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ISOLATION AND IDENTIFICATION OF COMMON BACTERIA THAT AFFECT BEER PRODUCED FROM RICE AND SORGHUM MALTS DURING FERMENTATION

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Abstract: Analytical studies were carried out to isolate and identify common bacteria that affect beer produced from mass rice and white sorghum malts during fermentation. Rice and sorghum malts were used in the course of this research to produce beer using a commercial yeast (Saccharomyces cerevisiae). Worts were obtained by infusion mashing and analysed for their physicochemical properties before wort boiling and subsequent pitching of the yeast (Saccharomyces cerevisiae) to commence fermentation that lasted seven days. Isolation of bacteria was done by culturing the beer samples on Nutrient, MacConkey and De Man Rogosa and Sharpe (MRS) agar plates and incubated for 48 hours. Morphological and microscopic analysis were used to identify the bacterial isolates. Results of wort analysis gave original gravity (1.030 and 1.032$^0\rho$), sugar (78.0 and 7.55)^{0}Brix, pH (5.6 and 5.4) and viscosity (1.21 and 1.13) cP for the wort samples from sorghum and rice malts, respectively. The beer after analysis gave specific gravity (1.005 and 1.004)$^0\rho$, sugar (1.03 and 1.29)^{0}Brix, pH (4.0 and 4.1) and %alcohol (3.66 and 3.27) v/v. the total bacterial counts was 1.2×10^4 and 1.1×104^7cfu/ml and the lactic acid bacterial count ranged from 1.3×10^4–$1.2. \times 10^4$cfu/ml for beer produced from rice and sorghum malts, respectively with no coliform count. The bacterial isolates identified included Streptococcus, Lactobacillus and Micrococcus species. This study confirmed the prevalence of contaminating bacteria on beer samples produced from rice and sorghum malts when fermentation is carelessly handled.
Keywords: Bacteria, beer, rice, sorghum, malt.

INTRODUCTION

Beer is a favourite and highly drunk beverage, since it holds desirable sensory attributes as well as nutritional/medicinal characteristics (Asano et al., 2009). Beer is a very stable beverage in terms of microbiological deterioration; nevertheless, any microbiological spoilage resulting from the malting, brewing, or storage process can negatively impact beer quality and have an adverse financial effect on the brewery industry. (Suzuki et al., 2008). Some Gram-positive and Gram-negative bacteria, wild yeast, and molds are examples of spoilage microorganisms. Many traditional and advanced biotechnological techniques have been applied for qualitative and quantitative determination of mentioned microorganisms (Vaughan et al., 2005). Beer is a brewed beverage consisting of malt, hop, water and yeast, which is drunk world-wide. Beer is the world's third-most consumed beverage, because of its pleasant sensory and health attributes (Sohrabvandi et al., 2010).

Surveys have shown that light to moderate consumption of beer can provide various impacts on humans‟ health, including nutritional benefits, anti-carcinogenic and anti-mutagenic effects, reduction of cardiovascular disease (cardioprotective effect), immune system stimulation, hypolipidemic effect, anti-osteoporosis effect and reduced risk of dementia (Sohrabvandi et al., 2010).

Microbial spoilage is a continuous challenge for the food industry, although beer is very restrictive to bacteria (due to its low pH, high acidity and different anti-microbial compounds) and the beer-spoiling organisms are limited to a few genera (Shabani and Devolli, 2010). The effects of the spoilage bacteria range from relatively minor changes in beer such as off-flavours and aroma defects (i.e. the buttery off-odour of diacetyl), turbidity problems, ropiness, abnormal attenuation rates and reduced yeast crops. These unwanted changes bring millions of dollars losses per year (March et al., 2005).

The majority of spoilage organisms are either obligate anaerobes of the species Pectinatus and Megasphaera, or lactic acid bacteria, primarily belonging to the genera Lactobacillus and Pediococcus. Out of the lactobacilli species, the certain number of strains is able to multiply in the beer medium and cause spoilage. The most common occurring spoilage bacteria in beer are Lactobacillus brevis and Lactobacillus lindneri, respectively (Anli and Alkis, 2010). In addition, there are many wild types of yeast causing spoilage in beer including, Saccharomyces cerevisiae and Candida pelliculosa. However, they cause spoilage problems with greater severity than bacteria. Additionally, malt and beer can be adversely affected by Fusarium infections in grains (Anli and Alkis, 2010).

