**ISOLATION AND CHARACTERIZATION OF INDUSTRIAL YEAST FROM PALM WINE IN LAGOS**

A PRACTICAL PROJECT AND SEMINAR PRESS BY

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YABA LAGOS

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*DECLARATION*

I hereby declare that this project was written by me and is a record of my research work out by Agunbiade, habeeb olamilekan with f/nd/20/3710121. It has not been presented in any previous application for a higher degree of this or any other institution. All citation and sources of information are clearly acknowledged by means of reference.

*CERTIFICATION*

This is to certify that this project entitled " ISOLATION AND CHARACTERIZATION OF INDUSTRIAL YEAST FROM PALM WINE IN LAGOS" was carried out by AGUNBIADE HABEEB OLAMILEKAN and submitted to the department of science laboratory technology, school of science.

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ABSTRACT KEYWORD: Palm wine, isolation, yeast, characterization, microbes.

Investigation was carried out on yeast isolated from palm wine obtained from Lagos, Nigeria. A total of 3 samples were analyzed and total yeast count was done on YDP, PDA & NA Agar using spread plate technique. The isolates were further characterized for certain attributes such as physiological and morphological presence. Palm wine considered as alcoholic beverages in Nigeria is known for microorganism and fungi. Recently bio mining of this local drink for high microbial strain have not gained much attention to scientist

*DEDICATION*

This project report is dedicated to Almighty Allah, the omnipotent, omniscience and omnipresence that has seen me through the period of my project.

*ACKNOLEGDEMENT*

I really must express my gratitude towards my supervisor "DR. ASHIRU WAIDI". I say a big thanks not only for guidance during this project but also for his patience and mentorship through my encounter in this research work. My heartfelt gratitude to the H.O.D, STAFF AND DEPARTMENT OF SCIENCE LABORATORY TECHNOLOGY for this opportunity to grow as a research scientist. My unending gratitude to my parents MR & MRS AGUNBIADE and my siblings for their endless love and support to my success.

**CHAPTER ONE**

*INTRODUCTION:*

The practice of isolating and evaluating local microbial strains for defined or potential commercial attributes is a common practice in the industrial microbiology and biotechnology. For isolating organisms, diverse environments such as water, soil, foods [including palm wine] are usually screened by using appropriate techniques. Saccharomyces cerevisiae is yeast [unicellular fungus]. The organism is usually isolated from sugary foods and beverages such as palm wine. Saccharomyces cerevisiae is common yeast of economic importance in food and beverage industries

Palm wine is a milky alcoholic beverage produced from palm wine. It contains diverse microbial population including bacteria, yeasts or moulds. Different species of yeast exist in palm wine. Yeast population among other organisms have been found to vary in palm wine depending on the source of the palm wine. The organism has fermentative and oxidative capabilities. Thus, it can convert the sugar content of the palm wine into alcohol [ethanol] and carbon dioxide under anaerobic conditions. The metabolic activities of the yeast in the palm wine normally create the physiochemical condition of the palm wine. Bechem et al. (2007) studied the physiological characteristics of some palm wine yeast isolates and some of these isolates showed to high concentration of sucrose and ethanol. Similarly, Chilaka et al. (2010) evaluated the efficiency of yeasts isolated from palm wine in diverse fruit wine production and concluded that acceptable wine could be produced from fruits using yeasts isolated from palm wine.

Palm wine yeast have been found to possess good sedimentation properties for high product recovery (Ukwuru and Awah, 2013). A milky juice containing initially well over 13% sucrose when it is collected fresh in the container from the palm tree, immediately after leaving the palm tree, yeast spores especially those of Saccharomyces cervisae infect the juice and start to ferment the fermentable sugars. Palm wine is predominantly fermented by yeast . Successful fermentations to produce ethanol using yeast requires tolerance to high concentrations of both glucose and ethanol. These characteristics are important because of high gravity fermentation, which are common in the ethanol industry, give rise to high sugar concentration at the beginning of the process and high ethanol concentration at end of the process. Saccharomyces cerevisiae is an important in bio-industries and its

tolerance to ethanol is one of the characteristics to decide if it can be used for bio fermentation resources

*BACKGROUND OF THE STUDY*

Palm wine is the fermented sap of the tropical plants of the palmae family. It is a popular traditional alcoholic beverage in Nigeria majorly produced and consumed in large quantities in the southern Nigeria. Some popular trees in Nigeria from which palm wine is tapped include Rafia Vinification, Elaeis Guineensis, Raphia hooked. This drink is consumed in several part of the world recognized as the natural white alcoholic drink. Various country and state have their unique name for palm wine; In yoruba, it is known as "Emu"; the hausas call it " bammi"; the igbo call it "mmanya"; the Chinese call it "panamcullo"; it is known as "tuba" in Mexico while "nsafufuo" or "doka" for the Ghanaian.

