



Research Article

Bioconversion of Sweet Sorghum Stalk Sugars into Ethanol by *Zymomonas mobilis* Isolated from Palm Wine

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Abstract

Background and objective: Sweet sorghum is a sugar-rich crop with considerable potential as a feedstock for bioethanol production. This study aimed to characterize sweet sorghum juice and evaluate its alcoholic bioconversion by *Zymomonas mobilis* isolated from palm wine, using *Saccharomyces cerevisiae* as a reference microorganism. **Material and methods:** *Z. mobilis* was isolated from fresh palm wine on Wallerstein Laboratory (WL) medium, purified, and cultivated on a standard medium. Its ethanol tolerance was compared with that of *S. cerevisiae* at different ethanol concentrations. Sweet sorghum juice was extracted by pressing and characterized for dry matter, ash, total and reducing sugars, nitrogen, and selected mineral elements. Fermentation was performed under non-aerated conditions at 30 °C in a 2-L fermenter using a *Z. mobilis* preculture corresponding to 10% of the final volume. **Results:** Colonies attributed to *Z. mobilis* on WL medium were lenticular and appeared after 4-5 days of incubation at 30 °C. The isolate showed growth at ethanol concentrations ranging from 2.5% to 10%. Sweet sorghum juice had a mean dry matter content of 25.97 g/100 mL and mean total and reducing sugar contents of 18.99 and 19.12 g/100 mL, respectively. Fermentation with *Z. mobilis* did not result in a significant decrease in °Brix, which declined from 17 °B initially to 16.5 °B after 3 days and 16.3 °B after 6 days. In contrast, the *S. cerevisiae* control produced an alcohol content of 5% with a reported yield of 50%. **Conclusion:** Under the experimental conditions investigated, the *Z. mobilis* strain isolated from palm wine did not efficiently ferment sweet sorghum juice. This failure may be associated with the nutritional characteristics of the substrate, preculture preparation conditions, or the physiological properties of the strain. Further studies involving more precise microbiological identification, optimization of the culture medium, and analytical monitoring of fermentation metabolites are warranted.

Keywords

Sweet Sorghum, Bioethanol, *Zymomonas Mobilis*, *Saccharomyces Cerevisiae*, Alcoholic Fermentation

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1. Introduction

The search for alternative energy sources to fossil fuels is a major challenge in many African countries. The development of biofuels is part of this effort, while also raising questions regarding competition between the food and energy uses of agricultural biomass. In this context, the use of sugar-rich feedstocks that can be valorized without primarily diverting resources intended for food production is of particular interest [1].

Bioethanol is produced by the fermentation of fermentable sugars by microorganisms. *Saccharomyces cerevisiae* is traditionally used because of its robustness and ability to ferment various sugar-rich feedstocks. *Zymomonas mobilis* is another promising ethanologenic bacterium; its ethanol productivity and yield can be high, but its substrate range is more limited, which may restrict its use depending on the composition of the feedstock [2, 3].

Sweet sorghum is particularly attractive for this application because of the high sugar content of its juice. Its sugar content is approximately 15-20%, and its juice could therefore potentially be used as a substrate for bioethanol production. The choice of *Z. mobilis* is also consistent with its reported role in the fermentation of palm-derived products and its ability to grow in the presence of ethanol [4, 5].

In addition, recent studies have highlighted the potential of sweet sorghum juice as a renewable feedstock for bioethanol production. However, optimizing fermentation conditions is a key factor in improving ethanol productivity [6]. Sweet sorghum also offers the advantage of containing fermentable sugars that can be directly extracted from the stalks, thereby reducing the need for biomass pretreatment. Furthermore, fermentation efficiency depends largely on the characteristics of the microbial strain used and its tolerance to osmotic stress and ethanol. In this context, *Zymomonas mobilis* is a particularly promising microorganism owing to its efficient sugar metabolism and high ethanol productivity. Nevertheless, its relatively limited ability to utilize a broad range of substrates derived from complex agricultural materials remains one of its main limitations [7]. However, advances in metabolic engineering offer promising opportunities to improve the utilization of alternative carbohydrate substrates, thereby broadening the range of feedstocks suitable for fermentation [8].

