Identification and Molecular Characterization of Probiotic Yeast with Anti - Fungal Activity

Hayder Idrees Hussein

Abstract: ***The investigation into the study is required to Obtain disconnects were additionally screened for their probiotic properties, for example, protection from low (pH 2.0, 2.5, 3.0), protection from bile salt (0.3%, 0.5%, 1.0%), antimicrobial test, anti-toxin protection test, haemolytic test and starch aging test. On plates the highest antifungal activity was observed with isolates HPoY8B9, HPoY9B10, HCoY2B6 which was found to be 71%, 65%, 60%. Isolates HPoY8B9, HPoY9B10, HCoY2B6 showed good antifungal activity and emulsification index percentage and hence were selected for further studies. Isolates HPoY8B9 showed highest emulsification index of 92% and 93% with coconut oil and engine oil as hydrocarbon source respectively. Whereas isolate HPoY9B10 showed 90%, 85% and isolate HCoY2B6 showed 90%, 91% of emulsification index with coconut oil and engine oil respectively after an incubation period of 5 days. The highest biosurfactant yield from isolates HPoY8B9, HPoY9B10, HCoY2B6was found to be 2.0, 1.8, 1.2 gm/L respectively after 5 days of incubation. Crude biosurfactant extracted from these three isolates was subjected to TLC for the identification of sugars and lipids present. All the Three isolates were then screened for their probiotic properties and were observed to have the capacity to endure against stomach environment(pH 2.0, 2.5, 3.0), they could make due against 0.3%, 0.5%, 1.0% centralization of bile salts. Isolates HPoY8B9, HPoY9B10, HCoY2B6 indicated protection against all the five anti-infection agents tried and were even observed to be non-haemolytic. Of the three isolates tried segregate HPoY8B9 acquired from coconut indicated great antifungal, biosurfactant and probiotics properties and consequently was sent for 18S rRNA sequencing and results uncovered it Saccharomyces cerevisiae HPoY8B9. Molecular Identification of Probiotic Strains Methods utilized for discovery of probiotics in human gastrointestinal tract are ID of province morphology, maturation designs, serotyping or some mix of these. In spite of the fact that these conventional techniques have constraints they are utilized for recognizable proof. With the creating innovation about the sub-atomic writing it is getting more solid to distinguish and separate bacterial strains. Classical microbiological systems are extremely critical for choice, list and biochemical portrayal (maturation profiles, salt-pH temperature resiliences) yet it isn't proficient to order a culture systematically. Atomic portrayal strategies are effective even between firmly related species. There are number of option ordered grouping strategies surely understood incorporating hybridization with species-particular tests and age of profile PCR candidates by species-particular preliminaries. Polymerase chain response based techniques (PCR-RFLP, REP-PCR, PCR ribotyping and RAPD) are predominantly utilized as atomic apparatuses. Examination between these techniques, the most intense and precise one is sequencing.***

Keywords: (0.3%, 0.5%, 1.0%), (pH 2.0, 2.5, 3.0), The highest biosurfactant yield from isolates HPoY8B9, HPoY9B10, HCoY2B6was found to be 2.0, HPoY8B9, HPoY9B10, HCoY2B6 HPoY8B9. (PCR-RFLP, REP-PCR, PCR ribotyping and RAPD).

