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ISOLATION AND IDENTIFICATION OF YEAST FROM MARINE ENVIRONMENT AND ITS ROLE IN ENZYME PRODUCTION

D.K.Usha[1*], S. Sivaranjani[1], Selvakumari J[3], G. Lakshmanan[2]*

[1]Bon Secours College for Women, Vilar bypass, Thanjavur - 613 006. TamilNadu, India.

[2]Bharath Institute of Higher Education and Research, Chennai – 600 073. TamilNadu, India.

[3]Integrated Disease Surveillance Program, Puducherry-605001, India.

***Corresponding author e-mail id: lakshmanang261988@gmail.com**

Abstract

The intense research on marine sources have brought many potential candidates that spanning variety of industries such as in various industries, such as baking, brewing, wine, bioethanol and pharmaceutical protein production. However, only little attention has been given to marine yeasts. The present study aimed to isolate the marine soil yeasts for the production of industrial important enzymes. Soil samples were collected from Dhanushkodi (9.152011°N 79.445851°E), Ramanathapuram Dist. Tamil Nadu was collected and screened for physiochemical parameters such as electron conductivity nitrogen, total phosphorous, total potassium, total calcium, magnesium, zinc, copper, iron and manganese and organic carbon etc., The yeast strains were isolated from the soil using Yeast Mannitol agar medium and biochemically they were identified. Screening for industrial important enzymes such as amylase, extracellular and L-asparaginase was done. The marine soil samples showed pH as 7.89 and the physiochemical properties similar to the previous studies. Totally six different types of yeast were isolated and showed white, red, yellow, white creamy and pale yellow pigmentation respectively. They were identified biochemically. The Saccharomycese sp. produced all of the three enzymes viz., amylase, L-Asparaginase and protease. Edomycosis sp. showed L-Asparaginase production only activity. The L-Asparaginase enzyme is produced by yeast strain of 2.4 IU/ml of Endomycosis sp. 2.8 IU/ml of Saccharomyces sp. and S. cerevisiae of 5.6 IU/ml. The isolated marine yeasts have great potential to be applied in various industries.

Keywords: yeasts, L-asparaginase, physiochemical parameters

1. Introduction

Yeasts are unicellular fungi that have mostly used in the fermentation process for the production of food and alcoholic beverages. Yeasts are identified through the physiological characters (Bekatorou, 2006). Yeasts are mostly found in natural habitats viz., plant leaves, flowers, soil and salt water or some skin surfaces and intestinal tracts of warm blooded animals, where they may live symbiotically or as parasites (Chandrasekaran, 1997). Yeasts are a polyphyletic group of basidiomycetous and ascomycetous fungi with a unique characteristic of unicellular growth. The term „yeast" is derived from the Old Dutchword gist and the German word gischt, which refers to fermentation. There are approximately 100 genera and 800 described species of yeasts and estimates suggest that these numbers represent only about 1% of the species that exist in nature, the rest being non-culturable (Kutty, 2008).

Yeasts have been used by the food industry principally for the production of ethanol and carbon dioxide, which are important to the brewing, wine distilling and bakingindustries. Their environmental role is similar to many other fungi, acting as saprophytes by converting plant and animal organics to yeast biomass and by-products, which may have commercial importance (Graham, 2007). Some yeast is pathogenic to plants and animals. Yeasts are rich with proteins, lipids and vitamins. Biotransformation of raw material into yeastbiomass (single-cell protein) is highly significant, due to the nutritional quality of yeast and its possible utilization as animal or aquaculture feed. Yeasts also have immunostimulatory properties by virtue of their complex carbohydrate and nucleic acid components. They can be produced very efficiently and economically because of their shorter generation time and use of inexpensive culture media. Lipids, pollutants and enzymes from yeasts are extracellular metabolites of commercial importance (Rosamma, 2008).