Palm wine contain nutritionally important components which includes sugars, protein, amino acids, alcohol and vitamins or minerals served in ceremonies such as traditionally weddings and funerals. They play an important role in medicines (herbal remedies) said to be useful for lactating mothers good for eyesight due to its high yeast content. Palm wine yeast have been found useful in baking, brewing and ethanol production.

Palm wine sap is collected from tapping the palm. This involves making a small incision in the bark of the palm tree about 15cm from the top of the trunk. A clean guord is tied around the tree to collect the sap which runs into it. The fresh palm wine juice is sweet, clear, colourless juice researched to contain 12-15% sucrose (by weight) and trace amount of reducing sugars including glucose, fructose, maltose and raffinose. However, these undergoes spontaneous fermentation by yeast and bacteria to produce a wide range of metabolites including ethanol, lactic acid, and acetic acid. This drink is a rich nutrient medium containing sugars, protein, amino acid, alcohol and minerals which makes this drink a veritable medium for growth in turn, change the physiochemical conditions of the wine giving rise to competition and sucession of organisms.

*STATEMENT OF THE PROBLEM*

The type of nature of yeast with leavened activity from palm wine samples from lagos state may have peculiar characteristics. Information in physiological studies of such indigenous yeast from Lagos is not available; this information is required for domesticating such yeast for industrial purposes.

Further studies on the yeast may give some good qualities and properties which will improve products in various industry.

*OBJECTIVES OF THE STUDY*

Therefore, the aim of this study was to evaluate the characterizing and isolating palm wine yeast for industrial process. These include for baking, ethanol production and single cell protein production.

*RESEARCH QUESTION*

This study asks the question if yeast posses certain attributes that could be necessary for efficient fuel ethanol production.

*JUSTIFICATION:*

Isolation and characterization of suitable microbes for use in production of economic importance are crucial in biotechnology. Indigenous strain must be isolated and subjected to rigorous studies they can be reliably used for these purposes as starter cultures. The research of this study would provide a basis for comparing palm wine yeast from Lagos (Nigeria) with those described in former reports and to confer Lagos palm wine yeast with different industrial useful properties.

*DEFINITION OF TERMS*

SACCHAROMYCES CERVISAE is a specie of yeast. It is perhaps the most useful yeast, isolated from sugary food and alcoholic beverages including local ones such as palm wine and beer. Yeast is rounded to void, about 5-10 micrometers in diameter. They reproduce by division processes known as budding. All strains of S. Cervisae can grow aerobically on glucose, maltose and tehalose and fail to grow on lactose and celliboise. However, growth on other sugars is variable Galactose and fructose are shown yo be two of the best fermenting sugars. The ability of yeast to use different sugars can differs depending on whether they are grown aerobically or anearobically. Some strains cannot grow anaerobically on sucrose and trehalose. All strains can use ammonia ad urea as the sole nitrogen source but cannot use nitrate since most amino acids, small peptides and nitrogen bases as a source. Yeast is used in viewing wine, when it is sometimes called top fermenting yeast because during fermentation process its hydrophobic surfa e causes the flows to adhere to CO2 and rise to the top of the fermentation vessel.

In Nigeria today, especially the southern, part palm wine, the fermented sap of oil palm tree is a popular beverage. The microbiology has been well studies and review of literature is available. S. Cerevisae is a component of palm wine and many Nigerian workers have explored both biological and economic as pectin of the species found in palm wine. For example, Ayanru, ovserves morp hological and physiological variants or races that could affect organoleptic qualities of palm wine. Other workers have described strain potentially useful as leavened agent for production of wine, beer and fuel ethanol.

