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Biochemical tests performed for the identification of the isolates collected from local rice beer (Kiad)

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Abstract
Alcoholic beverages have been used since many centuries BC. They were and are still one of the important parts of many human activities and endeavours including research. It is also being commonly consumed by various individual groups of the society. In this study, bacteria isolated and identified from six local rice beer (Local name- *Kiad*) samples collected from various localities of Meghalaya were found to contain gram positive and gram negative of different genus.

Keywords: Meghalaya, gram positive, gram negative, Kiad *etc*

Introduction
Alcoholic beverages have been used since many centuries BC. They were and are still one of the important parts of many human activities and endeavours including research. It is also being commonly consumed by various individual groups of the society. In the Indian context, wine and alcoholic beverages are important to many societies especially during festivities. These are usually produced and used locally. Hence the types and quality differ greatly with varying traditions. Scientific studies of such process are very important to highlight the various aspects related to the production process and quality of the product. The consumption of rice beer prepared from rice is a common practice among many tribal communities residing in the North-Eastern states of India and many of them have been preparing it since time immemorial. Rice beer (Kiad) is also commonly used by the people of Khasi (Khasi Hills) and Pnar (Jaintia hills) in Meghalaya during various religious festivals and ceremonies. Minimum consumption is considered to be good for health and acts as a remedy for various ailments or diseases such as urinary trouble and dysentry but maximum or excess consumption may be intoxicated and harmful [2]. Its nutritional and the medicinal properties need further detailed investigation as many of the tribal people have not carried out modern experiment as they still have an indigenous method of local liquor preparation coupled with a clear understanding of nature and how it works. The starter material for making Kiad is '*thiat'* (natural yeast). This starter is prepared by mixing the dried and powdered leaves of *Amomum aromaticum* (khaw-iang), powdered local red rice *Oryza sativa* (Khaw saw) with spring water (Umpohliew) to get a sticky paste [3].

Materials and Methods
1. Collection of sample *i.e.* local rice beer (Kiad)
The local rice beer was collected from various localities present in Meghalaya like Mylliem (Sample A), Sohbar (Sample B), Polo (Sample C), Nongmensong (Sample D), Laitumkhrah (Sample E) and Mawmluh located in Sohra (Sample F) based on the information collected from the producers predominantly involved in the process of making rice beer.

2. Isolation of microorganisms from local rice beer (Kiad)
About 25-30 µl of the collected samples i.e. local rice beer (Kiad) were pipette in Nutrient Agar plates. The plates were then spread out properly throughout. Incubation was done at 37°C for 1-2 days and the total number of colony forming units was determined. The individual colony formed was picked up using a sterile inoculation loop and then transferred into another NA plate containing 10-15% ethanol in order to select only the alcohol tolerant species.

3. Biochemical Characterization
The isolates were then subjected to the various biochemical tests and the individual results

were recorded. The biochemical tests that were used to identify the unknown cultures according to[1] were: Catalase test, Methyl Red (MR) test, Voges Proskauer (VP) test, Citrate utilisation test, Indole production test, Amylase Production Test (or demonstration of starch hydrolysis), Carbohydrate Fermentation Test, Hydrogen sulfide production test and Oxidase test.

Results and Discussion

The result of the various Biochemical tests performed for the identification of the isolates was shown in Table 1.

Table 1: Cumulative result of all Biochemical tests

Test	Sample A	Sample B	Sample C	Sample D	Sample E	Sample F
Catalase Test	+	-	+	+	+	+
Methyl Red test	+	-	+	+	-	-
Voges-Proskauer test	-	-	-	-	-	-
Citrate utilisation test	+	-	+	+	+	-
Indole production test	+	+	+	+	+	+
Amylase Production Test	-	-	-	+	+	-
Carbohydrate Fermentation Test	Glu (+)	Glu (+)	Glu (+)	Glu (+)	Glu (+)	Glu (+)
	Lac (+)	Lac (+)	Lac (+)	Lac (+)	Lac (+)	Lac (+)
	Suc (+)	Suc (+)	Suc (+)	Suc (+)	Suc (+)	Suc (+)
Hydrogen sulfide production test	-	-	-	+	+	+
Oxidase test	-	-	+	+	-	+

("+" indicates positive result and "-" indicates negative results).

Five isolates from six samples showed positive results for Catalase test, these isolates can break down H_2O_2 to O_2 and H_2O shown by the appearance of effervescence of the H_2O_2 solution or bubbles which result due to the release of free oxygen gas (O_2 ↑). Isolate from sample B showed negative results for this test (Figure 1).