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Today, there are about 500 species of yeasts in 60 genera and about 1,000 species of yeast-like organisms in the world. Although the fungi are multicellular, growing as filamentscalled hyphae, the yeasts or the yeast-like cells have morphological terms that refer to singlecelled fungi. The yeasts have already been used for various industrial purposes and basic studieson molecular biology, genetics in addition to traditional baking and alcoholic fermentations (Papagianni, 2004). Among the yeast species, Saccharomyces cerevisiae has been thought to be one of the most important micro-organisms for humans because most of the yeasts applying to various fermentation technologies were identified as the S. cerevisiae which had been secured as safe for foods by experience for a long period. The S. cerevisiae strains have the highest fermentative activities among the yeast species and they are found in various natural environments such as flowers, trees, animals, soils, hydrosphere and artificial environments such as foods, drinks. Since the beginning of the 21st century, various studies on marine-derived yeasts except for S. cerevisiae have been reported (Sreedevi et al., 2008; Chen et al., 2009; Mastuda et al., 2008; Zhang et al., 2010). Otherwise, only a few studies have been reported about isolation and application of marine-derived S. cerevisiae as a main target (Saravanakumar et al., 2013). A marine-derived S. cerevisiae C19 with the highest fermentative activity among many yeast isolates was isolated and applied to the ethanol production from various biomass (Obara et al., 2015; Obara et al., 2012). Enzymes play a definitive role in the production of wine, which could be seen as the product of enzymatic transformation of the grape juice. The enzyme activities do not only originate from the grape itself, but also from yeast and other microorganisms. The winemaker now reinforces and extends the action of these endogenous enzymes by the use of exogenous, industrial enzyme preparations (Fleet, 1993). The production of extracellular hydrolytic enzymes by indigenous yeast could be significant and needs to be understood and managed to the benefit of wine production. Moreover, wine yeast could bepotential sources for the commercial production of enzymes to be used in the process of winemaking (Charoenchai et al., 1997).

Enzymes are biological catalysts which are an indispensible component of biological reactions. The use of chemical catalysts has been followed for a very long time. Chemical catalysis though widely used was very cumbersome. The disadvantages that this method poses include need for high temperature and pressure for catalysis and the moderate specificity. These limitations were overcome by the use of enzymes. Enzymes work at milder conditions when compared to that required by chemical catalysts for operation. Also enzymes are highly specific and catalyze reactions faster than chemical catalysts (Prasad Nooralabettu Krishna, 2011). Enzymes are now being used in various sectors of industry. They are used in detergents; paper industry, textile industry, food industry and many others industrial applications. Enzymes have been in use since ancient times and they have been used in saccharification of starch, production of beverages like beer, treatment of digestive disorders and production of cheese from milk (Drauz et al., 2012). Among the many enzymes that are widely used α-Amylase has been in increasing demand due to its crucial role of starch hydrolysis and the applications of this hydrolytic action. The following sections elaborate on the types of amylases and their roles in enzymatic reactions.

Saccharomyces cerevisiae, the principal wine yeast, is not recognized as a significant producer of extracellular enzymes, although a few strains have recently been reported to degrade polygalacturonate (McKay, 1990). There is little informationon the production of extracellular enzymes by non-Saccharomyces wine yeast, although some strains of Kloeckera apiculata show extracellular protease activity (Laagace and Bisson, 1990; Dizy and Bisson, 2000). Various authors have reported glycosidase production by S. cerevisiae and the potential for these enzymes to enhance wine favor (Delcroix et al. 1994).

2. Materials and Methods

Soil samples were collected from Dhanushkodi (9.152011°N 79.445851°E), Ramanathapuram Dist. Tamil Nadu. Physiochemical parameters of soil analysis were done by adopting previous methods (Jackson, 1973). The electron conductivity nitrogen, total phosphorous, total potassium, total calcium, magnesium, zinc, copper, iron and manganese and organic carbon was estimated in the soil sample. The yeast strains were isolated from the soil using Yeast Mannitol agar medium (Waksman and Fred, 1922). The isolated yeast strains were identified up to genera (Barnett et al., 1990) based on microscopicappearance of the cell, mode of reproduction and certain biochemical and physiological characteristics. Amylase producing yeast were screened on Amylase activity medium (AAM, Fossi et al., 2009). Extracellular protease production was determined on YEPG medium (Strauss et al., 2001) and screened for L-asparaginase production by plate assay (Gulati et al., 1997). The isolated yeasts were also screened for their amylase producing activity (Fuwa, 1954).

