

International Journal of Innovative Research in Science, Engineering and Technology

(A High Impact Factor, Monthly, Peer Reviewed Journal)

Visit: www.ijirset.com

Vol. 6, Issue 12, December 2017

Isolation, Molecular Characterization of Probiotic Yeast with Biosurfactant

Hayder Idrees Hussein

Master's, Department of Microbiology Science College, Osmania University, Hyderabad, India

ABSTRACT: The present study was carried out to isolate probiotic yeasts from different sources and to evaluate them for their antifungal and biosurfactant property. A total of 30 isolates of yeasts were obtained from different sources (fruits, vegetables, dairy products). Their morphology was identified using staining technique. All the isolates were tried for their adversarial action against two fungal pathogens *Macrophomina phaseolina*, *Sclerotium rolfsii*. The biosurfactant property of all the isolates was likewise tried by performing emulsification index measure.

I. INTRODUCTION

Likewise, when the yeast cells are provided with just a single kind of carbon source, for example, glucose or n-alkanes, sophorolipid arrangement is watched. The creation is however considerably higher when two sorts of carbon sources, a hydrophilic (glycidic) and a hydrophobic (lipidic) one, are given. As a rule, glucose is utilized as the hydrophilic carbon source. Sucrose can likewise go about as substrate, yet the acquired sophorolipid level is lower. While trying to lessen substrate costs, cheddar whey was proposed as hydrophilic carbon source. Zhou and Kosaric (1993, 1995) first explored the maturation with galactose and lactose, the principle sugar parts of whey. *C. bombicola* was not ready to develop when just lactose was available, however when likewise olive, canola, or safflower oil was supplemented, development and sophorolipid arrangement were watched. Daniel et al. (1998a) at that point explored the generation of sophorolipids in a medium with deproteinized whey focus and rapeseed oil.

They got abnormal amounts of sophorolipid generation (280 g/l), yet for some odd reason, the lactose was not devoured, nor could β -galactosidase action in the supernatant or rough cell remove be recognized. In any case, similar scientists figured out how to completely utilize the deproteinized whey as a substrate for sophorolipid generation by a two-organize development process. They first cultivated the oleaginous yeast *Cryptococcus curvatus* on the whey. The cells, which accumulated a high single cell oil level, were harvested and disrupted and then served as lipidic substrate for the *C. bombicola* cells. Moreover, additionally lowcost soy molasses can go about as glucose substitute, yet once more, bring down yields were watched. As *C. bombicola* was initially disconnected from nectar, a few specialists have tried it as a substrate. It was however just supplemented toward the finish of the maturation procedure when the at first included glucose was devoured. A considerable measure of substrates can go about as hydrophobic carbon source: oils, unsaturated fats, and their comparing esters, alkanes, and so on. The level of sophorolipid development amid maturations in light of alkanes as hydrophobic bolster stock generally relies upon the chain length of the utilized substrate. Hexadecane and octadecane give the best generation yields. They have all the earmarks of being specifically changed over into hydroxy unsaturated fats and fused into the sophorolipid atoms, along these lines emphatically affecting the unsaturated fat structure of the sophorolipid blend. Davila et al. (1994), for instance, identified more than 70% of hydroxylated hexadecanoic and octadecanoic corrosive for maturations on hexadecane and octadecane, individually. Shorter alkanes are just to a minor degree consolidated, while most by far is either lengthened to C16 or C18 unsaturated fats, or used by means of β -oxidation. Pretty much the same is valid for eicosane (n-C20) or longer alkanes. A couple of rates of eicosane could be distinguished in the sophorolipid unsaturated fat moiety, though none were watched for the more drawn out alkanes. The alkanes are utilized by β -oxidation, either totally or somewhat to offer ascent to shorter unsaturated fats which can be joined into the sophorolipid atoms. The yields are however higher when contrasted with the shorter lkanes and even equivalent to those acquired for n-C16 and n-C18. The same trend is observed for fatty acids or fatty acid methyl esters. The best results are obtained for oleic acid (C18:1). It turns out that

International Journal of Innovative Research in Science, Engineering and Technology

(A High Impact Factor, Monthly, Peer Reviewed Journal)

Visit: www.ijirset.com

Vol. 6, Issue 12, December 2017

the fatty acid chain length determines the rate and position of hydroxylation and consequently governs the incorporation into the sophorolipid molecule. Pentadecanoic acid is too short for hydroxylation, but palmitic acid (22.55 Å) is not. It is predominantly hydroxylated at the terminal position. The more drawn out the chain, the more the terminal/subterminal oxidation proportion decays; for stearic corrosive (25.05 Å) for instance, no terminal oxidation is watched while this occurs for oleic (24.22 Å) and heptadecanoic corrosive (23.80 Å).