Beer when carelessly handled during the production processes, is affected by countless microorganisms during fermentation. Hence, contaminated beer is dangerous to the consumers. There have been very little studies on the isolation and identification of microorganisms that contaminate beer during fermentation. Hence, this study.

The aim of this study is to isolate, characterize and identify common bacteria that affect beer produced from rice and sorghum malts during fermentation.

MATERIALS AND METHODS

Sources of materials

Rice (Mass rice) and sorghum (white varieties) were purchased from Eke-Agbani in Nkanu-West of Enugu state while hops, yeast (Saccharomyces cerevisiae), Nutrient agar, MacConkey agar, De Man, Rogosa and Sharpe (MRS) agar and other materials/equipment used to carry out this project research were supplied by the laboratory section of the Department of Applied Microbiology and Brewing, ESUT.

Methods

The methods of analysis employed in the evaluation of malt, wort and beer were based on the recommended methods of analyses of the Institute of Brewing (IOB) and American Society of Brewing Chemists (ASBC) (Agu, 2006).

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Malting process

Malting was done by selecting the grain first, followed by steeping (the steep liquor was changed at 6 hr interval after which the grains were allowed to have a 2 hr air rest) for 40 hr, casting (the grains were drained off water and heaped on a jute bag) for 24 hr, germination (the grains were spread out on the jute bag very well for uniform aeration and germination) for 3 days and kilning (the malt is dried to reduce the moisture content). After kilning, the rootlets were removed by using fiction (abrasion).

Milling

The rice and sorghum malts were milled using milling machine to obtain moderately coarse grits used for mashing.

Mashing process

Two conical flasks were washed properly and labelled according to the malted grain used (50g of white sorghum malt and 50g of Rice malt). Distilled water (360ml) was added into each of the conical flasks containing ach malts. One millilitre (1ml) of exogenous enzymes; Amyloglucosidase, Fungamyl (β-amylase), thermamyl (α-amylase) and neutrase (proteinase) were added into each of the conical flask containing the samples and shaken properly. The conical flasks were covered with aluminium foil and placed in a water bath to commence mashing process. The temperature was raised to 35^0C and maintained for 30mins. The temperature was raised again to 45^0C and maintained for 30mins for Beta-glucanase activities. The temperature was raised again to 50^0C and maintained for 30^oC for proteolysis. The temperature was further raised to 63^0C and maintained for 1 hour for Beta-amylase activities. Finally, the temperature was raised to 72^0C and maintained for 30mins to allow for the activities of alpha-amylase. One (1) drop of iodine solution was added to check for saccharification and vigorously boiled for 10mins after a complete saccharification with yellow colouration as evidence. The essence of vigorous boiling for extra 10mins is to mash off. The samples (mashes) were allowed to cool and filtered using filter cloth to obtain a clear solution known as worts.

Wort analysis

The parameters determined were original gravity (O.G) ($^o\rho$), sugar (^{o}Brix), pH, flow rate (sec), viscosity (cp), temperature (^{o}C) and reducing sugar (glucose and maltose). This was done using the method of the Methods of Analysis of the Institute of Brewing.

Wort boiling

This was carried out before fermentation to sterilize the worts, inactivate the enzymes and extract the hop constituents. The worts were poured in a 500ml conical flask and arranged in a pot on a burning gas cooker. Hops (isomerised) was added and boiled for $1^{1/}{}_2$ hr.

Wort cooling and filtration

The hopped boiled worts were cooled to room temperature using heat exchanger technique by placing the conical flasks in a big basin containing cold water. The separation of hop debris and the coagulant protein (trub) from the wort was done with the help of sterile muslin cloth and filter.

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Wort fermentation

The cooled and aerated worts were now ready for yeast fermentation. Strain of Saccharomyces cerevisiae was employed for the fermentation process. The yeast was first reconstituted by mixing 20g of yeast, 10g of glucose with water in a container. It was shake vigorously and checked for pressure which signified that the yeasts were back to life. Ten (10ml) of yeast inoculum was added to each wort sample (pitching), the container was open to allow contamination by bacteria. At the end primary fermentation which last for 7days, the green beer samples were analysed for physicochemical properties and possible contamination by bacteria.

Beer analysis

Determination of alcoholic content

The percentage alcohol by weight of each green beer sample was determined by subtracting the final gravity of the beer from the original gravity of wort and multiplying by 0.129.

