol.

Keywords: Indigenous microbial species, Exotic plants, Fruits, Bioethanol, Fermentation.

Introduction

The incessant global population growth, coupled with industrialization, is increasing the energy demands all over the world. Biomass-derived fuels are known to provide promising alternative; they are renewable, non-toxic and their combustion do not release carbon dioxide (CO_2) into the atmosphere. It is estimated that biomass will contribute up to 50% of the world's primary energy consumption by the year 2050 (Kumar, 2010); it is one of the world's largest sustainable energy source (Kour *et al*., 2019). The global community has acknowledged bioethanol for providing energy security worldwide. This has to do with the fact that bioethanol has a great potential of becoming a future fuel of choice due to its renewable nature and efficiency. Bioethanol is a liquid biofuel that it can be produced from the microbial fermentation of various biodegradable feedstock, including fruits (Kour *et al*., 2019). Of great interest in the present study are the fruits of some exotic ornamental plants such as scarlet jungle flame (*Ixora coccinea*), golden newdrop (*Duranta repens*) and malmo (*Syzygium guineense*). The spread of these plants in our residential and institutional areas is at an increase; they produce many seeds of which their management becomes worrisome.

Microorganisms ferment a vast array of carbohydrates from fruits to produce bioethanol. The use of microorganisms to produce biofuels provide a double environmental benefit in addition to reducing greenhouse gases emissions. The production processes also reduce environmental waste by harnessing the ability of various microorganisms to utilize and break down complex carbohydrate into fermentable sugars (El-Imam, 2012). Microorganisms varied in their abilities to degrade sugars; microbial species have some types of sugars they can hydrolyze, not necessarily all; thus, different organisms are needed in fermentation medium with a variety of sugars, so that absolute degradation of these substrates can be fully attained to achieve highest bioethanol yield (Al-Makishah, 2017). These organisms can be isolated from their various habitats and identified; their abilities to produce bioethanol from different sugars can be determined. This study determined the bioethanol production ability of some indigenous microbial isolates using the fruits of some selected exotic plants, namely *I. coccinea, D. repens* and *S. guineense*.

MATERIALS AND METHODS

Sample Collection

Samples of the fruits from the selected exotic plants, namely *I. coccinea*, *D. repens* and *S. guineense*, were collected in clean polythene bags from New Campus, Bayero University Kano, Nigeria. Samples were washed with water to remove any dirt.

Isolation and Identification of the Fermenting Organisms

The organisms isolated and used in the study were *Bacillus subtilis*, *Zymomonas mobilis* and *Saccharomyces cerevisiae*. *B. subtilis* was isolated from garden soil samples, while *Z. mobilis* and *S. cerevisiae* were isolated from palm wine according to the method employed by Amin *et al*. (2015). Ten (10) garden soil samples and 10 palm wine samples were collected from different locations in clean polythene bags and plastic bottles, respectively. One milligram (1g) of the soil sample was dissolved in 10 ml of nutrient broth and incubated at 35^{o}C for 24 hours. After incubation, 1ml of the cultured broth was streaked on fresh nutrient agar and incubated under the same conditions as above. In the same manner, one milliliter (1ml) of wine sample was introduced into 10 ml of nutrient broth and incubated anaerobically at 35^{o}C for 24 hours. After incubation, 1ml from the broth culture was streaked on fresh nutrient agar and incubated anaeroically to isolate *Z. mobilis*. To isolate *S. cerevisiae*, 1ml of the wine sample was aseptically transferred into 10 ml of Sabouraud Dextrose Broth and incubated at 35^{o}C for 48 hours, and after incubation, 1ml was streaked on fresh Sabouraud Dextrose Agar (SDA) and incubated under the same conditions (Sukirtha and Abinaya, 2019). The colonies expected to be the target organisms were sub-cultured on fresh media to produce the axenic culture of the individual cells. These were used for molecular analysis to confirm the identification of the isolates.

Molecular Analysis of the Isolates for Identification

The molecular analysis was carried out by genomic study of the 16S rRNA and 18S rRNA regions of the bacterial and fungal (yeast) DNAs, respectively. The microbial DNAs were extracted using

Nucleo-pore gDNA Fungal/Bacterial Mini Kit (Genetix Biotech Asia Manual, 2018), amplified using universal primers for bacteria (27F/1492R) and fungi (NS1/NS4), and identified using Blast programme, as described in Alhassan *et al*. (2020).

Pretreatment of the Samples and Reducing Sugars Determination

The clean fruit samples were separately blended with a blender (Preethi Taurus, India) to accelerate microbial degradation and enhance fermentation. A duplicated setups of seven (7) conical flasks (500ml-capacity) were arranged for each fruit and a known quantity (20g) of the blended fruit sample was accordingly placed in each flasks and diluted with 250ml of distilled water. The conical flasks were covered appropriately with cotton wool and aluminum foil, and then placed in ultrasonicator (SW 30H, Nickel-Electro Ltd., Switzerland) to enhance pretreatment. The samples were then sterilized in an autoclave (STV-I, Bioevopeak, China) at a pressurized temperature of $121^{O}C$ for 15 minutes. Reducing sugars concentrations in the pretreated fruit samples were determined using DNSA-UV-visible spectrophotometry as described in Alhassan (2023).