Linolenic acid (C18:3), however, does not follow this rule, no sophorolipids with this fatty acid tail were ever observed. It is recommended that the 15– 16 twofold bound is excessively near the enzymatic response site. In both sophorolipid blends on alkanes and unsaturated fats with coordinate joining, desaturation is watched.

Moreover, oils (particularly those of vegetable cause) are broadly utilized as lipidic carbon source. The most widely recognized vegetable oils are involved soaked or unsaturated fats with chain engths of 16 or 18 carbon particles, making them a perfect substrate for coordinate consolidation and the subsequent high sophorolipid creation and yield. Cases of tried oils are canola, corn, safflower, sunflower, olive, rapeseed, grape seed, palm, coconut, fish, and soybean oil. As vegetable oils are inexhaustible assets, sophorolipid amalgamation with those carbon sources adds to the ecological agreeable character of this surfactant. Moreover, squander streams, for example, biodiesel result streams, soybean dull oil and waste broiling oil can be utilized. It is hard to analyze the impacts of different oil sources in light of the fact that distinctive media and culture conditions were utilized.

Casas and Garcia-Ochoa (1999), in any case, analyzed olive, grape seed, sunflower, corn, and coconut oil and inferred that sunflower oil gave the best outcomes. Coconut oil is less reasonable, as it in reality contains high measures of lauric and myristic corrosive. Davila et al. (1994) attempted palm, sunflower, edge, and rapeseed oil. The last one ended up being most reasonable for sophorolipid creation because of its high oleic corrosive content. These experts could also grow the age and yield from 255 g/l and 0.53 g/g to 340 g/l and 0.65 g/g while using methyl or ethyl esters of the rapeseed oil. Non-melded substrates, either alkanes, unsaturated fats, or esters, are primarily oxidized to CO₂.

Brakemeier et al. (1995, 1998a) built up a technique to evade the length-needy and confined fuse. They used assistant alcohols (C12 to C16) as the lipophilic carbon source. A large portion of the substrate was combined into the glycolipids with no further modifications, though little rates of (ω -1)- alkandiol and hydroxy unsaturated fats were viewed. The consequent blends indicate better surface dynamic properties when appeared differently in relation to nearby sophorolipids. Another striking event is the possible coupling of sophorose units at the two regions of the alkandiol, provoking a glycolipid with two sophorose units separated by a hydrocarbonic spacer; these particles are however just to some degree dissolvable in water. The best detriment of this technique is the high cost of auxiliary alcohols. PEssential alcohols are considerably less expensive substrates, however are in all probability changed over to unsaturated fats and used in the β -oxidation course. The utilization can by the by be stayed away from for the consolidation in sophorolipids by expanding the level of glucose (150 g/l) and yeast separate (4 g/l). Tests with 2-, 3-, or 4 dodecanones demonstrated that those ketones were lessened into their relating alcohols by *C. bombicola* and in this manner joined into sophorolipid particles. This hypothesis is supported by the findings of Hommel et al. (1994), regarding the biosynthesis of the sophorose moiety, which resembles the trehalose synthesis of *Saccharomyces cerevisiae* under anaerobic conditions. It was also demonstrated that sophorolipids can be used as sole carbon source. As *C. bombicola* and *C. apicola* by nature occur in environments with high osmotic strength, sophorolipid production may be a way of dealing with the high sugar concentrations by converting, storing, and making them less available for other organisms. Furthermore, sophorolipids display anti- microbial activity against certain yeasts such as *Candida* and *Pichia* species and Gram-positive bacteria. The mono- and diacetylated lactones have the most grounded inhibitory impact. We trust that the physiological part of sophorolipid amalgamation is extracellular carbon source stockpiling, joined with managing a high-sugar specialty and protecting this against other contending microorganisms.

II. MATERIALS AND METHODS

The experiment was conducted at the laboratory of the Department of microbiology, University College of science, Osmania University. Materials or equipment were sterilized to avoid contamination. Aseptic conditions were maintained carefully, and controlled experiments were also carried out to avoid false positive and false negative results.

International Journal of Innovative Research in Science, Engineering and Technology

(A High Impact Factor, Monthly, Peer Reviewed Journal)

Visit: www.ijirset.com

Vol. 6, Issue 12, December 2017

Replications of some important steps in different experiments were also done to obtain more accurate results. Various modern materials and methods were used very carefully in this experiment as follows:

Materials

Collection of samples

Random samples of fresh ripe fruits, dairy products, were obtained from different retail sellers and supermarkets and that of spoiled fruits was obtained from different vegetable markets of Hyderabad and was carried to lab in zip lock covers. These samples included fruits of papaya, apple, chikoo, grapes, tomato, pineapple, banana, figs, kiwi, pomegranate, dates, watermelon and the dairy products included wheat toasts, cheese, curd, butter, milk cream.