Calculation

(Original gravity of wort – final gravity of beer) x 0.129

Determination of apparent fermentability

The apparent fermentability is determined by subtracting the original gravity from the specific gravity divided by the original gravity and multiplying by 100%

Calculation

$$\frac{\text{Original gravity} - \text{specific gravity}}{\text{Original gravity}} \times \frac{100}{1}$$

Isolation of bacteria from the beer samples

All the media used were produced according to their manufacturer's instruction. About 0.5ml of each serially diluted beer samples (10^{-4}) were dropped on molten Nutrient, MacConkey and MRS agar plates and spread on the surface of the medium with a sterile wire loop. Nutrient and MacConkey agar plates were incubated at 30^{o}C for 24 hr. MRS agar plates were incubated at 37°C for 48 hr. Colonies exhibiting a surrounding clear zone were observed and counted. Distinct colonies observed in the incubated medium were transferred into freshly prepared Nutrient agar plates respectively. This was done to obtain a pure culture of each isolates.

Identification of bacterial isolates

Characterization and identification of bacterial isolates were based on standard microbiological methods including gram staining, morphological and cultural characteristics on media plates. Biochemical tests such as catalase, coagulase, indole, oxidase, citrate and urease tests were also carried out to determine their biochemical properties.

RESULTS

Wort analysis

This study showed bacteria that affect beer fermentation. Table 4.1 shows the result of wort analysis with original gravity of 1.030 and 1.032$^{0}\rho$ for worts produced from mass rice variety and white sorghum variety, respectively.

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Table 1: Wort analysis

Samples	Original Gravity ($^0\rho$)	Sugar (^{o}Brix)	pH	Temp (^{o}C)	Flow rate (sec)	Viscosity (cp)	Reducing sugar (mg/l) Glucose	Maltose
A	1.032	8.04	5.6	28	21.40	1.21	54.73	88.79
B	1.030	7.55	5.4	29	20.11	1.13	54.73	88.79

Key: A = Wort produced from sorghum malt, B = Wort produced from rice malt.

Beer analysis after primary fermentation

Table 4.2 showed result of beer analysis after primary fermentation with specific gravity of 1.005 and 1.004$^0\rho$ and alcohol of 3.27 and 3.66%v/v for beer produced from mass rice variety and white sorghum variety, respectively.

Table 2: Beer analysis after primary fermentation

Samples	Specific Gravity ($^o\rho$)	Sugar (^{0}Brix)	pH	Temp (^{o}C)	% Alcohol	Apparent Fermentability (%)
A	1.004	1.03	4.0	25	3.66	87.5
B	1.005	1.29	4.1	25	3.27	83.5

Key: A = Wort produced from sorghum malt, B = Wort produced from rice malt.

Mean total bacterial counts from beer samples

Table 4.3 shows the mean total bacterial counts from beer samples, the total bacterial counts was $1.2x10^4$ and $1.1x104^7$cfu/ml and the lactic acid bacterial count ranged from $1.3x10^4$ – $1.2.x10^4$cfu/ml for beer produced from mass rice variety and white sorghum variety, respectively with no coliform count.

Table 3: Mean total bacterial counts from beer samples (cfu/ml)

Samples source	Total Bacterial Count (Nutrient Agar)	Total Coliform counts (MacConkey Agar)	Lactic Acid Bacteria Count (MRS Agar)
A	$1.1x10^4$	-	$1.2 x10^4$
B	$1.2x10^4$	-	$1.3 x10^4$

Key: A = Wort produced from sorghum malt, B = Wort produced from rice malt.

Identification scheme of the bacterial isolates

Table 4.4 indicates the colony characteristics of the isolates identified along with their biochemical, Gram reaction and microscopic examination. The isolates identified includes Streptococcus, Lactobacillus and Micrococcus species.

Table 4: Identification Scheme of the Bacterial Isolates

Isolates type	Growth Appearance of Media	Biochemical test				Sugar fermentation					Possible organisms
		Gram stain	Cat	Ind	Oxi	Gl	F	Ml	Ma	La	
A	Large mucoid creamy colonies on MRS Agar	+ short rod in chains	—	—	—	AG	A	A	A	A	Lactobacillus sp.
B	Small creamy mucoid colonies on MRS Agar	+ cocci in chains	+	—	—	A	A	AG	A	A	Streptococcus sp.
C	Large creamy mucoid colonies on Nutrient Agar	+ short rod in chains	—	—	—	A	A	AG	AG	A	Lactobacillus sp.
D	Milky, round, raised, medium, buttery, opaque, smooth colonies on MRS Agar	+ cocci in clusters and pairs	—	—	—	A	A	A	A	A	Micrococcus sp

KEY: MRS = Man Regosa Sharpe Agar, Cat = Catalase test, Ind = Indole test, Oxi = Oxidase test, Gl = Glucose, F = D-Fructose, Ml= Maltose, Ma = Mannitol, La = Lactose, + = positive, — = negative, A = Acidic, AG = Acidic and Gas, G = Gas, +ve = positive, —ve = negative.