Bioethanol Production

The production of bioethanol from the pretreated fruit samples was conducted through fermentation as described in Alhassan (2023). Each sample in the first setup was inoculated with 25ml of the suspension of a pure broth culture of *Saccharomyces cerevisiea*; while the second was inoculated with that of *B. subtilis*, the third with *Z. mobilis*, the 4^{th} with a mixed culture of *S. cerevisiea* and *B. subtilis*, the 5^{th} with a mixed culture of *S. cerevisiea* and *Z. mobilis*, the 6^{th} with a mixed culture of *B. subtilis* and *Z. mobilis*, and the last (7^{th}) was inoculated with a mixed culture of *S. cerevisiea, Z. mobilis* and *B. subtilis*. The flasks were incubated in shaking incubator (SI – 200, Major Science Co. Ltd., Singapore) at $45^{o}C$ for seven (7) days (Ziemlewska *et al*., 2021), and bioethanol produced was recovered from the fermentation media using distillation (Alhassan, 2023).

Concentrations of Bioethanol Produced

The concentrations of bioethanol produced were determined using UV-Spectrophotometry as described in (Alhassan, 2023).

Statistical Analysis

One-way statistical analysis of variance (ANOVA) was used to determine whether there is a significant difference in reducing sugars and bioethanol yield between the fruits samples and control.

RESULTS AND DISCUSSION

Results

Table 1 below shows the result of the microbial isolation from the soil and palm wine samples. *B. subtilis* was isolated in four out of the 10 soil samples investigated. *Z. mobilis* was isolated from two out of 10 samples, while *S. cerevisiea* was isolated from six (6) out of 10 samples. The identification of these organisms was confirmed by molecular study of the16S and 18S RNA examination.

Table 1: Results of the Microbial Isolation and Identification

Organism	**Yield**	**Cultural features**	**Morphological features**	**Molecular confirmation**
B. subtilis	40%	White rounded colonies with flat and dry surfaces.	Short chains Gram positive large rod-shaped cells	Confirmed
Z. mobilis	20%	Whitish convex colonies	Gram negative rod-shaped cells	Confirmed
S. cerevisiea	60%	Smooth creamy colonies with circular margin	Circular budding yeast cells	Confirmed

The results of the reducing sugars determination after pretreatment is presented in Figure 1 below. It was found that *I. coccinea* had the highest reducing sugars concentration (28.6%) with significant difference ($p < 0.05$) compared to *S. guineese* (23.2%) and *D. repens* (with 5.3%).

Figure 2 presents the results of the bioethanol concentrations, where it was observed that *I. coccinea* had the highest yield of bioethanol with significant difference ($p > 0.05$) when compared to *S. guineense* and *D. repens,* but there was no significant difference regarding the fermentation ability of the microbial isolates. Moreover, there was no significant difference in bioethanol yield observed between the single culture and the mixed culture of the microbial isolates.