Fermented fruits and fruit juices: All the above mentioned twelve types of fruits were washed properly by tap water. Juice was prepared from sugarcane and watermelon. All the items were fermented anaerobically at room temperature (~30°C) for several days.

Media and composition

Media used for enrichment of biomass: Enrichment is done by using an enrichment medium which encourages the growth of yeasts and inhibit the growth of undesired microorganisms.

YMPD Agar (gm/L)

Peptone - 5.0

Yeast extract - 3.0

Malt extract - 3.0

Dextrose - 10

Agar - 20

pH - 6.0

YMPD broth

Peptone - 5.0

Yeast extract - 3.0

Malt extract - 3.0

Dextrose - 10

pH - 6.0

Potato dextrose agar

Potatoes - 200gm

Yeast extract - 3.0

Dextrose - 20

Agar - 20

pH - 6.0

Potato dextrose broth

Potatoes - 200gm

Yeast extract - 3.0

Dextrose - 20

pH - 6.0

Nutrient agar medium

Peptone - 5.0

yeast extract - 3.0

NaCl - 5.0

Dextrose - 10

Agar - 20

International Journal of Innovative Research in Science, Engineering and Technology

(A High Impact Factor, Monthly, Peer Reviewed Journal)

Visit: www.ijirset.com

Vol. 6, Issue 12, December 2017

Media related components and chemicals from Hi-media

Bacteriological agar powder, safranin, Ethanol (50%, 70%, absolute), Glycerol, Phenol red, Proteose peptone, Sodium chloride, Sodium hydroxide, Sugar (Glucose, Fructose, Maltose, Galactose, Lactose), Yeast extract, malt extract, oleic acid, methanol, chloroform, ethylacetate, hydrochloric acid, ox bile. Antibiotic disks (Gentamycin, Erythromycin, Nalidixic acid, Vancomycin, Chloramphenicol), coconut oil, engine oil.

Glassware and other accessories

Beakers (50ml, 100ml, 250ml, 500ml, 1000ml), Conical flasks (50ml, 100ml, 250ml, 500ml, 1000ml), Centrifuge tube and bottle, Durham's tube, Eppendorf tube 1.5 ml, 2.0 ml, Measuring cylinder (50ml, 100ml, 250ml, 500ml, 1000ml), Micropipettes (5-50 μ l, 20-200 μ l, 100- 1000 μ l), Pipette pump, Pipettes (1ml, 5ml, 10 ml), Petri dishes, Screw cap tubes, Test tubes, Test tube holder, round bottom flasks, microscopic slides,

Methods

Sampling

a) **Collection of samples:** Samples of various sources were obtained from different retail sellers and supermarkets and were carried to lab in zip lock covers. At that point were brought into research facility, washed by both flushing faucet water and refined water and that of ruined natural products were acquired from various vegetable markets of Hyderabad.

b) **Sample processing:** Collected samples were allowed to ferment at room temperature ($\sim 30^{\circ}\text{C}$) for 2-4 days in laboratory.

Isolation of yeast

a) Enrichment of the samples

Total procedure of enrichment process is as follows:

1. At first, 100 ml of YEPD broth was prepared homogeneously. pH was adjusted to 6.5, sealed them by non absorbant cotton. Then the medium was sterilized by autoclave at 121°C for 15 minutes in 15 psi.
2. The collected samples were then added to these conical flasks and labeled with the name of the sample.

b) Preparation of samples for inoculation.

1. At first, a set of ten test tubes for each sample were filled with 9ml of distilled water and were cotton plugged.
2. Then required amount of YMPD agar media was prepared and pH was adjusted to 6.5.
3. Then all of these were sterilized by autoclave.
4. Autoclaved YMPD agar media was filled disinfected Petri dishes and permitted to set.
5. Each set of test tubes were then serially diluted with 1ml of their respective enriched samples. And labelled accordingly with the dilution and sample name.

c) Inoculation and incubation of the samples

Spread/pour plate technique as described by Harley and Prescott, (2008) was followed throughout this study. 0.1ml of each of these six dilution (10⁻², 10⁻³, 10⁻⁴, 10⁻⁵, 10⁻⁶, 10⁻⁷) of serially diluted tubes was transferred with sterile pipette and spreaded on YMPD plates containing 50 $\mu\text{g}/\text{mL}$ of ampicillin antibiotic with the help of sterile bent glass rod. Then all

YMPD plates were incubated in an incubator at 30°C for 48 hours.

d) Selection of yeast isolates

After 48 hours of incubation at 30°C , single morphologically well-formed colonies were isolated and preserved for further test.