The overall objective of this study was therefore to characterize sweet sorghum juice and evaluate its bioconversion into ethanol by *Z. mobilis* isolated from local palm wine. Fermentation with *S. cerevisiae* was used as an experimental control to assess the performance of the bacterium under study.

2. Material and Methods

2.1. Biological Material and Sampling

The biological material consisted of a sample of fresh palm wine at 17 °Brix, with a whitish color, and sweet sorghum

stalks. The palm wine, collected approximately one hour after tapping, was collected in a sterile 250-mL flask, transported to the laboratory, and stored at 4 °C before analysis. Sweet sorghum stalks were collected at Pointe Sarène, approximately 100 km from Dakar, for juice extraction.

2.2. Culture Media

Wallerstein Laboratory (WL) medium was used for isolation. Its reported composition was as follows, in g/L: yeast extract (4), tryptone (5), glucose (50), potassium dihydrogen phosphate (0.55), potassium chloride (0.425), calcium chloride (0.125), magnesium sulfate (0.125), ferric chloride (0.0025), manganese sulfate (0.0025), bromocresol green (0.022), and cycloheximide (0.004). The pH was adjusted to 4. The media were sterilized by autoclaving at 120 °C for 15 min.

For the growth and maintenance of *Z. mobilis*, a standard medium at pH 5 containing 0.5% yeast extract and 2% glucose was used, according to the protocol attributed to Swings and De Ley (1977). For the preculture intended for fermentation, the document reports enrichment of the medium with 2% yeast extract and 20% glucose.

2.3. Isolation and Purification of *Z. mobilis*

Three inoculation procedures were used to inoculate palm wine onto WL medium: mixing the sample with the medium before solidification, depositing the sample before adding the medium, and surface inoculation of the solidified medium. Presumptive *Z. mobilis* colonies were subsequently streaked onto standard medium solidified with 2% agar. Cultures were incubated at 30 °C for 72 h and then stored at 4 °C, with renewal every 2-3 weeks.

2.4. Evaluation of Ethanol Tolerance

The ethanol tolerance of *Z. mobilis* and *S. cerevisiae* was studied separately in media containing 2.5%, 5%, 7.5%, 10%, 12%, and 15% ethanol. Ethanol was added aseptically to the sterile medium. After 24 h of incubation at 30 °C, growth was estimated by measuring optical density at 600 nm, using uninoculated broth as the control [4].

2.5. Juice Extraction and Characterization

The stalks were cut into pieces of approximately 5 cm, chopped, and then pressed using a hydraulic press. The first pressing yielded approximately 20 L of juice at 19 °Brix. The bagasse was then moistened at a ratio of 1 L of water per 5 kg and pressed again, yielding approximately 18 L of juice at 11 °Brix. The juices were characterized for moisture, ash, sugars, nitrogen, and selected mineral elements.

2.5.1. Moisture and Ash

Moisture was determined using 5 g of sample in triplicate, after drying at 105 °C and weighing before and after desiccation. Ash content was determined using 5 g of sample after evaporation on a hot plate and incineration at 550 °C for 2 h. The calculation formulas are presented below.

$$H = \{(M1-M2) \times 100\} / PE$$

H = Moisture percentage

M1 = Mass in g of the raw sample + capsule

M2 = Mass in g of the dried sample + capsule

PE = Test portion in g

$$\text{Ash content} = \{(M1-M2) \times 100\} / PE$$

M1 = Mass in g of the raw sample + capsule

M2 = Mass in g of the dried sample + capsule

PE = Test portion in g

2.5.2. Sugar Determination

After extraction with 40% ethanol (v/v) and clarification using Carrez solutions, total sugars were determined by the Luff-Schoorl method after acid hydrolysis. Reducing sugars were determined according to the same principle without the hydrolysis step using 4 M HCl. The results were expressed in g/100 mL in the experimental table.

2.5.3. Mineral Determination

Minerals were determined by atomic absorption spectrometry after treatment of 5 g of sample with nitric acid, ammonium, and a vanadate-molybdate solution. Standard solutions of magnesium, copper, and potassium at 1000 ppm were used for measurement.