I. INTRODUCTION

A. Yeast Metabolism

Yeasts are heterotrophic living beings, implying that vitality digestion and carbon digestion are personally interconnected. Adenosine triphosphate (ATP) is gave by oxidation of natural 12 atoms that additionally go about as carbon hotspots for biosynthesis, and is at last utilized as the vigorous transitional for all intents and purposes every single cell movement. Yeasts have moderately straightforward dietary necessities, a carbon source, a nitrogen source (ammonium salt, nitrate, amino acids, peptides, urea, purines, pyrimidines), phosphate, sulfate, bring down groupings of potassium, magnesium, calcium, iron, zinc, and by and large a vitamin, for example, biotin, thiamine, or pantothenic corrosive making up a total development medium. It is notable that the key carbon source utilized by yeasts is starch, fundamentally hexose sugars as monosaccharides (glucose, fructose, galactose, or mannose) or disaccharides (maltose or sucrose).In expansion, an extensive variety of other carbon sources (e.g., alcohols, natural acids) can be used under vigorous conditions. Van Dijken and Scheffers (1986) arranged yeasts physiologically as indicated by the sort of vitality creating process engaged with sugar digestion, in particular non-fermentative, facultatively fermentative, or obligately fermentative. It was later discovered that basidiomycetous yeasts, for example, Cryptococcus, Rhodotorula, and others are non fermentative and entirely oxygen consuming.

Not even the commit fermentative species can get by for long under strict anaerobic conditions, since the union of certain film constituents (i.e., sterols) requires oxygen. Yeast digestion and physiology are in this manner firmly subject to sugar and oxygen accessibility. Yeast high-impact breath has been characterized by Dawes (1986) as the entire oxidation of carbon-containing particles to CO2 and H2O by the interrelated procedures of the tricarboxylic corrosive (TCA) cycle and the electro transport bind coupled to phosphorylation with oxygen as the terminal electron acceptor. In yeast anaerobic digestion, frequently called "alcoholic aging," pyruvate created by glycolysis is part into ethanol and CO2 in a redox-impartial process. At last, there are three regularly observed impacts related with the sort of vitality creating forms engaged with sugar digestion or potentially oxygen accessibility; Pasteur, Cabtree, and Custer impact. Because of its modern significance, better comprehension of yeast digestion is required keeping in mind the end goal to give understanding into.

Revised Version Manuscript Received on December 16, 2017.

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The development of essential and optional metabolites and their effect on human health.

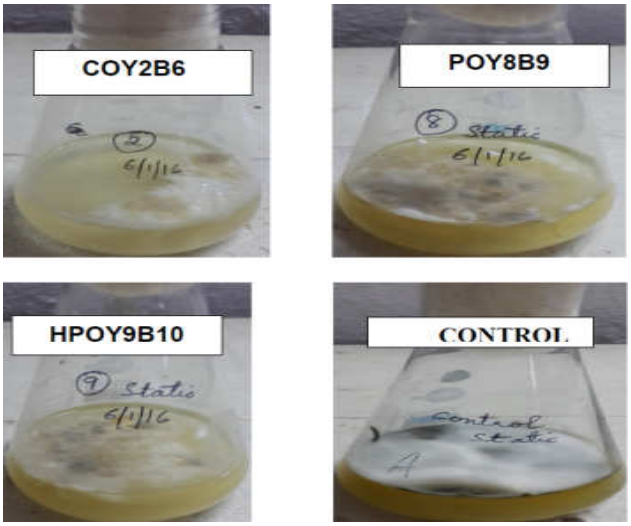

Figure 1: Antifungal Activity by Yeast Isolates Against M. Phaseolina in Broth under Stationary Condition.

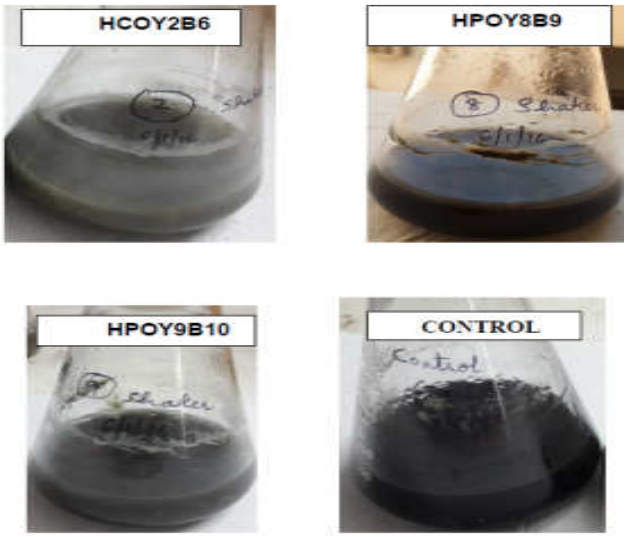

Figure 2: Antifungal Activity by Yeast Isolates Against M. Phaseolina in Broth under Shaking Conditions.