International Journal of Innovative Research in Science, Engineering and Technology

(A High Impact Factor, Monthly, Peer Reviewed Journal)

Visit: www.ijirset.com

Vol. 6, Issue 12, December 2017

e) Culture purification

Then the selected isolates of yeast species were sub cultured by streak plate technique on YMPD plates to check its purity and incubated at 30°C for 48 hours.

f) Stock culture preparation

The purified isolates were sub cultured into two sets of YMPD slants in test tubes and incubated at 30°C for 48 hours. Then one set was taken to apply sterile 60% glycerol above the cells grown in MEAB slants to keep the cells wet, second set had no glycerol. Then both the sets were preserved at -20 and 4°C as stock culture. All these stocks were sub cultured every month to check viability.

c) Biochemical characterization

The biochemical strategies depended on the usage of carbon and nitrogen sources, as beforehand. The capacity of strains to use glucose, fructose, maltose, galactose, lactose as a sole carbon source and generation of gas was resolved in Durham's tubes on sugar aging medium. A positive response was identified by perception of shading change to yellow and gas arrangement in Durham tubes (for starch maturation) in the arrangement.

i) Carbohydrate fermentation

Preparation of sugar solution

One gm of Bactopeptone (Hi Media, India) and 0.5 gm of sodium chloride was dissolved in 100 ml distilled water to prepare 1% peptone water. The medium was boiled for 5 minutes and pH was adjusted to 7.0, cooled and then filtered through filter paper. Phenolred indicator was used and added to peptone water and 5 ml was dispensed into cottonplugged test tubes containing inversely placed Durham's tubes. These were then sterilized in the autoclave at 121°C maintaining a pressure of 10 pounds/sq. inch for 15 minutes.

The sugar solutions were sterilized in tyndalization at 100°C for 30 minutes during their successive days. An amount of 0.5 ml of sterile sugar solution was added aseptically in each culture tubes containing sterile peptone water. Before use, the sterility of the sugar media was examined by incubating it 37°C for 24 hours (Cook, 1958)

Procedure

The carbohydrate fermentation test was performed by immunizing a loopful of yeast culture into the tubes containing diverse sugar media (five essential sugars e.g. glucose, fructose, maltose, galactose, lactose) and hatched at 30°C for 48 hours. The changing of shading from yellow to pink showed the corrosive generation via starch maturation and amassing of gas rises in upset Durham's tube demonstrated CO₂ gas creation. No shading change demonstrated negative outcome and moderate shading change showed as frai

III. RESULTS

Isolation of yeast from different sources

A total of 30 colonies were isolated and purified from different fruits, vegetables, dairy products by plating serial dilutions on YMPD. Representative colonies on the YMPD plates were picked up and purified by repeated sub culturing and preserved in 20% glycerol at -80°C. All the isolates were assigned the code numbers. Isolates were selected on the basis of distinct colony size, shape, color and elevation of colonies. These were subjected for the screening of various probiotic and biosurfactant properties.

Morphological distinguishing proof of the acquired disconnects by microscopy With a specific end goal to decide morphology of yeast cells, the way of life were analyzed minutely.

International Journal of Innovative Research in Science, Engineering and Technology

(A High Impact Factor, Monthly, Peer Reviewed Journal)

Visit: www.ijirset.com

Vol. 6, Issue 12, December 2017

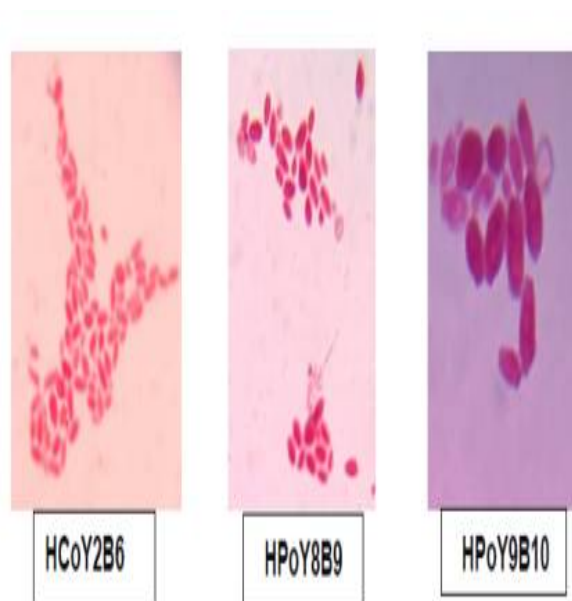


Figure 1: Microscopic images of isolates HCoY2B6, HPoY8B9, HPoY9B10

Characterization of yeast isolates for its antimicrobial activity

Antifungal activity of the yeast isolates

All the isolates obtained were tested for their antifungal activity against two phytopathogenic fungus *Macrophomina phaseolina*, *Sclerotium rolfsii*.