2.6. Alcoholic Fermentation

Isolated *Z. mobilis* colonies were cultivated at 30 °C for 24 h without agitation in 50 mL of enriched standard liquid medium. A fraction of this culture was transferred into 180 mL of sweet sorghum juice, whose pH was adjusted to 5 with 0.1 N NaOH, to obtain a 200-mL preculture. The preculture was incubated for 12-18 h at 30 °C.

Fermentation was carried out in a 2-L fermenter. The preculture represented 10% of the final volume, and the fermenter was then filled with sweet sorghum juice pasteurized at 77 °C for 1 min. Fermentation was conducted at 30 °C under non-aerated conditions. Changes in °Brix were used as an indirect indicator of sugar consumption.

3. Results

3.1. Microscopic Observation and Isolation of *Z. mobilis*

Microscopic examination of fresh palm wine revealed a

mixture of oval-shaped yeasts and bacteria with various morphologies. Small bacilli with regular margins, sometimes motile, were observed and could be attributed to *Z. mobilis*. On WL medium, presumptive *Z. mobilis* colonies were lenticular, measured approximately 1-4 mm in diameter, and appeared after 4-5 days of incubation at 30 °C.

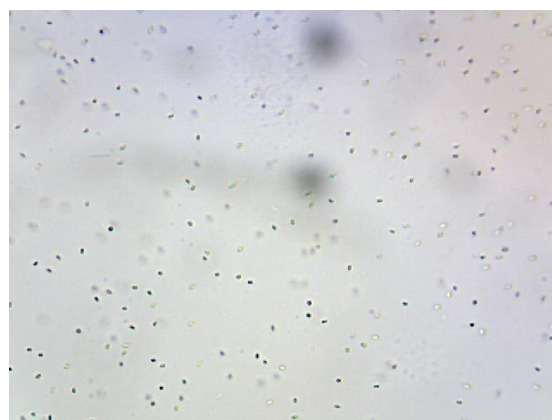


Figure 1. Microscopic observation of a suspension attributed to the rosette form of *Z. mobilis* (40× objective).

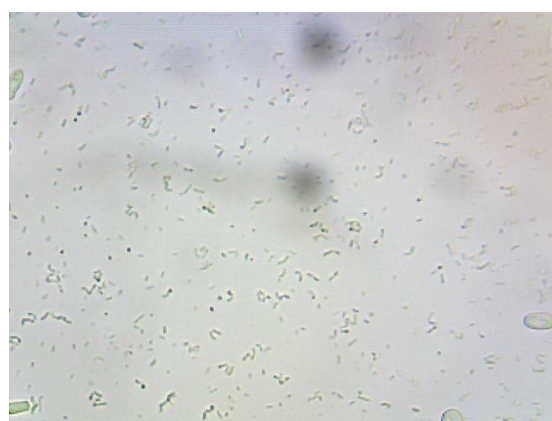


Figure 2. Microscopic observation of a suspension attributed to the chain form of *Z. mobilis* (40× objective).

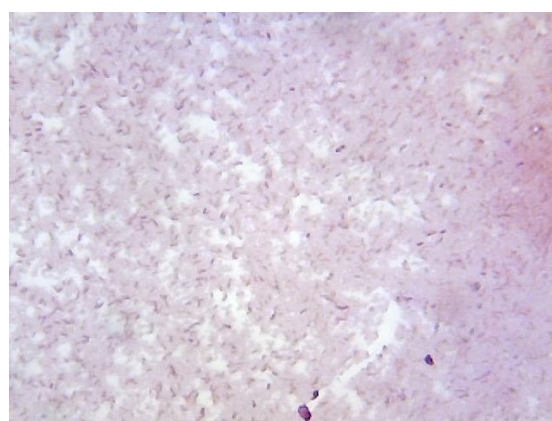


Figure 3. Microscopic observation after Gram staining of a suspension attributed to the filamentous form of *Z. mobilis* (100× objective).