Since yeast cell is having organism, it has numerous needs, and it is only if these are met that it will grow vigorously and produce a large quantity of carbon dioxide. Food and moisture are needed for growth. S. Cerevisae differs from other yeast species. It has more aerobic growth habit, maximum yielding capability during storage. It has usually derived from special selection of fast-growing occurring yeast strains.

**INDUSTRIALIZATION OF YEAST**

*ISOLATION OF YEAST*

The utilization of isolated strains of *S. cerevisiae* is an important strategy for keeping the quality and assuring the reproducibility of wine features. The utilization of strains isolated from specific regions could be even more interesting because of their high adaptation to their own climatic conditions and grapes. Even more, these strains are usually associated to wine characteristics that frequently identify specific wines and regions.

Isolating yeast at this point is as simple as taking a very small amount of your culture and rubbing (streaking) it on to an agar plate. Because of the stable, non-liquid agar medium, once streaked, single colonies of microbes are essentially stranded by themselves. After a few days or weeks (depending on incubation temperature and microbe population), they’ll multiply and grow large enough to be seen with the naked eye. At that point, it’s a matter of selecting the colonies you like and growing them up to larger amounts. It’s impossible to stress enough how important cleanliness and sanitation are at this point in the process. If you truly want a pure strain, you need to ensure you’re not contaminating what you’re trying to isolate with other cultures.

This process involves the identification of the physiological and morphological feature of yeast. Microbes can be identified physically and broadly using microscope after staining for its visual or presence. Due to the fungi presence in most yeast lactophenol blue staining is best for its viewing under microscope. Yeast is identified under category such as; color, elevation, shape, surface, opacity & so on.

Result part; YDP is beige color, NA is light yellow and PDA is yellowish brown

**CHAPTER TWO**

*LITERATURE REVIEW:*

Several research mentioned to isolation and identification of yeast strains for palm wine production:

J.D. Atputharajah et al. (1986) investigated the Microbiology and biochemistry of natural fermentation of coconut palm sap. A total of 166 isolates of yeasts and 39 isolates of bacteria were identified. Seventeen species of yeasts belonging to eight genera were recorded. The largest number of isolates (72%) belonged to genera Candida, Pichia and Saccharomyces. Saccharomyces chevalieri was the most dominant yeast species and accounted for 35% of the total isolates. Seven genera of bacteria were isolated. The predominant Genera was Bacillus. Others included Enterobacter, Leuconostoc, Micrococcus and Lactobacillus. The major physical, chemical and microbiological changes occurring in the fermenting sap indicated that a natural fermentation of coconut sap consist of an initial lactic acid fermentation, a middle alcoholic fermentation and a final acetic acid fermentation. It also appeared that activities brought about by micro-organisms of early phase helped the activities of the micro-organisms in each of the later phases. T.R Shamala, K.R Sreekantiah (1988) isolated and identified microorganisms that are responsible in fermenting wild date palm (Phoenix sylvestris) sap into wine (toddy). Saccharomyces cerevisiae, Schizosaccharomyces pombe, Acetobacter aceti, Acetobacter rancens, Acetobacter suboxydans, Leuconostoc dextranicum, Micrococcus sp., Pediococcussp., Bacillus sp. and Sarcina sp. were encountered in the freshly tapped sap. Most of these microorganisms were also isolated from the traditionally fermented fresh toddy samples. In a comparitive study on the fermentation of fresh sap and fresh toddy, certain variations in the growth pattern of these microorganisms were noticed. In addition to this, the amount of ethanol, volatile acid, non-volatile acid and esters produced during these fermentations also varied.