Table 1: Antifungal Activity of Yeast Isolates Against Pathogenic Fungi.

ISOLATE	% Inhibition	
	MACROPHOMINA PHASEOLINA	SCLEROTIUM ROLFSII
HCoY1L7	-ve	-ve
HCoY2B6	60%	-ve
HKiY3B4	56%	-ve
HCoY4B8	59%	-ve
HPoY5B5	-ve	-ve
HCoY6L6	-ve	-ve
HPoY7L9	52%	-ve
HPoY8B9	71%	-ve
HPoY9B10	65%	-ve
HCoY10L8	-ve	-ve
HKiY11L4	-ve	-ve
HCoY12B7	50%	-ve
HPoY13L5	-ve	-ve
HPoY14L10	-ve	-ve
HPaY15S11	-ve	-ve
HSaY16L1	-ve	-ve
HMaY17S6	43%	-ve
HCuY18S8	26%	-ve
HCuY19L5	-ve	-ve
HChY20L7	-ve	-ve
HCkY22L7	23%	-ve
HBaY30L4	39%	-ve

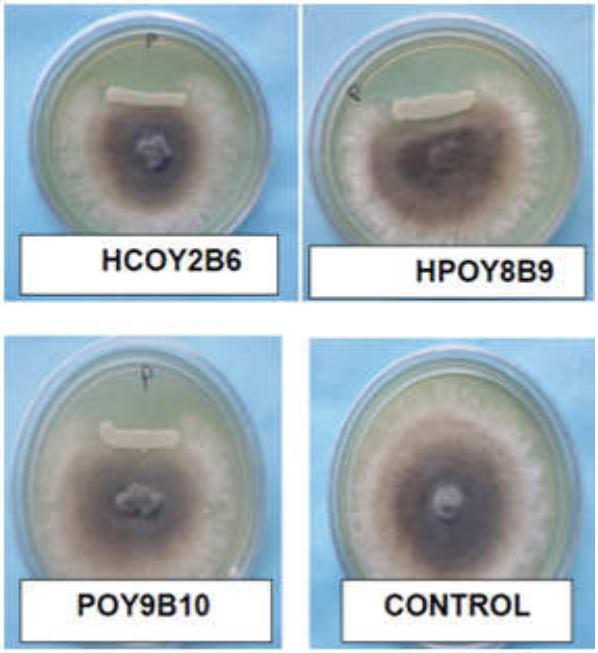

Figure 3: Antifungal Activity on Plates

II. ANTAGONISTIC CHARACTERISATION OF YEASTS

The utilization of antagonistic microscopic organisms to repress pathogenic microbes has been considered broadly finished the years, while little consideration has been given to yeasts in a comparative part. The examination and potential uses of antibacterial mixes discharged by yeasts are in this manner still at a beginning time of advancement. Threat of microorganisms by yeasts has been credited essentially to (1) rivalry for supplements, (2) pH changes in the medium because of development coupled particle trade or natural corrosive production,(3)production of high groupings of ethanol, (4) discharge of antibacterial mixes and arrival of antimicrobial mixes, for example, executioner poisons or "mycocins". Mycocins are extracellular proteins or glycoproteins that disturb cell layer work in vulnerable yeasts, which bear receptors for the compound. Their movement is coordinated principally against yeasts firmly identified with the maker strain, which has a defensive factor. The principal mycocins were recognized in relationship with S. cerevisiae in the fermenting business. A few have since been separated, much of the time where yeast populaces exist in high thickness and in profoundly aggressive conditions.