3. Result

The marine soil samples analyzed in physicochemical properties of the pH was 7.89, electrical conductivity 0.48 dsm^{-1}, organic carbon was 0.12 %, organic matter 0.24%, available nitrogen 112.2 mg/kg,

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potassium 118 mg/kg, zinc 0.89 ppm, copper 0.48 ppm, iron 4.89 ppm, manganese 2.16 ppm, cation exchange capacity 23.6 C.mole proton/kg, calcium 10.6 C. mole proton/kg, magnesium 6.8 C. mole proton/kg, sodium 1.26C. mole proton/kg, potassium 0.24 C. mole proton/kg was recorded respectively (Table 1).

The isolation of yeast from the marine soil samples was totally 35 colonies at 10^{-3}, 22 colonies at 10^{-4}, 9 colonies at 10^{-5} and 3 colonies at 10^{-6} dilution recorded respectively (Table 2; Plate II and Fig. 1). Totally six different types of yeast were isolated and identified in the marine soil sample. The isolates are white, red, yellow, white creamy and pale yellow colour. Surface are round, smooth, wrinkle. In 1, 2, 4 and 6 yeast are elongation margin and 4 and 6 are undulating margin. All isolated are convex especially 1 is slightly convex. All yeast colonies are ascospore and true mycelium is absent and pseudomycelium in present, particularly 1 and 6 strain of yeast (Table 3 and Plate III and IV). In biochemical characterization of different yeast isolates were using carbon assimilation study. All strain was assimilating in glucose, sucrose and mannitol. Same waystarch is no assimilating in all the yeast strain (Table 3 and 4; Plate IV). The enzymes amylase, L-Asparaginase and protease were screened by yeast isolates. The Saccharomycese sp. is produce in three enzymes of amylase, L-Asparaginase and protease and Edomycosis sp. is only seen in L-Asparaginase activity and S. cerevisiae is active in ability of three enzymes. The results were recorded and tabulated (Table 5 and Plate V). The L-Asparaginase enzyme is produced by yeast strain of 2.4 IU/ml of Endomycosissp. 2.8 IU/ml of Saccharomyces sp. and S. cerevisiae of 5.6 IU/ml was detected (Table 6; Plate VI and Fig. 2).

Table 1: Physico-Chemical Analysis of Soil Sample

S. No	Name of the parameter	Soil sample
1.	pH	7.89
2.	Electrical conductivity (dsm^{-1})	0.48
3.	Organic Carbon (%)	0.12
4.	Organic Matter (%)	0.24
5.	Available Nitrogen (mg/kg)	112.2
6.	Available Phosphorus (mg/kg)	3.75
7.	Available Potassium(mg/kg)	118
8.	Available Zinc (ppm)	0.89
9.	Available Copper (ppm)	0.48
10.	Available Iron (ppm)	4.89
11.	Available Manganese (ppm)	2.16
12.	Cation Exchange Capacity (C. Mole $Proton^{+}$/kg)	23.6
13.	Calcium (C. Mole $Proton^{+}$/kg)	10.6
14.	Magnesium (C. Mole $Proton^{+}$/kg)	6.8
15.	Sodium (C. Mole $Proton^{+}$/kg)	1.26
16.	Potassium (C. Mole $Proton^{+}$/kg)	0.24

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Table 3: Morphological and Microscopical Characteristics of Yeast Isolates

Characteristics	**1**	**2**	**3**
Colour	Wh	Red	W/cr
Surface	Ro	Sm	Wri
Margin	En	En	Undu
Elevation	Sl co	Co	Co
Ascospore	A	A	A
Pseudomycelium	A	P	P
True mycelium	A	A	A

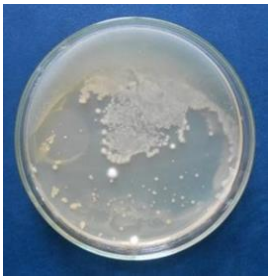
Wh =White; Wh/cr = White/cream; R- red, Y-yellow, Py- pale yellow, Rou = Rough; Sm = Smooth; Wrin = Wrinkled; En =Entire; Undu = Undulating; Sl. Co = Slightly convex; Co =Convex; R = Round; O = Oval; A = Absent; P = Present

Plate I: Isolation of Yeast from Marine Soil Environme

10^{-3}

10^{-4}

10^{-5}



10^{-6}

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Table 4: Biochemical Characteristics of Different Yeast Isolates Using Carbon Assimilating Tests

Characteristics	**1**	**2**	**3**
Glucose	+	+	+
Lactose	-	+	+
Maltose	-	+	+
Sucrose	+	+	+
Citrate	+	-	-
Mannitol	+	+	+
Starch	-	-	-