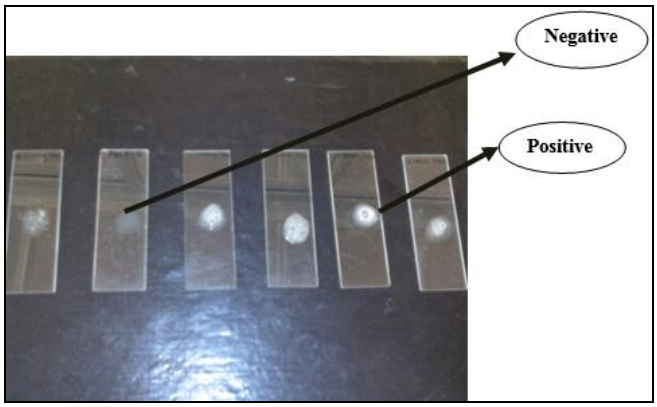


Fig 1: Glass slides showing positive and negative result for Catalase test.

Three isolates showed positive results to MR test while others are negative. When compared to the control, development of yellow colour was seen in these isolates due to the presence of a base (as Methyl red is an acid-base indicator) (Figure 2).

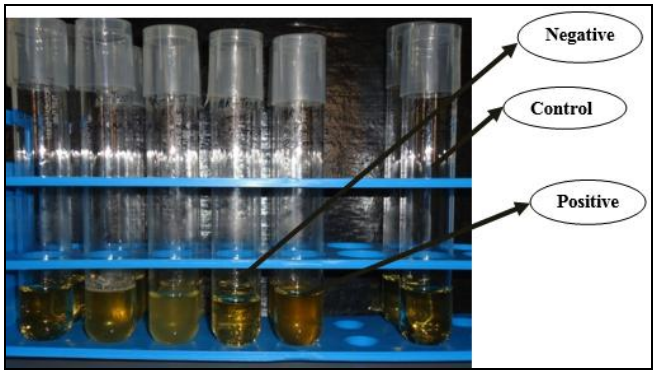


Fig 2: Positive and negative results marked for MR test.

In VP test, all the isolates showed negative results. No colour change, compared with that of the control, was observed in all the tubes (Figure 3).

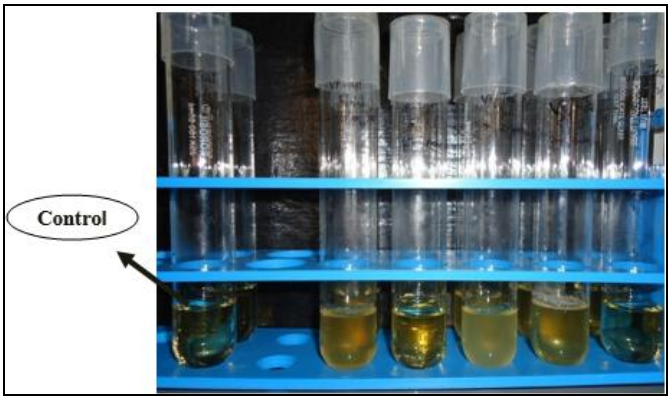


Fig 3: Negative results of VP test

Most of the isolates are able to utilize/ferment the compound Citrate as a source of carbon. Compared to the control, blue coloration of the medium was observed in 4 isolates which indicate the presence of an enzyme citrate permease, hence, citrate-positive while others showing green color are citrate-negative (Figure 4).

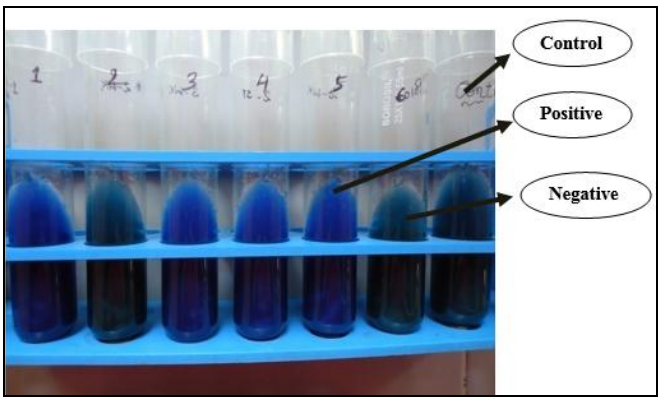


Fig 4: Blue coloration of the medium marked positive result while green coloration marked negative result for Citrate utilisation test.

A cherry red reagent layer is observed in all the tubes, hence, the isolates showed positive Indole production test which

indicated that they can oxidize tryptophan due to the presence of tryptophanase enzyme to produce indole, pyruvic acid and ammonia (Figure 5).

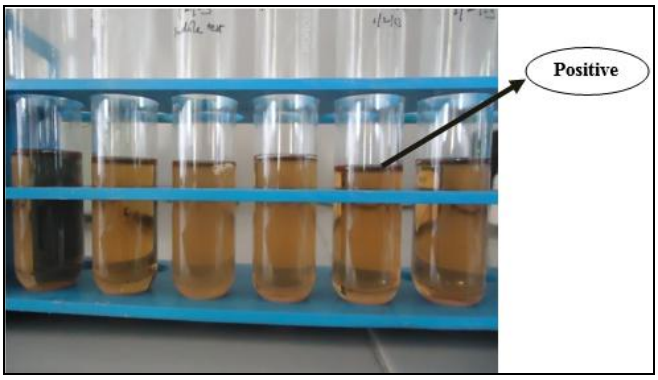


Fig 5: A cherry-red reagent layer seen in the tubes marked positive Indole production test.