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Mycocin generation happens among numerous yeast genera including Saccharomyces, Candida, Cryptococcus, Debaryomyces, Kluyveromyces, Pichia, Torulopsis, Williopsis, and Zygosaccharomyces. Genetic and molecular studies and investigations have demonstrated that the executioner poison characteristic might be carried on extrachromosomal components as twofold stranded RNA infections, on doublestranded direct DNA, or on a chromosome. The notable instruments of the executioner poison are the interference of cell division by obstructing the DNA amalgamation, restraint of combination of the cell divider segment β1,3-glucan, and particle spillage caused by the development of diverts in the cytoplasmic layer.

Not at all like yeast-against-yeast threat, the antibacterial properties of yeast are substantially less reported. Truly, the main positive signs of the opposing movement of yeasts distributed right on time in the twentieth century by Hayduck (1909) who announced an unstable thermolabile lethal concentrate from yeast most likely an amine that hinders the development of Escherichia coli and Staphylococci. Fatichenti et al. (1983) demonstrated that the antibacterial action of Debaryomyces hansenii against Clostridium tyrobutyricum and Clostridium butyricum was identified with its capacity to create both 14 extracellular and intracellular antimicrobial mixes. Bilinski and Casey (1989) revealed restraint of the development of the lager decay microscopic organisms Bacillus megaterium and Lactobacillus plantarum because of the change of methylene blue into a pharmacologically dynamic shape by Kloeckera apiculata and Kluyveromyces thermotolerans. Dieuleveux et al. (1998) thusly portrayed restraint of Listeria by a strain of Geotrichum candidum confined from French red smear cheese. The two anti-listerial compounds (d-3-phenyllactic and d-3-indollactic acids) are stable over a wide pH range and can be heated to 120°C for 20min. Also, Cavalero and Cooper (2003) demonstrated that Candida bombicola produces extracellular glycolipids called sophorosides, which have proven antibacterial activity against Staphylococcus aureus and also inhibit Candida albicans. Having tested hundreds of dairy yeasts, Goerges et al. (2006) reported a strain of Candida intermedia capable of reducing viable Listeria counts by 4log CFU/cm2 in co-culture on agar, while three C. intermedia and one Kluyveromyces marxianus suppressed L.monocytogenes growth by 3logCFU/cm2.

The same group more recently found a strain of Pichia norvegensis (WSYC 592) able to reduce L. monocytogenes counts by 7 log cycles, while numerous strains of Issatchenkia orientalis, Candida krusei, and K. marxianus reduced Listeria counts by 4–5 log units in co-culture on agar. However, strainWSYC592 decreased Listeria counts on Tilsit cheese by only one log cycle. More recently, Hatoum et al. (2012) characterized anti-listerial hydrophobic peptides extracted from cultures of four wild dairy yeasts identified as D. hansenii, P. fermentans, C. tropicalis, and W. anomala. In experiments using a Camembert curd model, the anti-listerial compounds of D. hansenii and W. anomalus were found to reduce L. monocytogenes counts by 3log units during the first 9 days of ripening. The active principles are thermostable and apparently peptides and appear to induce leakage in bacterial cells and ultimately cause bacterial lysis.

III. IDENTIFICATION OF YEAST ISOLATE

Identification of yeasts up to species level was carried on the basis of standard cultural, morphological and physiological /biochemical tests.

(Harrigan and McCance, 1982; Barnett et al., 1990; Vaughan-Martini and Martini, 1993; Sanni and Lonner, 1993; Kurtzman and Fell, 1998; and De Maristela et al., 2006).

A. Cultural Characterization

Cultural characteristics of yeast isolates were performed by streak culturing of the isolates on YMPD plates and incubated at 30°C for 48 hours and then the colonies were observed for following characteristics:

i) **Shape**: Shapes of the isolated colonies were recorded as circular or irregular.

ii) **Color**: Color was recorded as creamy, off white, white, orange, greenish black or black.

iii) **Edge**: Edge of the isolated colonies was recorded as entire, dentate, lobate, cottony or undulate.

iv) **Opacity**: Opacity of the isolated colonies was recorded as opaque or translucent.

v) **Elevation**: Elevation was recorded as raised, convex or spread.

vi) **Surface:** Surface was recorded as rough or smooth.

vii) **Consistency**: Consistency was recorded as viscid, non viscid, solid, oily or liquid.