3.2. Ethanol Tolerance

Comparison of optical densities indicated a progressive decrease in the growth of *S. cerevisiae* as the ethanol concentration increased. Clear growth was still observed up to 7.5%, after which biomass decreased markedly, with almost no

growth from 12.5% onward. *Z. mobilis* grew at ethanol concentrations between 2.5% and 10%, but growth was poor above 10% under the conditions tested. These observations are consistent with the ethanol tolerance attributed to the species in the literature [4, 5, 9].

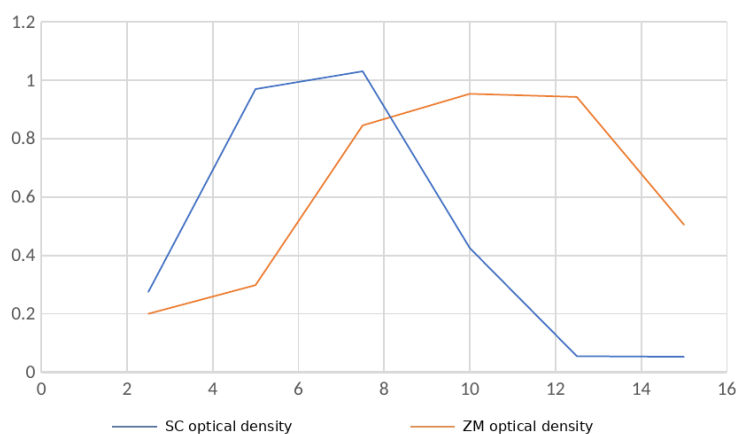


Figure 4. Comparison of the growth of *S. cerevisiae* and *Z. mobilis* in the presence of different ethanol concentrations.

3.3. Characterization of Sweet Sorghum Juice

The mean results of the physicochemical analyses of the unfermented juice are presented in Table 1. The mean dry matter

content was 25.97 g/100 mL. The mean total and reducing sugar contents were 18.99 and 19.12 g/100 mL, respectively. The mean nitrogen content was 0.12%.

Table 1. Mean composition of unfermented sweet sorghum juice.

Parameter	Trial 1	Trial 2	Trial 3	Mean	Unit
Ash	1,45	1,44	1,44	1,44	g/100 mL
Dry matter	25,90	25,81	25,81	25,97	g/100 mL
Total sugars (sucrose)	19,33	18,55	18,55	18,99	g/100 mL
Reducing sugars (glucose + fructose)	19,17	18,54	18,54	19,12	g/100 mL
Nitrogen	0,12	0,13	0,13	0,12	%
Phosphorus	27,70	29,21	29,08	28,66	g/100 mL
Magnesium	46,17	/	46,87	46,52	g/100 mL
Copper	0	0	0	0	g/100 mL
Potassium	442,91	460,60	492,03	465,18	g/100 mL

3.4. Fermentation of the Juice by *Z. mobilis*

At T0, the fermentation medium was homogeneous. After approximately 1 h 30 min, phase separation was observed, with a dark-green upper phase and a lighter, denser lower

phase associated with fiber sediment. After 24 h and again after 6 days of incubation, no macroscopic signs characteristic of active fermentation by *Z. mobilis* were observed in the experimental system. The Brix value decreased from an initial 17 °B to 16.5 °B after 3 days and 16.3 °B after 6 days.

For comparison, fermentation with *S. cerevisiae* was accompanied by bubble formation after 24 h and clarification of the medium during incubation. This control also showed an alcohol content of 5% and a yield of 50%.

4. Discussion

The isolation of a bacterium attributed to *Z. mobilis* from fresh palm wine is consistent with the documented presence of this species in certain alcoholic fermentations of palm sap. The use of WL medium containing cycloheximide was intended to limit yeast growth and favor the detection of fermentation-associated bacteria. Colony morphology and microscopic observations are consistent with historical descriptions of the species, but they are not sufficient on their own for definitive taxonomic identification [5]. Molecular identification, particularly by sequencing an appropriate marker, would therefore be necessary in future studies. Recent studies consider *Z. mobilis* a particularly promising microbial platform for bioethanol production, while emphasizing the importance of genetic and physiological characterization of the strains used [10, 11].