Two isolates were found to hydrolyze starch, hence, amylase positive while the others are all negative i.e. the bacteria cannot hydrolyze starch as indicated by the total blue colouration of the medium (Figure 4.6).

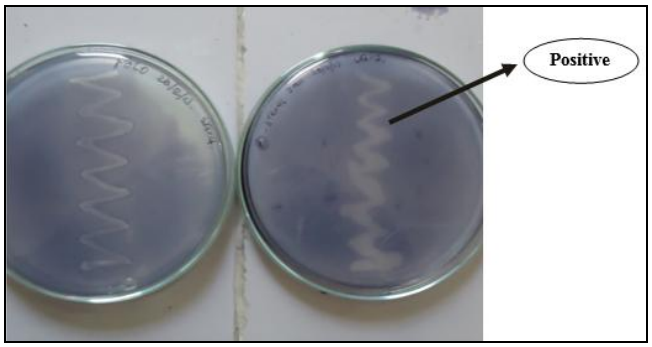
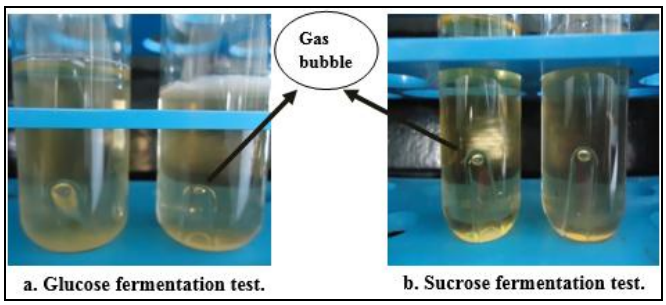
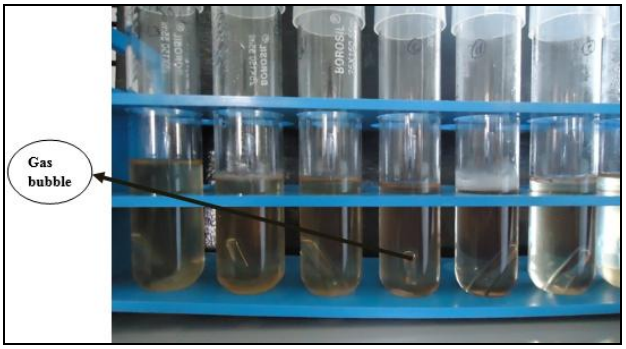


Fig 4.6: A clear zone round the colony showed positive Amylase production test.

Comparing to the uninoculated tubes (control), all isolates showed fermentative metabolism. They were able to ferment Glucose, Sucrose and Lactose. Yellow coloured medium due to the production of acid was seen in all the tubes while gas bubble in the Durham tube was seen only in lactose-*Sample C*, glucose-*Sample B and E* and sucrose-*Sample B and C*. Hence, this indicates that the isolates are positive to Carbohydrate fermentation test (Figure 4.7).





c. Lactose fermentation test.

Fig 4.7: Yellow coloured medium seen in all the tubes of (a) Glucose, (b) Lactose and (c) Sucrose broths. Gas bubble formed in some of the Durham tube indicates a positive fermentation test.

Three species were able to reduce sulfur-containing compounds to sulfides and found to produce H_2S gas. Blackening of the culture medium was also seen which indicates a positive test for H_2S production. The other three species showed negative for H_2S gas production as there is no black precipitate formation (Figure 4.8).

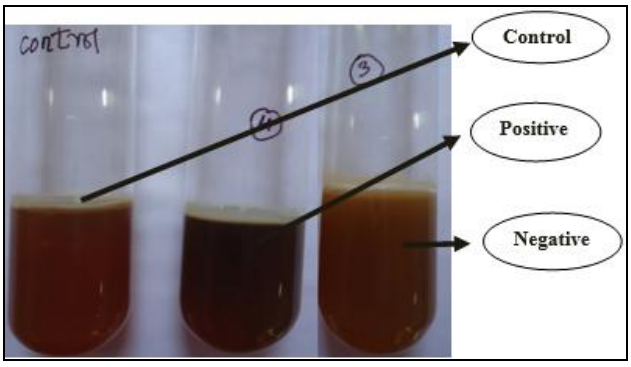


Fig 4.8: Blackening of the culture medium seen indicates positive Hydrogen sulfide production test.

It was also found out that the species of samples C, D and F showed positive oxidase test by the development of blue colour while the rest are negative. The clear oxidase reagent turns blue when cytochrome oxidase adds electrons to the chemical instead of to the oxygen, as it normally does. The positive results indicate the presence of the enzyme cytochrome oxidase. Absence of cytochrome oxidase is identified by no colour change in the strips, hence negative.

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