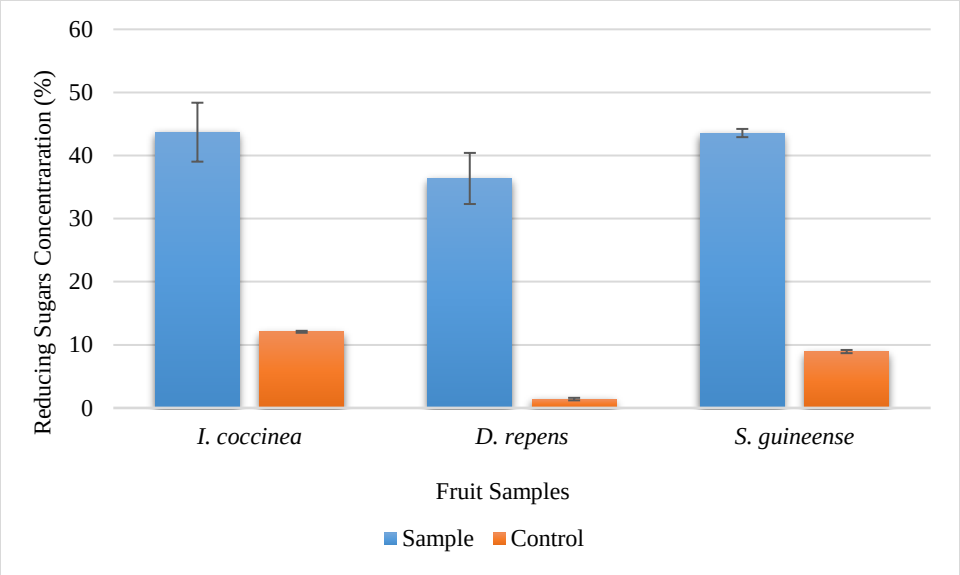

Figure 1: The Result of the Reducing Sugars Concentrations in the Fruit Samples

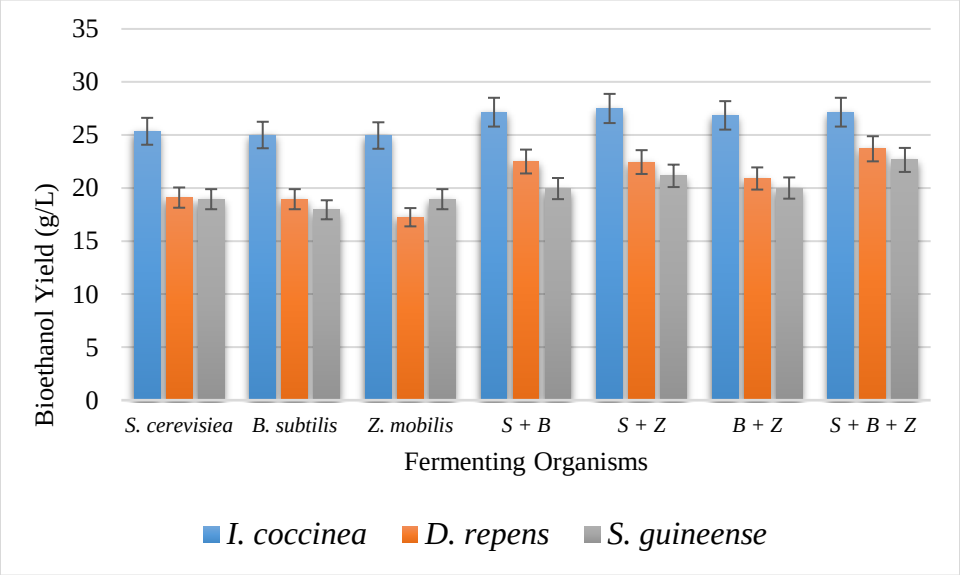

Figure 2: The Result of bioethanol yield from the fermentation of fruit samples using various combinations of fermenting organisms

Discussion

Microorganisms can ferment a vast array of carbohydrates to produce numerous fuel compounds, including bioethanol; they varied in their ability to digest sugars in the process of fermentation. Fermentative microorganisms can be found in different environment, including soil, fermented beverages and fruits, among others. As in the preset study, *S. cerevisiea* is usually isolated from alcoholic beverages, and is extensively used in the production of bioethanol. Some species of bacteria such as bacillus and clostridium are usually isolated from soil samples and they are

capable of producing a variety of alcohols such as ethanol and butanol. *B. subtils* and *Z. mobilis* isolated in this study have been described as promising organisms in bioethanol production.

The proliferation of exotic plants is on the increase in Kano and other Northern states in Nigeria. These plants produce a considerable amount of fruits that are usually wasted; their consumption is not common. Of particular interest in this study are *I. coccinea*, *D. repens* and *S. guineense*; their fruits are described to have high sugar content (Jordaan and Downs, 2012). Considering the results of fermentation using the identified organisms in this study, the utilization of these fruits in the production of bioethanol will have a great economic and sustainable impact.

Ultrasonication has been used in the recent times to enhance pretreatment in bioethanol production processes. The use of ultrasonication in the pretreatment of the fruit samples was found to be very effective, as it increased the yield of reducing sugars in the samples, higher than what was found in Alhassan (2023), in which autoclave was employed as the only pretreatment method. Similar findings were reported in Subhedar and Gogate (2016), where ultrasonication was fond to enhance pretreatment of various biomass in bioethanol production processes. With regards to bioethanol yield, the mixed cultures containing two fermenting organisms produced the higher ethanol concentrations compared to individual isolates. Moreover, the mixed culture containing the three organisms was found to have the highest yield, though the differences in each case were not statistically significant (at $p > 0.05$). This finding is similar to that of Chibuzor *et al*. (2016) and Na-Allah *et al*. (2021), where a combination of the fermenting organisms was found to produce higher bioethanol yield compared to individual microbial cells.

CONCLUSION

The fruits of exotic plants, specifically *I. coccinea*, *D. repens* and *S. guineense*, are produced in large quality every season, and usually found everywhere in the environment around cities of developing countries, including Kano (Nigeria) without proper management and utilization. These fruits can be fermented by a variety of microorganisms including yeast (such as *S. cerevisiea*) and bacteria (such as *B. subtils* and *Z. mobilis*) to produce bioethanol. If commercial production of this fuel and other important bioproducts will be considered from these fruits, it will have a great socioeconomic and environmental impact, especially in developing countries.

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