a. Antifungal activity using plate assay

Of the 14 isolates tested, seven isolates namely HCoY2B6, HKiY3B4, HCoY4B8, HPoY5B5, HPoY8B9, HPoY9B10, HCoY12B7 showed inhibition against *Macrophomina phaseolina* growth with percentages being, 60%, 56%, 59%, 52%, 71%, 65%, 50% respectively. Whereas none of the isolates showed inhibition against *Sclerotium rolfsii*.

Subjective Analysis Of Sophorolipid Mixture By Thin Layer Chromatography (TLC) Thin layer chromatography (TLC) is the most straightforward, speediest and least expensive of the different explanatory procedures that have been utilized widely for the subjective examination of mixes. Favored adsorbent utilized for TLC is silica gel. Commercially available standard silica gel coated on aluminium plates (Kiesel-gel 60F254, Merck, Darmstadt, Germany, silica layer thickness 2 mm) are used for the analysis of SL mixture.

Glass micro-capillary is used for the spot application of samples. The simplest, staining with iodine vapours works best for compounds with double bonds. The ethyl 81 acetate extract of SL mixture was used for the determination of number of components present in the mixture by means of thin layer chromatography. Developing system used was CHCl₃:CH₃OH:H₂O in the ratio of 65:15:0.5, v/v/v. Spots were visualized by exposure to iodine vapour.

Antifungal activity in broth

The antifungal activity of three isolates showing highest antifungal activity on plates was further confirmed using broth experiments under stationary and shaking conditions. Isolate HPoY8B9 showed highest percentage inhibition of 97% under stationary condition and 48% under shaking conditions. Whereas isolates HPoY9B10 showed 93% of inhibition under stationary conditions and 30% of inhibition under shaker conditions and HCoY2B6 showed 91% and 25% of inhibition under stationary and shaking conditions.

International Journal of Innovative Research in Science, Engineering and Technology

(A High Impact Factor, Monthly, Peer Reviewed Journal)

Visit: www.ijirset.com

Vol. 6, Issue 12, December 2017

Table 1. Inhibition of *Macrophomina phaseolina* growth in broth under stationary and static conditions.

Isolate	% inhibition of <i>Macrophomina phaseolina</i> In broth	
	stationary	shaker
HCoY2B6	91% 25%	
HPoY8B9	97% 48%	
HPoY9B10	93% 30%	

Screening for biosurfactant activity

Emulsification activity

All the 30 isolates were screened for their biosurfactant activity by checking for itsemulsification index (EI24). Of the 30 isolates tested isolate HPoY8B9, HPoY9B10, HC oY2B6 gavehigh emulsification index with coconut and engine oil. Their emulsification index percentages withcoconut oil and engine oil are represented in table 4.5 and table 4.6 respectively.

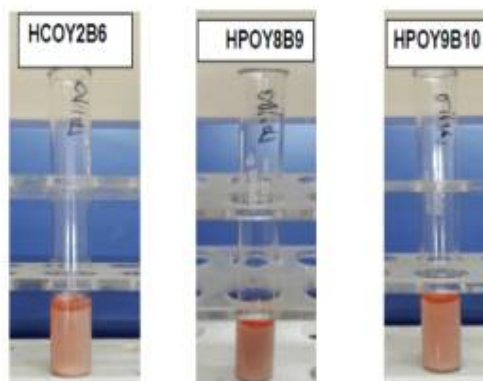


Figure 2: Emulsification activity of isolates using engine oil as hydrocarbon source

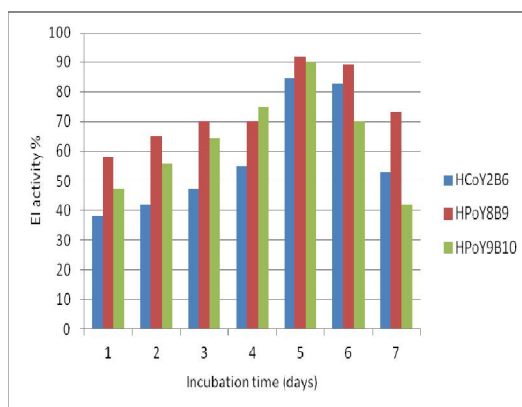


Figure3: Emulsification activity of isolates using coconut oil as hydrocarbon source

International Journal of Innovative Research in Science, Engineering and Technology

(A High Impact Factor, Monthly, Peer Reviewed Journal)

Visit: www.ijirset.com

Vol. 6, Issue 12, December 2017

Table 2: Emulsification index (percentage) with coconut oil as hydrocarbon source.