T.E. Ayogu et al. (1999) evaluated the performance of a yeast isolate from Nigerian palm wine in wine production from pineapple fruits. Saccharomyces cerevisiae species were isolated from the fermenting sap of Elaesis guineansis (palm wine) as a source of yeast for wine making from pineapple fruits. One of these isolates was used to pitch a pineapple must prepared as the fermenting medium. A high ethanol yield of 10·2% (v/v) was obtained when compared with a commercial wine yeast (control) which gave 7·4% (v/v), indicative of higher ethanol tolerance by this isolate. Ezeronye OU et al (2001) defined the genetic and physiological variants of yeast selected from palm wine. Genetic screening of 1200-palm wine yeasts lead to the selection of fourteen isolates with various genetic and physiological properties. Nine of the isolates were identified as Saccharamyces species, three as Candida species, one as Schizosaccharomyces species and one as Kluyveromyces species. Five of the isolates were wild type parents, two were respiratory deficient mutants (rho) and nine were auxotrophic mutants. Four isolates were heterozygous diploid (alphaa) and two were homozygous diploid (aa/alphaalpha) for the mating a mating types were further identified on mating with type loci. Four Mat alpha and four Mat types were further identified on mating with standard haploid yeast strains. Fortyfive percent sporulated on starvation medium producing tetrads. Fifty-two percent of the fourspored asci contained four viable spores. Maximum specific growth rate [micromax] of the fourteen isolates range from 0.13-0.26, five isolates were able to utilize exogenous nitrate for growth. Percentage alcohol production range between 5.8-8.8% for palm wine yeast, 8.5% for bakers' yeast and 10.4% for brewer’s yeast. The palm wine yeast was more tolerant to exogenous alcohol but had a low alcohol productivity. Hybridization enhanced alcohol productivity and tolerance in the palm wine yeasts. Nwachukwu et al. (2006) carried out on yeasts isolated from palm wines obtained from Southeastern Nigeria. The isolates were characterised for certain attributes necessary for ethanol production. Isolations were made from 600 hour-aged wines. The attributes investigated included ethanol tolerance and sedimentation rates. The effect of certain locally available supplements on ethanol tolerance was also investigated. Nine strains of Saccharomyces cerevisiae, two strains of S. globosus, and two strains of Hanseniaspora uvarum were isolated in this study. Results of the ethanol tolerance revealed a range of 10-20% (v/v), ethanol tolerance for the isolates. The sedimentation rates varied from 55.5 to 93.1%. Addition of local supplements enhanced ethanol tolerance of the isolates. Amoa-Awua WK et al. (2007) investigated the microbiological and biochemical changes which occur in palm wine during the tapping of felled oil palm trees. Microbiological and biochemical contents of palm wine were determined during the tapping of felled oil palm trees for 5 weeks and during the storage. Saccharomyces cerevisiae dominated the yeast biota and was the only species isolated in the mature samples. Lactobacillus plantarum and Leuconostoc mesenteroides were the dominated lactic acid bacteria, whilst acetic acid bacteria were isolated only after the third day when levels of alcohol had become substantial. The pH, lactic and acetic acid concentrations during the tapping were among 3.5-4.0%, 0.1-0.3% and 0.2-0.4% respectively, whilst the alcohol contents of samples collected within the day were between 1.4% and 2.82%; palm wine which had accumulated over night, 3.24% to 4.75%; and palm wine held for 24 h, over 7.0%. Stringini M et al. (2009) surveyed yeast diversity during tapping and fermentation of palm wine from Cameroon. They have investigated the occurrence of yeast flora during tapping and fermentation of palm wine from Cameroon. The yeast diversity was investigated using both traditional culture-dependent and culture-independent methods. Moreover, to characterize the isolates of the predominant yeast species (Saccharomyces cerevisiae) at the strain level, primers specific for delta sequences and minisatellites of genes encoding the cell wall were used. The results confirm the broad quantitative presence of yeast, lactic acid bacteria and acetic acid bacteria during the palm wine tapping process and highlight a reduced diversity of yeast species using both dependent and independent methods. Together with the predominant species S. cerevisiae, during the tapping of the palm wine the other species found were Saccharomycodes ludwigii and Zygosaccharomyces bailii. In addition, denaturing gradient gel electrophoresis (DGGE) analysis detected Hanseniaspora uvarum, Candida parapsilopsis, Candida fermentati and Pichia fermentans. In contrast to the progressive simplification of yeast diversity at the species level, the molecular characterization of the S. cerevisiae isolates at the strain level showed a wide intraspecies biodiversity during the different steps of the tapping process. Indeed, 15 different biotypes were detected using a combination of three primer pairs, which were well distributed in all the samples collected during the tapping process, indicating that a multistarter fermentation takes place in this particular natural, semicontinuous fermentation process. A. I. Elijah et al. (2010) investigated the effect of S. gabonensis (0.625%) and A. boonei (0.50%) on the kinetics of Saccharomyces cerevisiae isolated from palm wine (PW). Concentrations of the preservatives used in this study were optimal concentrations of the preservatives that had preservative effect on fermenting palm sap. The fermentation rate constant, k, of 2.79 × 10-4 mol-1s-1 obtained for untreated PW was higher than the k values for PW treated with A. boonei (1.7 × 10-4 mol-1s-1) and S. gabonensis (1.1 × 10-4mol-1sec-1). Both preservatives enhanced yeast growth. The specific growth rates (µmax) for the yeast were 0.43, 0.76 and 0.88 for the control, samples treated with A. boonei and S. gabonensis, respectively. However, the sedimentation rate of the yeast was reduced by both preservatives, but A. boonei produced the greatest effect [1].