(-) =Absent; (+) =Present

Table 5: Production of L-Asparaginase Enzyme by Yeast Strain

S. No	**Name of the yeast**	**L-Asparaginase activity (IU/ml)**
1	Endomycosis sp.	2.4
2	Saccharomyces sp.	2.9
2	S. cerevisiae	5.6

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4. Discussion

The marine soil plays a significant role in determining the metabolic ability such as growth, replication and metabolic actions of biotic components including microbes. Climate changes had attracted a research priory due to ocean acidification and urge us to use green-energy sources. The most important challenge of research is to identify the key marine species that has enough and boundless tolerance and adaptation capability against the global climatic change (Vaijayanthi and Vijayakumar, 2014; Guinotte and Fabry, 2008). In the present study, the physico-chemical factor including pH, Electrical conductivity, Macronutrients (organic carbon, nitrogen, phosphorus, and potassium), Micronutrients (iron, manganese, zinc, copper) and others Caution exchange capacity, Magnesium and Sodium were analyzed in the soil. The results showed that the sand clay loam soil was predominately found in the sampled area. The pH of the sampled soils was ranged from 6.3 to 6.9 and mimicked the slight acidic nature. The pHof soil is one of the most important parameter. At basic or low acidic pH soils usually used for rice cultivation (Chandra Sharma, 2015). The correlation coefficient analysis between the physical and chemical nature of the samples soils and isolated actinobacterial species were performed. The soil parameters like texture, calcium carbonate content, electrical conductivity (EC), pH, organic carbon (OC), available nitrogen, available phosphorus, available potassium iron, manganese, zinc, copper and cation exchange capacity were studied by thestandard methods and had been correlated to the nature of the actinobacterial isolates previously (Manikandan and Vijayakumar 2015). In contrast, Karthikeyan et al., (2013) also reported that the variation could be attributed to the actinobacterial content and the soil parameters. Further, they had showed that the seasonal variation paly the main role in determining the ratio of actinobacterial species in marine ecosystems. Salinity is the amount of total soluble salts present in the soil and it usually helps for plant growth. In contrast, while its level increased, the plants unable to extract the water and micronutrients from the soils and end with retarded growth. In addition to, Water with too few salts can lead to surface soil dispersion and soil crusting. Excess salinity may also cause moisture stress within the plant and too be detrimental. Madhava Sarma (2015) studied degree of sensitivity and tolerance levels of crops to salts and showed that most crops may tolerate efficiently up to level 1.1 or less that had no effects on total yields.

In the present study, the salt concentrations in soil samples were ranged from 2.5 to 3.6%. The pH ranges was between 8.1 and 8.3. Chen et al., (2009) reported similar range of pH and salt concentration in their study. The high salinity and moderately alkaline conditions indicated that the collected waters rarely mixed with riverine water. Relatively few studies had investigated marine yeasts from those type soils and those groups of Mycota are still poorly understood (Kutty and Philip, 2008). In the current study, totally, 6 actinomycetal cultures were isolated from the soil samples. The soil was rich in fungal genera that consist of four different types such as Saccharomycese and Endomycosis sp. The presence of Saccharomyces and Pichia in marine soils had been reported by various authors. Our study showed that Candida tropicalis was the most frequent species in the sampled area and such kind of similarity had been reported from Taiwanese sea shore previously (Tien and Wang, 2004). In addition, few fungal genera such as C. tropicalis and Rhodotorula rubra has been frequently found Taiwanese marine and concordant with our results. Many of the previous studies already reported the presence of C.tropicalis in Indian seashores. They also present as predominant intestinal microbiota of marine animals of Indian, Pacific and Atlantic Oceans (Kutty and Philip 2008, Wang et al. 2007). Candida sp. has ubiquitous distribution and reported to be the dominant genera in both Arabian Sea and Bay of Bengal (Kriss, 1967). Fell et al. (1963) also reported that Candida sp. found abundantly in coastal waters that had a close proximity with the urbanized regions due to domestic pollutions. Those results were concordant with our present study.