Isolate	Emulsification index percentage with coconut oil						
	24hrs	48hrs	72hrs	96hrs	120hrs	144hrs	168hrs
HCoY1L7	71.42%	45%	30%	---	---	---	---
HCoY2B6	38.23%	42.85%	47%	55%	85%	83%	53%
HKiY3B4	78.78%	81.25%	20%	35%	---	---	---
HCoY4B8	85.71%	57.145	10%	15%	---	---	---
HPoY5B5	57.4%	14.28%	15%	10%	---	---	---
HCoY6L6	62.5%	38.23%	25%	45%	---	---	---
HPoY7L9	57.14%	8.82%	50%	85%	---	---	---
HPoY8B9	58.82%	65%	70%	70%	92%	89%	73%
HPoY9B10	47.64%	56%	64%	75%	90%	70%	42%
HCoY10L8	17.14%	22%	45%	40%	---	---	---
HKiY11L4	9%	35%	5%	---	---	---	---
HCoY12B7	52.94%	48%	5%	20%	---	---	---
HPoY13L5	22.85%	71%	40%	40%	---	---	---
HPoY14L10	11%	45%	75%	---	---	---	---
HPaY15S11	11.9%	27%	46%	49%	42%	---	---
HSaY16L1	09%	19%	31%	48%	36%	29%	-
HMaY17S6	12%	24%	39%	46%	53%	51%	32%

Table3: Emulsification index percentage with engine oil as hydrocarbon source.

Isolate	Emulsification index percentage with engine oil						
	24hrs	48hrs	72hrs	96hrs	120hrs	144hrs	168hrs
HCoY1L7	30%	33%	47%	55%	57%	34%	25%
HCoY2B6	36%	47%	54%	59%	91%	83%	61%

International Journal of Innovative Research in Science, Engineering and Technology

(A High Impact Factor, Monthly, Peer Reviewed Journal)

Visit: www.ijirset.com

Vol. 6, Issue 12, December 2017

HKiY3B4	26%	29%	32%	37%	51%	39%	41%
HCoY4B8	37%	45%	49%	52%	57%	51%	47%
HPoY5B5	28%	32%	41%	46%	49%	54%	40%
HCoY6L6	29%	31%	39%	47%	51%	44%	-
HPoY7L9	17%	39%	44%	49%	56%	63%	74%
HPoY8B9	39%	47%	59%	66%	93%	90%	75%
HPoY9B10	38%	44%	53%	61%	90%	87%	67%
HCoY10L8	19%	32%	39%	47%	51%	55%	45%
HKiY11L4	26%	36%	49%	53%	57%	58%	56%
HCoY12B7	14%	29%	34%	39%	48%	52%	40%

International Journal of Innovative Research in Science, Engineering and Technology

(A High Impact Factor, Monthly, Peer Reviewed Journal)

Visit: www.ijirset.com

Vol. 6, Issue 12, December 2017

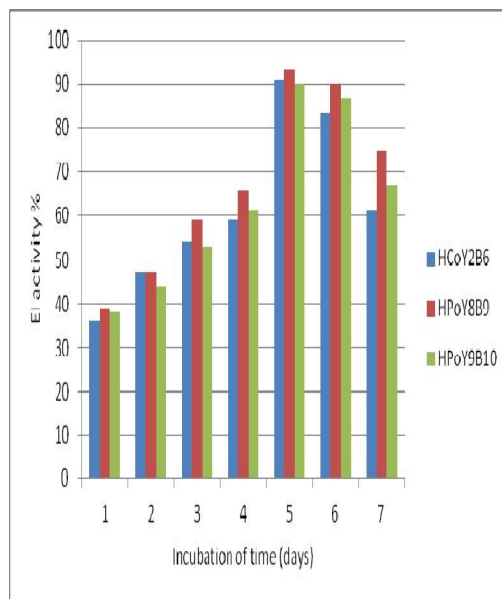


Figure 4: EI24 % of isolates using engine oil as hydrocarbon source

Production of biosurfactant

Three isolates namely HPoY8B9, HPoY9B10, HC oY2B6 showing highest EI activity were further employed for the production of biosurfactant using glucose as hydrophilic carbon source and oleic acid as hydrocarbon source. Samples were harvested for every 24hrs till five days, the compound was extracted and the yield was noted. . Of the three isolates , isolate HPoY8B9 gave highest yield of 2.0 gm/L for 120hrs. whereas yield of biosurfactant produced by isolates HPoY9B10, HC oY2B6 was found to be 1.8 gm/L and 1.2 gm/L respectively.