B. Morphological Characteristics

Keeping in mind the end goal to decide morphology of yeast cells and multiplication sort, the way of life were analyzed minutely (Barnett et al., 2000). Vegetative cells were seen following 3 days of brooding at 30°C in YMPD medium. Morphological qualities were controlled by minute examination.

i) Slide preparation: a small drop of sterile deionized water was placed upon the center of the slide. With proper aseptic technique, the inoculating loop was used to smear a small amount of the culture into the drop and a proper thin smear was made out of it.

Then the slide was allowed to air dry and then heat fixed

ii) Gram staining: Heat fixed the slide by ignoring it the fire a few times. The warmth settled slides were overflowed with precious stone violet and kept for 60 seconds, at that point flush tenderly with tap water. At that point overflowed the slides with the Gram's iodine and kept for 60 seconds, at that point flushed with tap water. Next, the slides were held at an inclination and squirt with the decolorizing operator ethanol or CH3)2CO for three seconds, letting the decolorizer and overabundance color keep running off the slides. At that point the slides were flushed promptly with tap water. These were then counterstained by flooding the slides with safranin for two minutes. At that point flush with tap water and smear dry with the tissue paper.

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At the point when the cells are seen with the magnifying instrument, ascospores will be Gram-negative (pink) and the vegetative Saccharomyces cells will be Gram-positive (violet).

IV. ANTIMICROBIAL ACTIVITY OF YEAST

A. Antibacterial Activity

Five strains of bacteria used were Bacillus subtilis, Staphylococcus aureus, Pseudomonas aeruginosa, Klebsiella pneumoniae and Escherichia coli. The stock culture was maintained on nutrient agar medium at 4°C. In vitro antibacterial activity was determined by using nutrient agar mediumobtained from Himedia Ltd. All the five bacterial strains were aseptically inoculated into sterile nutrient broth and incubated over rotary shaker at 180rpm, for 24 hrs at 37°C. The agar well diffusion method was followed for antibacterial susceptibility test. Petriplates were prepared by pouring sterile nutrient agar medium and allowed to solidify. These plates were inoculated with 0.1ml of overnight grown bacterial cultures, spread properly and allowed to dry. Well were made with the help of sterile borer. 20µl of active cultures of yeasts were inoculated into the wells and incubated at 30°C for 48 hrs. Plates were observed for zone of inhibition after 48 hrs of incubation.

B. Antifungal Activity

Plate Assay by Dual Cuture Method

Three fungal pathogens namely *Sclerotium rolfsii, Macrophomina phaseolina, were* used to test the antifungal activity of the yeasts obtained. The stock culture was maintained on potato Dextrose Agar medium at 4°C. In vitro antifungal activity was determined by using potato dextrose agar medium obtained from Himedia Ltd. Potato dextrose agar media plates were prepared and allowed to solidify. *Sclerotium rolfsii, Macrophomina phaseolina,* fungal plugs were carefully placed in the centre of potato dextrose agar plates and incubated at 28±2°C for 24 hrs. After 24 hrs of incubation, plates were checked for vegetative growth of mycelium and active culture of yeast was streaked towards one end of the plate. Plates were incubated at 28±2°C for 96 hrs. Control was maintained with only fungus. Percentage of inhibition was calculated against control.

$$\text{Percentage of inhibition} = \frac{\text{diameter of fungal mycelium} - \text{diameter of test mycelium}}{\text{diameter of fungal mycelium}} \times 100$$

C. Broth Assay

100ml Potato dextrose broth was prepared in 250ml erlynmeyer conical flask and was inoculated with fungal plugs. All the flasks were incubated at 28±2°C for 24 hrs. After 24 hrs of incubation, flasks were inoculated with 2% of yeast inoculum and incubated at 28±2°C for 96 hrs. Biomass reduction was calculated by filtering the fungal mycelium through whattmann NO.1 filter.