In the present study the extracellular enzymes were screened in yeast strain. The maximum enzyme activity was observed in S. cerevisiae strain. Though, this finding was in contrast with the previous report and production of extracellular enzymes was particularly attributed to the intrinsic properties of the microorganism (Oliveira et al., 2007). The beer and wine industry mainly utilize the proteases from yeasts and many such studies had addressed to their production potentials (Bilinski and Stewart 1990; Dizy and Bisson 2000; Strauss et al., 2001; Ray et al., 1992). Pectinases have peculiar industrial applications and they are always being in the research thrust. The search for efficient candidates from the groups of moulds and bacteria for pectinases is in current lime lights (Sakai et al., 1993). The present study also evaluated the potential of pectinase production in the isolates, but, the pectinolytic activity of yeasts had, however, been studied with contrasting results (Charoenchai et al., 1997; Strauss et al., 2001). Our results were concordant the findings of Hostinova (2002). Thus, Chi et al. (2009) had already reported the characteristic gluco-amylase producing marine yeasts previously. L-Asparagine serves as significant source of carbon and nitrogen for microbial metabolism. In intracellular environment, it is being deamidated only by L-asparaginase. In our study, its production reached the highest on 6th day and these results were concordant with previous findings (Meena et al., 2015). Thus, the further molecular level analyses on those isolates would be helpful for identifying the potential candidates for food and fermentation industrial applications.

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5. Conclusion

The yeast samples were confirmed by biochemical activity and morphological foridentification. The potential group of yeast such as Saccharomyces cerevisiae screened for industrial important enzymes such as amylase, L-asparaginase and protease were performed.S.cerevisiae showed higher production of L-Asparaginase enzyme and the further analyses would be help in identifying the potential candidates for industrial applications.

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Ethical approval: The study was approved by the Institutional Ethics Committee

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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TABLE 1: Physico-Chemical Analysis Of Soil Sample

S. No	Name of the parameter	Soil sample
1.	pH	7.89
2.	Electrical conductivity (dsm^{-1})	0.48
3.	Organic Carbon (%)	0.12
4.	Organic Matter (%)	0.24
5.	Available Nitrogen (mg/kg)	112.2
6.	Available Phosphorus (mg/kg)	3.75
7.	Available Potassium(mg/kg)	118
8.	Available Zinc (ppm)	0.89
9.	Available Copper (ppm)	0.48
10.	Available Iron (ppm)	4.89
11.	Available Manganese (ppm)	2.16
12.	Cation Exchange Capacity (C. Mole $Proton^{+}$/kg)	23.6
13.	Calcium (C. Mole $Proton^{+}$/kg)	10.6
14.	Magnesium (C. Mole $Proton^{+}$/kg)	6.8
15.	Sodium (C. Mole $Proton^{+}$/kg)	1.26
16.	Potassium (C. Mole $Proton^{+}$/kg)	0.24

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TABLE 2: ISOLATION OF YEAST FROM MARINE SOIL ENVIRONMENTS

S. No	Dilution	Soil samples
		TNC
1	10^{-3}	35
2	10^{-4}	22
3	10^{-5}	9
4	10^{-6}	3

TABLE 3: MORPHOLOGICAL AND MICROSCOPICAL CHARACTERISTICS OF YEAST ISOLATES

Characteristics	1	2	3
Colour	Wh	Red	W/cr
Surface	Ro	Sm	Wri
Margin	En	En	Undu
Elevation	Sl co	Co	Co
Ascospore	A	A	A
Pseudomycelium	A	P	P
True mycelium	A	A	A

Wh =White; Wh/cr = White/cream; R- red, Y-yellow, Py- pale yellow, Rou = Rough; Sm = Smooth; Wrin = Wrinkled; En =Entire; Undu = Undulating; Sl. Co = Slightly convex; Co =Convex; R = Round; O = Oval; A = Absent; P = Present

TABLE 4: BIOCHEMICAL CHARACTERISTICS OF DIFFERENT YEAST ISOLATES USING CARBON ASSIMILATING TESTS

Characteristics	1	2	3
Glucose	+	+	+
Lactose	-	+	+
Maltose	-	+	+
Sucrose	+	+	+
Citrate	+	-	-
Mannitol	+	+	+
Starch	-	-	-

(-) =Absent; (+) =Present

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TABLE 5: IDENTIFICATION OF YEAST ISOLATES

Strain No	Name of the yeast isolates
1	Saccharomyces sp.
2	Endomycosis sp.
3	S. cerivisiae