Discussion

Bacteria are isolated from beer samples produced from rice and local sorghum malts. The detection of microbial contamination in the food and beverage industry, specifically brewing, is vital for quality control purposes (Condina et al., 2019). The detection of beer spoilage bacteria in the brewery is done using PCR or conventional cultivation on selective media, according to current standard microbiological quality control procedures. Cultivation on selective agar is still used since PCR is expensive and requires known primers to identify a limited and specific number of microorganisms.

The sweet worts produced from local sorghum and rice malts showed good physicochemical properties when analysed, as required for good for brewing. The results of wort analysis as recorded in Table 4.1 showed original gravity of 1.032 and $1.030^{0}\rho$, sugar level (8.0 and $7.55)^{0}$Brix, pH (5.6 and 5.4) and viscosity (1.21 and 1.13)cp for worts produced from sorghum and rice malts, respectively. The results showed rice and local sorghum malts possess good brewing properties. This result is similar to the result of Lyumugabe et al. (2015).

The worts were allowed to ferment and analysed after primary fermentation. The results of beer analysis after primary fermentation showed the gravity, sugar and pH to reduce and alcohol produced. The specific gravity of the beer samples were 1.004 and $1.005^{0}\rho$, sugar (1.03 and 1.29^{o}Brix) and alcohol (3.66 and 3.27) %v/v. This indicates the activities of yeasts on the wort (Lyumugabe et al., 2012).

Determination of bacterial contamination on the beer samples was positive as discrete colonies were found on the Nutrient agar, MacConkey agar, De Man, Rogosa and Sharpe (MRS) agar plates after incubation. The total bacterial counts was $1.2x10^{4}$ and $1.1x104^{7}$cfu/ml and the lactic acid bacterial count ranged from $1.3x10^{4}$ – $1.2.x10^{4}$cfu/ml for beer produced from mass rice variety and white sorghum variety, respectively with no coliform count. This result agrees with the findings of Suzuki et al. (2008)

Streptococcus, Lactobacillus and Micrococcus species are the bacteria identified after being characterized morphologically, examined microscopically (gram stain) and biochemical tests. This result is similar to the findings of Bischoff et al. (2009). The presence of this organism in beer indicates contamination which can be detrimental to the finished beer. LAB, among beer-spoilers, have been reported as the most prevalent which has been estimated for 60–70% of all spoilage incidents (Jespersen and Jakobsen, 1996; Iijima et al., 2007; Weber et al., 2008; Haakensen et al., 2009).

Despite the low pH, moderate ethanol content and hop antimicrobials present in beer, microorganisms are responsible for most beer defects. However, hygienic conditions should be observed/practiced to minimize entry and growth of microbial contaminants throughout the beer-making process and ensure consistent manufacturing of high-quality beer.

Conclusion

In conclusion, results obtained from this study showed rice and local sorghum to be a good raw material for beer production and possible replacement of barley in brewing. The results also indicated the presence bacterial contamination in the beer samples under study and this is due to careless handling of fermentation processes, use of unsterilized equipment, poor storage, non-use of pure strains of brewing yeasts and unhygienic production process. Various bacteria identified included lactic acid bacteria such as Streptococcus, Lactobacillus and Micrococcus species with different morphological and microscopic characteristics. The prevalence of bacteria species in this study justifies a basic requirement for use of a single strain of microorganisms (pure yeast culture) in fermentation industry to produce desirable products. Good and adequate storage facility should be provided

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during and after fermentation of beer. Using a pure culture of yeast strain is recommended for fermentation. Sterilized equipment should be used during beer production. Good and hygienic manufacturing practices should be maintained while producing beer. Further studies should be carried out towards harnessing these bacteria species for a better purpose and also to see if they can be genetically modified to make them suitable for beer fermentation and production of useful metabolites.

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