The fungal biomass was died at 60°C for 6 hrs to remove moisture. Biomass reduction was calculated using the formula

$$\text{Percentage inhibition} = \frac{\text{diameter of fungal mycelium} - \text{diameter of test mycelium}}{\text{diameter of fungal mycelium}} \times 100$$

D. Screening for Biosurfactant Activity

Emulsification test (E24): 1% inoculum of overnight grown yeast culture was inoculated into 250ml erynmeyer conical flask containing 100mL of YMPD and incubated on rotary shaker at 180 rpm at 30 °C for 24 hrs. After the incubation, 2mL of culture was centrifuged at 10000rpm for 10 mins. 2 mL hydrocarbon (oil) was added to the supernatant and vortexed for 1min and allowed to stand for 24 h. the emulsification index was maeasured for every 24 hrs till 7 days. The emulsion index (E24) is the height of the emulsion layer (cm) divided by total height (cm), multiplied by 100.

$$\text{Emulsification index (E24)} = \frac{\text{Height of the emulsion layer}}{\text{Total height}} \times 100$$

E. Inoculums Preparation

Inoculum was developed by growing yeast cells at 30 °C in 100 ml YMPD medium ontaining (g l-1): malt extract, 3.0; glucose, 100; yeast extract, 3.0; peptone, 5.0; pH 6.0 and shaken at 180 rpm for 24 h.

F. Production of Biosurfactant

Production of sophorolipids from the selected strains HPoY8B9, HPoY9B10, HCoY2B6 was carried out in 1 liter Erlenmeyer flasks containing 250 ml of optimized media of composition (g l-1): malt extract, 3.0; glucose, 100; yeast extract, 3.0; peptone, 5.0. Media pH was adjusted to 6.0. All the flasks containing 250 ml of media were supplied with 1 ml of oleic acid. Total 4 ml of fatty acid was used for 1 liter of media. The flasks were then inoculated separately with 10 % (v/v, 25 ml inoculum to each 250 ml media) 24 h grown inoculum and incubated on rotary r shaker at 180 rpm for 168 h at 30 °C for sophorolipid production.

G. Extraction of Sophorolipids

After incubation of 168 h it was observed that brownish oily viscous layer of SL settled down at the bottom of the culture broth. Culture medium was centrifuged at 10000 rpm for 20 min. The supernatant was separated and extracted twice with equal volumes of ethyl acetate. To ensure the complete isolation of sophorolipids, the cells were also washed with ethyl acetate so as to remove the glycolipids and fatty acid adhered to cells. The solvent was removed by rotary-evaporation at 40 °C. The brownish viscous product was obtained, which was washed twice with n-hexane to remove the unreacted fatty acid and was dried under vacuum. Sophorolipid yield was calculated gravimetrically. The product was stored at 4 °C till further use.

V. RESULT

A. Screening for Probiotic Properties

Resistance to low pH

Isolates HPoY8B9, HPoY9B10, HC oY2B6 were tested for its resistance against low pH (2.0, 2.5, 3.0). The viability of the isolates was tested by measuring the absorbance at 660 nm for 0 hr, 2hrs, and 4hrs.

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One isolates from coconut and two isolates from pomogranate showed high acid tolerance at pH (2.0, 2.5, 3.0) after an exposure up to 3 hrs. with a survival rate of 56 to 100%. Even after increasing the exposure time to 4 hrs. there was no significant decline in the survival rate.

Table 2. Resistance of Isolates Against Low Ph

Isolate	pH	0.hr	2.hrs	4.hrs
HCoY2B6	2.0 2.5 3.0	0.458 0.407 0.351	0.486 0.448 0.710	0.344 0.446 0.954
HPoY8B9 2.0	2.5 3.0	0.460 0.491	0.355 0.414	0.314 0.381
HPoY9B10 2.0	2.5 3.0	0.486 0.453	0.333 0.344	0.358 0.371

B. Tolerance Against Bile

Isolates HPoY8B9, HPoY9B10, HC oY2B6 were tested for its tolerance against bile (0.3%, 0.5%, 1.0%). The viability of the isolates was tested by measuring the absorbance at 660 nm for 0 hr, 2hrs, and 4hrs, 6hrs. All the three isolates were found to be tolerant to different bile concentrations tested. When the concentration of oxbile was increased to 1%, there was practically no decline in the survival rate.