Table 4: Biosurfactant yield at different time intervals

isolate	Biosurfactant yield gm/L						
	16 8 hr s	24 hr s	48 hr s	72 hr s	96 hr s	12 0h rs	144h rs
HCoY 2B6	0. 5g m	0.7 g m	0.7 g m	1.1 g m	1.2 g m	1.1 gm	0.8g m
HPoY 8B9	0. 7g m	0.9 g m	1.4 g m	1.6 g m	2.0 g m	1.0 gm	0.7g m
HPoY 9B10	0. 5g m	1.1 g m	1.3 g m	1.5 g m	1.8 g m	1.1 gm	0.8g m

International Journal of Innovative Research in Science, Engineering and Technology

(A High Impact Factor, Monthly, Peer Reviewed Journal)

Visit: www.ijirset.com

Vol. 6, Issue 12, December 2017

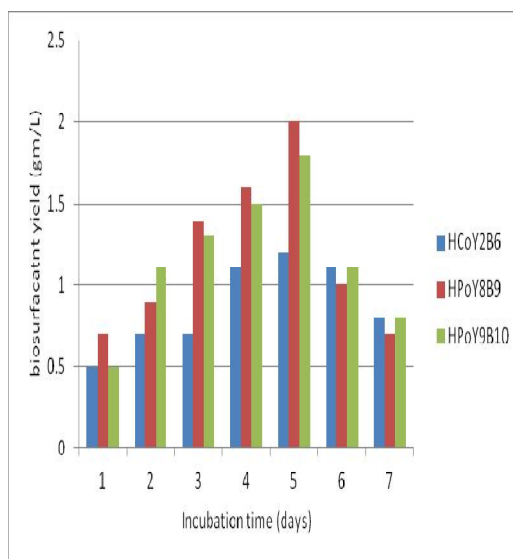


Figure 5: Biosurfactant yield at different time intervals

Identification of compound by Thin layer chromatography

The biosurfactant produced was subjected to TLC for the identification of different lipids and sugars present. Sugar detection: Detection of sugars from the compound extracted for isolates HPoY8B9, HPoY9B10, HC oY2B6 single spot with Rf value was observed with the extracted compound obtained from three isolates.

Table 5: Rf values of spot obtained on TLC for detection of sugars

Isolate	Number of spots	Distance travelled	Rf value
HCoY2B6	1	3.9	0.65
HPoY8B9	1	3.9	0.65
HPoY9B10	1	4.0	0.66

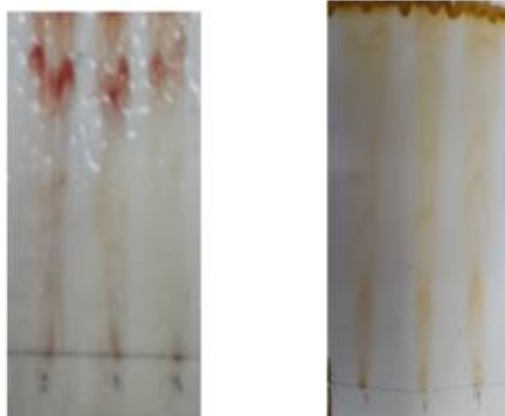


Figure 6: TLC for detection of sugars and lipids

International Journal of Innovative Research in Science, Engineering and Technology

(A High Impact Factor, Monthly, Peer Reviewed Journal)

Visit: www.ijirset.com

Vol. 6, Issue 12, December 2017

Detection of lipids: Detection of lipids from the compound extracted from isolates HPoY8B9, HPoY9B10, HCoY2B6. Four spots were observed with the extracted compound obtained from three isolates. The distance travelled and the Rf values are mentioned in table 4.9 and table 5.0 respectively.

Table 6: List of isolates, number of spots obtained and the distance travelled by spots

Isolate	Number of spots	Distance travelled
HCoY2B6	4	1.5 ; 2.9 ; 3.8 ; 5.7 cm
HPoY8B9	4	1.5 ; 2.9 ; 3.9 ; 5.8 cm
HPoY9B10	4	1.7; 3.5 ; 4.2 ; 5.6 cm

Table 7: Rf values of spots obtained on TLC for detection of lipids.