TABLE 6: SCREENING OF YEAST BY ENZYME PLATE ASSAYMETHOD

Name of the yeast	Amylase	L-Asparaginase	Protease
Endomycosis sp.	+	+	-
Saccharomyces sp.	-	+	-
S. cerivisiae	+	++	+

(+) positive activity, (-) absent (++) high activity

TABLE 7: PRODUCTION OF L-ASPARAGINASE ENZYME BY YEASTSTRAIN

S. No	Name of the yeast	L-Asparaginase activity (IU/ml)
1	Endomycosis sp.	2.4
2	Saccharomyces sp.	2.9
2	S. cerevisiae	5.6

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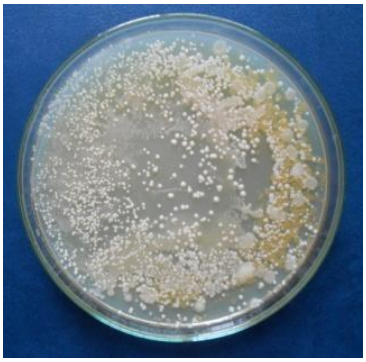
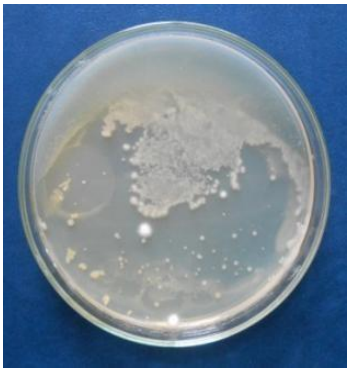
PLATE II: ISOLATION OF YEAST FROM MARINE SOILENVIRONMENTS



10^{-3}

10^{-4}

10^{-5}



10^{-6}

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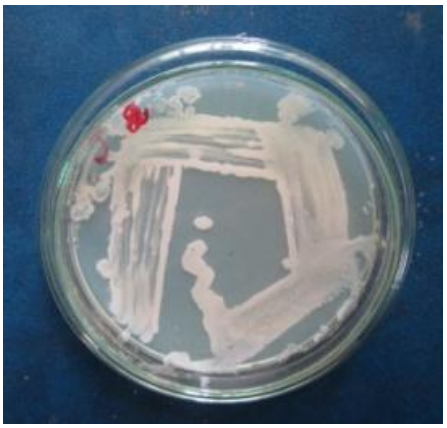
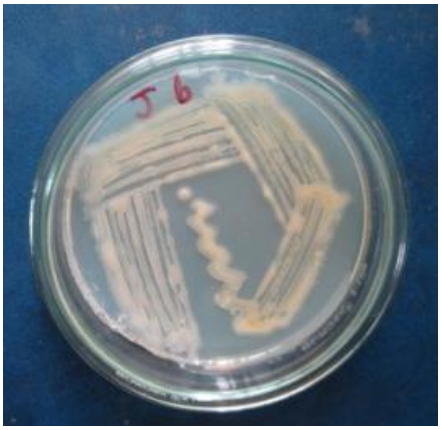
PLATE III: PURIFICATION OF THREE DIFFERENT SPECIES OF YEAST FROM MARINE SOIL ENVIRONMENTS

Endomycosis sp.



S. cerivisiae



Saccharomycetes sp.