Table 3. Tolerance of Isolates against Different Bile Concentrations.

Isolate	bile concentration	0 hr	2 hrs	4 hrs	6 hrs
HCOY2B6	0.3%	0.461	0.473	1.081	1.595
	0.5 %	0.463	0.518	0.933	1.504
	1.0%	0.474	0.357	0.369	0.682
HPOY8B9	0.3%	0.471	0.524	1.008	1.835
	0.5%	0.465	0.553	1.220	1.827
	1.0%	0.462	0.310	0.564	0.756
HPOY9B10	0.3%	0.487	0.678	0.937	1.634
	0.5%	0.479	0.529	0.959	1.609
	1.0%	0.477	0.331	0.388	0.543
HChY19	0.3%	0.459	0.682	0.720	0.970
	0.5%	0.437	0.543	0.636	1.094
	1.0%	0.453	0.385	0.546	0.884

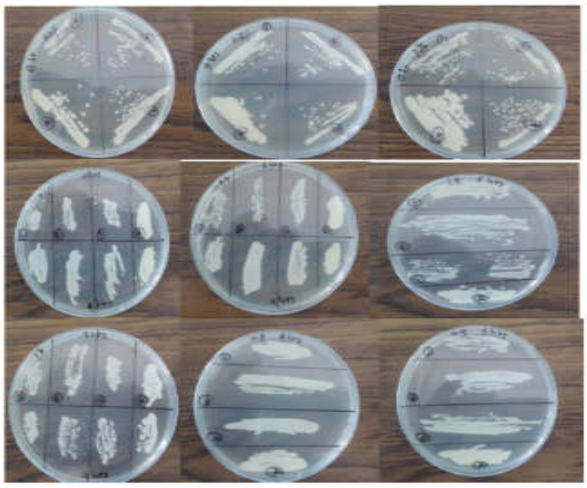

Figure 4: Tolerance of isolates HPoY8B9, HPoY9B10, HC oY2B6 against bile concentrations (0.3 %, 0.5 % and 1.0 %).

C. Antimicrobial Activity

Isolates were tested for its antibacterial activity against Escherichia coli, staphylococcus aureus, klebsiella sp, Pseudomonas sp. Bacillus sp, All the three isolates did not show any inhibition against the bacteria tested.

D. Antibiotic Suceptibility Test

Isolates HPoY8B9, HPoY9B10, HC oY2B6 were tested for its susceptibility against the antibiotics gentamycin, vancomycin, erythromycin, nalidixic acid and ciprofloxacin. All the three isolates showed resistance to all the five antibiotics tested and zone of inhibition was observed around the disk.

Table 4

Isolate	genta mycin	erythro mycin	Nalidixic acid	Ciprofl oxacin	vanco mycin
HCoY2 B6	Resist ant	Resista nt	Resistant	Resista nt	Resist ant
HPoY8 B9	Resist ant	Resista nt	Resistant	Resista nt	Resist ant
HPoY9 B10	Resist ant	Resista nt	Resistant	Resista nt	Resist ant
HChY1 9	Resist ant	Resista nt	Resistant	Resista nt	Resist ant

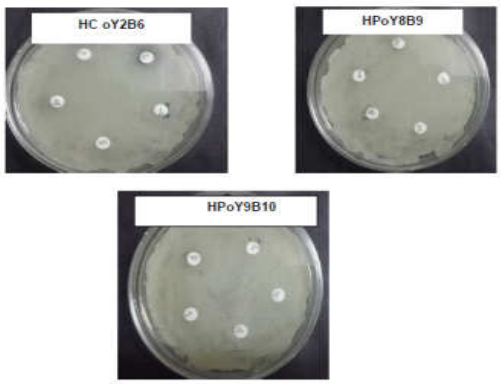

Figure 5: Antibiotic Suceptibitity Test

E. Haemolytic Activity

Isolates HPoY8B9, HPoY9B10, HC oY2B6 were tested for its haemolytic activity on blood agar plates and all the three isolates were found to be non haemolytic.