The biotechnological interest in the use of *Z. mobilis* is based in particular on its distinctive hexose metabolism. In contrast to *Saccharomyces cerevisiae*, *Z. mobilis* preferentially uses the Entner-Doudoroff pathway, which directs carbon toward ethanol production. This may explain the high yields reported for some *Z. mobilis* strains [12, 13].

The ethanol tolerance observed in the isolated strain is an interesting finding. Growth of *Z. mobilis* between 2.5% and 10% ethanol is consistent with published data on the ability of the species to tolerate relatively high ethanol concentrations [4, 9]. However, ethanol tolerance in culture medium does not necessarily imply the ability to efficiently ferment a complex substrate. *Z. mobilis* has a more limited substrate range than some yeasts, and medium composition can strongly influence its fermentation performance [2, 3]. Likewise, other studies distinguish stress resistance from overall fermentation performance and show that metabolic engineering can be used to improve ethanol tolerance, sugar utilization, and productivity simultaneously [10, 14].

The sweet sorghum juice analyzed was rich in sugars, with approximately 19 g/100 mL of total sugars. This high sugar content gives the juice considerable potential as a substrate for ethanol production. The absence of significant fermentation by *Z. mobilis* despite this high sugar concentration is a central finding of the present study. Several factors may be considered.

First, pasteurization at 77 °C for 1 min may have altered the microbiological balance and certain characteristics of the substrate.

Second, the preculture was prepared in a medium highly enriched with yeast extract and glucose and then transferred into juice whose reported nitrogen content was only 0.12%. An abrupt change in nutritional conditions may have limited

the physiological adaptation of the cells.

Conversely, recent studies specifically conducted on sweet sorghum juice show that *Z. mobilis* can efficiently convert this type of substrate when the strain and operating conditions are appropriately adapted. Charoenpunthuwong et al. [16] reported, using a recombinant strain expressing *groESL* genes, an ethanol production of 41.66 g/L at 40 °C, with a theoretical yield of 88.9%.

The result obtained with *S. cerevisiae* confirms that the juice was fermentable by a yeast under the conditions tested. It therefore suggests that the failure of *Z. mobilis* was not solely due to the absence of fermentable sugars. Studies on fermentation technologies also show that the performance of *Z. mobilis* strongly depends on the substrate, strain physiology, and operating conditions [2]. For example, recent studies have demonstrated faster glucose utilization than fructose utilization by *Z. mobilis*, with different percentages of ethanol production [15].

5. Conclusion

This study enabled the isolation, from fresh palm wine, of a strain attributed to *Zymomonas mobilis* that exhibited ethanol tolerance and the characterization of sweet sorghum juice as a sugar-rich substrate. However, significant fermentation of this juice by *Z. mobilis* was not observed under the experimental conditions used, whereas *S. cerevisiae* yielded a reported alcohol content of 5% with a yield of 50%. The use of *Z. mobilis* for the valorization of sweet sorghum juice therefore requires optimization of culture and fermentation conditions, particularly the nutritional composition of the preculture, pH, physiological state of the inoculum, and monitoring of sugars and metabolites. More robust taxonomic identification of the strain and replication of the experiments using a controlled experimental design would be necessary to better confirm the results.

Abbreviations

WL	Wallerstein Laboratory
°C	Degrees Celsius
L	Liter
%	Percent
°B	Degrees Brix
°Brix	Degrees Brix
pH	Potential of Hydrogen
h	Hour
nm	Nanometer
cm	Centimeter
Kg	Kilogram
g	Gram
v/v	Volume/volume
M	Molarity
HCl	Hydrochloric Acid

mL	Milliliter
ppm	Parts per Million
N	Normality of the Solution
NaOH	Sodium Hydroxide
T0	Initial Time Point (time zero)
min	Minute

Author Contributions

Babacar Ngom: Conceptualization, Methodology, Validation, Writing – original draft, Writing – review & editing

Lat Souk Tounkara: Data curation, Methodology, Project administration, Resources

Papa Gueye: Data curation, Formal Analysis, Investigation

Serigne Fallou Wade: Formal Analysis, Investigation, Visualization

Mady Ndiaye: Supervision

Conflicts of Interest

The authors declare no conflicts of interest.

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