Nguyen Van Thanh et al (2012) conducted based on survey selecting of yeast for making high quality palm wine. There are 18 yeast trains were obtained from palm juice at different treatment conditions. The treatment conditions did not affect the ability of yeast isolation. However, the ability of the presence of yeast in palm juice could be affected by harvesting time. Selected yeast train, which was isolated from palm juice harvested in afternoon without treatment, showed the best yeast strain for making palm wine with high alcohol content (13-14% v/v) [7]. Ho Kim Vinh Nghi et al (2013) study on the selection of Saccharomyces cerevisiae strains for production of wine from palmyrah palm flower’s saps. Palmyrah palm wine was fermented.

from Palmyrah palm flower’s saps, which was a special product of A Giang province. Natural Palmyrah palm wine fermenting process was related to Saccharomyces cerevisiaes, lactic acid fermenting bacteria and acetic acid fermenting bacteria. Naturalal uncontrolled fermenting process with multiform microorganisms led to unstableness and easy spoilage of this product quality. This research focused on the selection of Saccharomyces cerevisiae strains for wine fermentation from Palmyrah palm flower’s saps. Extract from Palmyrah palm flower’s saps included total sugar of 108.38 ± 11.74g/l, in which glucose was 30.24 ± 3.95g/l, protein was 1.59 ± 0.35 g/l and minerals were 1.6 ± 0.05g/l. Saccharomyces cerevisiae CNTP 7028 was selected, which was able to achieve 15.3%v/v, furfurol did not appeared, methanol content was low at 0.145g/l.

In our study, differenent yeast strains are isolated and characterized before palm wine fermentation. 3605 International Journal of Engineering Research & Technology (IJERT) Vol. 2 Issue 11, November - 2013 IJER.

Many other workers have indeed carried out studies aimed at isolating and exploiting palm wine yeasts for industrial processes. These include for baking, portable ethanol production and single cell protein production. Ogbonna (1984) and Onyedinma (1983) used palm wine isolates of Saccharomyces cerevisiae to produce artificial palm wine and beer, respectively. Very few efforts have been made at characterizing these yeasts fouel ethanol production. Despite the continuing researchefforts at utilising bacteria for ethanol production (Ingram and Burttke 1984), the yeast is still the primary choice for fermentation (Chandra and Panchal, 2003). In selecting yeasts for the efficient production of fuel ethanol (as opposed to portable ethanol), workers have set out certain requirements for these yeasts. These include being ethanol tolerant, osmotolerant, acid tolerant, and possession of flocculating properties depending on process requirements (Stewart et al.,1984). This work aims at investigating palm wine for yeasts possessing certain attributes that could be necessary for efficient fuel ethanol production.

**CHAPTER 3**

*MATERIAL AND METHODS*

Total of three (3) fresh palm wine samples were collected in sterile container from three (3) different location in lagos. Sources and codes of the wine are as shown in table 1. The palm wine sample collected where then taken to the environmental biology in Yabatech labelled A, B, and C respectively and were cooled in a cool refrigerator for 24hrs.

TABLE 1

|  |  |  |
| --- | --- | --- |
| Sample code | Type of palm wine | Sources |
| HB1 | Raffia palm wine | Akoka, lagos |
| HB2 | Raffia palm wine | Bariga, lagos |
| HB3 | Raffia palm wine | Pako bustop, lagos. |

Cool at 4⁰C.

PREPARATION OF MEDIA (AGAR)

Three (3) different agars which includes potatoes dextrose agar (PDA), Nutrient agar (NA), and YDP agar were used for the analysis with three conical flask containing distilled water. Xg of potatoe dextrose agar was measured using a weighing and was transferred in a conical flask containing 100ml of distilled water which was shaken vigorously for proper mixture. Another Xg of Nutrient agar was also measured and poured in a conical flask containing 100ml of distilled water, well shaken for mixture and Xg of YDP agar was also measured and transferred in a conical flask of 100ml distilled water, well shaken. These flasks were then plucked with cotton wool and foil paper and taped using a paper tape around the tip for sterilization using the autoclave.