F. Sugar Fermentation Test

Three isolates HPoY8B9, HPoY9B10, HCoY2B6 were tested for their sugar fermentation abilities. All the three isolates showed production of gas, which was evitable by the formation of gas bubble in durhams tube. could not ferment xylose. Isolates HC oY2B6 , HPoY8B9 showed acid production as a result of fermentation of dextrose, fructose, maltose and mannose, whereas isolate HPoY9B10 could ferment dextrose, fructose and mannose and no acid production was observed in case of maltose though there was formation of gas bubble in Durhams tube.

Table 5: Sugar Fermentation Ability

Isolate	Dextrose		Xylose		Maltose		fructose		Mannose	
	acid	gas	acid	gas	acid	gas	acid	gas	acid	gas
HCoY2B6	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve
HPoY8B9	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve
HPoY9B10	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve

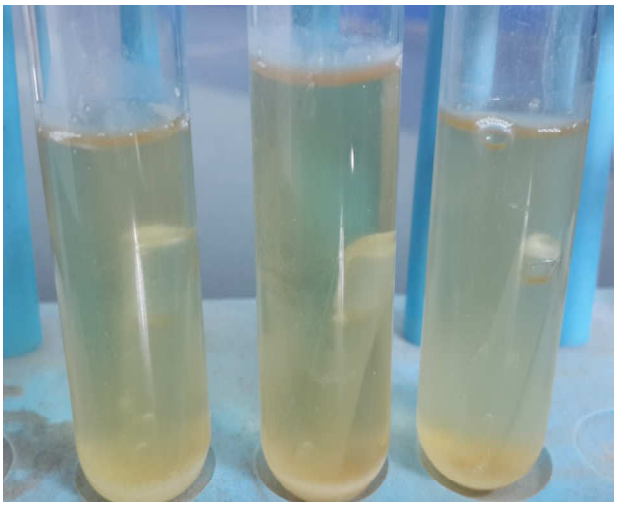

Figure 6: Sugar Fermentation Test

VI. CONCLUSION

Three yeast isolates have been selected from this study. Of the three isolates, based on biochemical and microscopic it is identified as Saccharomyces spp. The isolate HPoY8B9 has been identified as Sacharomyces cerevisiae and has been confirmed as new biosurfactant producers with ability to produce assayable biosurfactant activity. These isolates have the potential to produce biosurfactants with new properties and applications. Further studies are required to establish optimum culture conditions, develop purification strategies and elucidate the chemical structure and characterize the nature of the biosurfactants produced by each isolate. Isolates HPoY8B9, HPoY9B10, HCoY2B6 were further tested for their probiotic nature and were confirmed as probiotic strains by performing various test e.g staining, Resistance to low pH (3.0 &1.0), Bile salt tolerance, Antimicrobial activity test, Carbohydrate fermentation test (Glucose, fructose, xylose, maltose and mannose), haemolytic test, antibiotic resistance test. Major trait of probiotics is their capability to survive in stomach pH (1.5-3.0) and intestine pH(7.1-7.4), bile concentration (0.3%). Percentage inhibition of the three isolates HPoY8B9, HPoY9B10, HCoY2B6 against fungal pathogen Macrophomina phaseolina was also found be good. From The above information, we can concluded that, isolated yeasts from coconut and pomegranate are probiotics. Use of probiotics has long history and are used for various healing purpose. So, by proper evaluation and investigation, isolated strains can be used as probiotics. For solving antibiotic resistance and health hazard, now probiotics are main intention of researcher and scientist.

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