Isolate	Spot 1	Spot 2	Spot 3	Spot 4
HCoY2B6	0.23	0.46	0.60	0.90
HPoY8B9	0.23	0.46	0.61	0.92
HPoY9B10	0.26	0.55	0.66	0.88

IV. CONCLUSION

The investigation finishes up the yeast isolates HPoY8B9, HPoY9B10, HCoY2B6 have been affirmed as new makers of biosurfactant. Additionally examines are required to build up ideal culture conditions for biosurfactant generation and to describe the biosurfactant delivered by each disconnect. Improvement of cleansing methodologies and explanation of biosurfactant structure are basic to decide any curiosity in the biosurfactant sorts. Since, the present condition of information on common biosurfactants is outstanding to be blocked by an absence of accessibility of monetarily delivered items, future investigations ought to likewise be coordinated toward the utilization of shoddy substrates and the advancement of productive biosurfactant refinement frameworks.

REFERENCES

1. Abe Y, Inoue S, Ishida A (1981) Cosmetic composition of skin and hair treatment. US patent 427340.
2. Breithaupt TB, Light RJ (1982) Affinity-chromatography and further characterization of the glucosyltransferases involved in hydroxydocosanoic acid sophoroside production in *Candida bogoriensis*. J Biol Chem 257:9622-9628.
3. Lin, M., Savaiano, D. and Harlender, S. 1991. Influence of nonfermented dairy products containing bacterial starter cultures on lactose maldigestion in humans. Journal of Dairy Science 74: 87-95.
4. Isoda H, Kitamoto D, Shinmoto H, Matsumura M, Nakahara T (1997) Microbial extracellular glycolipid induction of differentiation and inhibition of the protein kinase C activity of human pro-myelocytic leukemia cell line HL60. Biosci Biotechnol Biochem 61:609-614.
5. Çakır, İ. 2003. Determination of some probiotic properties on Lactobacilli and Bifidobacteria. Ankara University Thesis of Ph.D.

International Journal of Innovative Research in Science, Engineering and Technology

(A High Impact Factor, Monthly, Peer Reviewed Journal)

Visit: www.ijirset.com

Vol. 6, Issue 12, December 2017

7. De Koster C, Heerma W, Pepermans HAM, Groenewegen A, Peters H, Haverkamp J (1995) Tandem mass spectrometry and nuclear magnetic resonance spectroscopy studies of *Candida bombicola* sophorolipids and product formed on hydrolysis by cutinase. *Anal Biochem* 230:135–148.
8. Akari S, Akari Y (1987) Method of modifying quality of wheat flour product. Japanese patent 61205449.
9. Hirayama, K., and Rafter, J. 2000. The role of probiotic bacteria in cancer prevention. *Microbes and Infection* 2:681-686.
10. Kimura, T., Kitamoto, N., Matsuoka, K., Nakamura, K., Iimura, Y., and Kito, Y. (1993). Isolation and nucleotide sequences of the genes encoding killer toxins from *Hansenula mrakii* and *H. saturnus*. *Gene* 137, 265–270.
11. Bulut, Ç. 2003. Isolation and molecular characterization of lactic acid bacteria from cheese. IYTE Thesis of Ms.
12. Buts, J.-P. (2009). Twenty-five years of research on *Saccharomyces boulardii* trophic effects: updates and perspectives. *Dig. Dis. Sci.* 54, 15–18.
13. Çakır, İ. 2003. Determination of some probiotic properties on Lactobacilli and Bifidobacteria. Ankara University Thesis of Ph.D.
14. Cardinal, M.J., Meghrou, J., Lacroix, C., Simard, R.E., 1997. Isolation of *Lactococcus lactis* strain producing inhibitory activity against *Listeria*. *Food Biotechnology* 11:129.
15. Deak, T. (2006). "Environmental factors influencing yeasts," in *Biodiversity and Ecophysiology of Yeasts (The Yeast Handbook series)*, eds G. Péter and C. Rosa (Berlin: Springer), 155–174.
16. Hillion G, Marchal R, Stoltz C, Borzeix CF (1998) Use of a sophorolipid to provide free radical formation inhibiting activity or elastase inhibiting activity. US patent 5756471
17. Hodgson, V. J., Button, D., and Walker, G. M. (1995). Anti-candida activity of a novel killer toxin from the yeast *Williopsis mrakii*. *Microbiology* 141, 2003–2012.
18. Isoda H, Shinmoto H, Kitamoto D, Matsumura M, Nakahara T (1997) Differentiation of human promyelocytic leukemia cell line HL60 by microbial extracellular glycolipids. *Lipids* 32:263–271. doi:10.1007/s11745-997-0033-0