*ISOLATION OF PALM WINE SAMPLE*

Work bench was sterilized first using ethanol and heat set up was also done at both bench end for avoid contamination. About seven (7) test tube labelled 10¹, 10², 10³, 10⁴, 10⁵, 10⁶, 10⁷ each used were rinsed well with distilled water and isolation of yeast from the sample was performed by serial dilution. 1ml of the palm wine sample (A) was transferred into 9ml of Sterilized distilled water inside the test ttubelabelled 10¹ using a sterilized syringe and was shaken well for mixture, 1ml of test tube 10¹ was drawn and transferred in test tube 10², this process continued till testube 10⁷. After dilution, basic test tube (like 10², 10⁴ & 10⁷) were picked for further culturing. From the diluents, 1ml was pipetted and released into the sterilized petri dish containing the culture agar of Nutrient agar. Same procedure was done for potatoes dextrose agar (PDA) and YDP agar then taken for incubation of 48hours at 28⁰C. Upon the next day, colonies were observed and counted, then stored in a refrigerator for 12hours. The isolated yeast was then streaked on another agar culture preparation and incubated for growth.

A glass container with a group of bottles in it

Description automatically generated with low confidence

A group of white candles

Description automatically generated with low confidence

*YEAST IDENTIFICATION*

Isolation and identification of yeast was done using standard morphological and physiological test of identification key described by Barnet et al., (1990). Incubation was at 28⁰C. The morphological and physiological characteristics of the yeast were studied which include colour, shape, elevation, number of visible colonies, edges opacity, surface and size.

*MICROSCOPY EXAMINATION*

After yeast are being identified physiological for above features such as color, shape etc. Microscopically, staining using lactophenol cotton blue (LPCB) was done by adding a drop of 70% ethanol on a clean microscopic glass slide, then the fungal specimen was added to the drop of alcohol using a sterile mounter such as innoculating loop. The fungal sample of the alcohol was teased using a needle mounter to ensure the sample mixes well with the alcohol. Using a dropper, two drops of lactophenol cotton blue solution (prepared above) was added before the ethanol dries off and carefully, the stain was covered with a cover slip without making air bubble to the stain and observed with a light microscope under ×10, ×40 and ×100 Magnification.

**CHAPTER FOUR**

*RESULTS:*

*PREPARATION OF CULTURE*

Preparation of culture has allowed the visibility of yeast. Upon mixture, YDP agar, and NA agar shows a \_\_\_\_\_\_\_\_\_ colour, while PDA agar is \_\_\_\_\_ in colour. After sterilization within some period of the time the agar begins to solidify.

TOTAL YEAST COUNT

Table 2 shows the total yeast counts from palm wine collected from this different location. YDP agar of test tube sample (10²) shows the highest number of colonies having 142CFU/mL obtained from \_\_\_\_\_\_\_, NA agar of test tube sample A (10²) shows the least number of colonies having 11CFU/mL and some NA agar like testube sample B (10⁷), C (10⁷) shows no bacteria colonies. Palm wine is enriched with nutrients and it is considered as excellent substrate for the growth and proliferation of micro organism such as bacteria yeast (Karamoko et al.., 2012; Santiago Urbina et al., 2013).

|  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Agar (PDA) | Color | Shape | Elevation | Visible colonies | Remark | Edges | Opacity | Surface | Size |
| 10⁷ (A) | White |  |  |  |  |  |  |  |  |
| 10⁷ (B) |  |  |  |  |  |  |  |  |  |
| 10⁷ (C) |  |  |  |  |  |  |  |  |  |
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*MORPHOLOGICAL & MICROSCOPIC IDENTIFICATION OF YEAST ISOLATES*

This is shown in table 3. Yeast was common to all the samples obtained from different locations. The morphological and physiological observation of the yeast isolates was seen to be cream or whitish color, raised, elevation, opaque, smooth in entire edges and only few were big and small. Some were bacteria and infected. The morphological feature of the yeast was confirmed by staining using phenol blue and mounting on a glass. On microscopic examination, blue colour having some round shape like structure were also observed.