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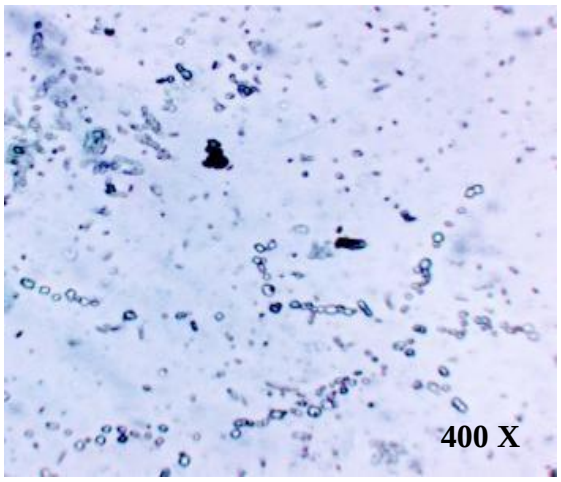
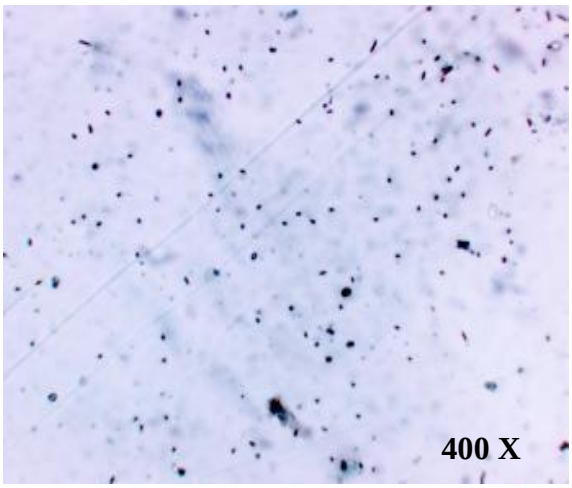
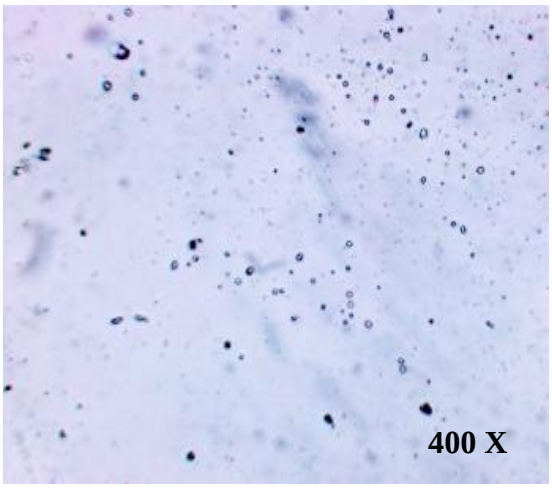
PLATE IV: MICROSCOPIC OBSERVATION OF YEAST ISOLATES



Endomycosis sp.



Saccharomycetes sp.



S. cerivisiae

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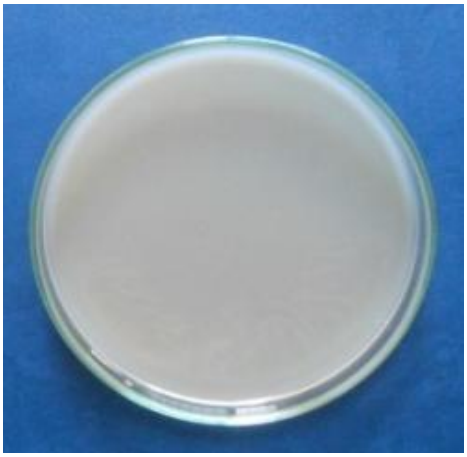
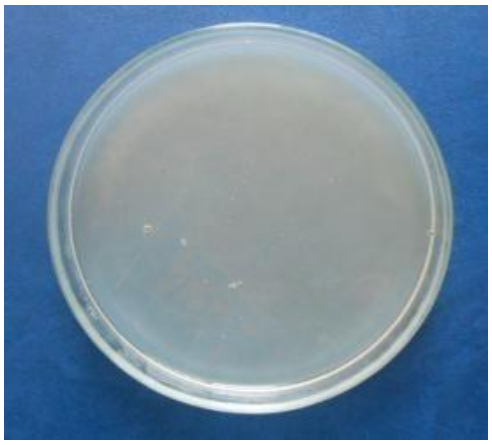
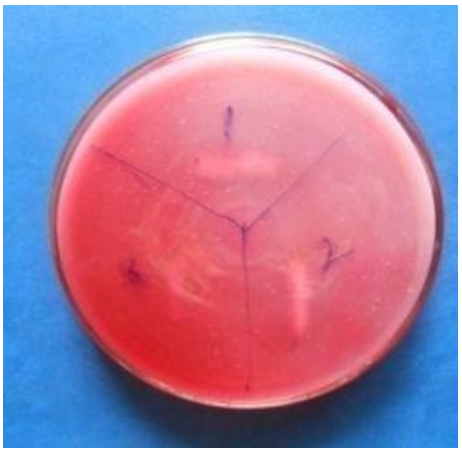
PLATE V: SCREENING OF ENZYME BY YEAST STRAIN



Amylase





Protease

L-asparaginase

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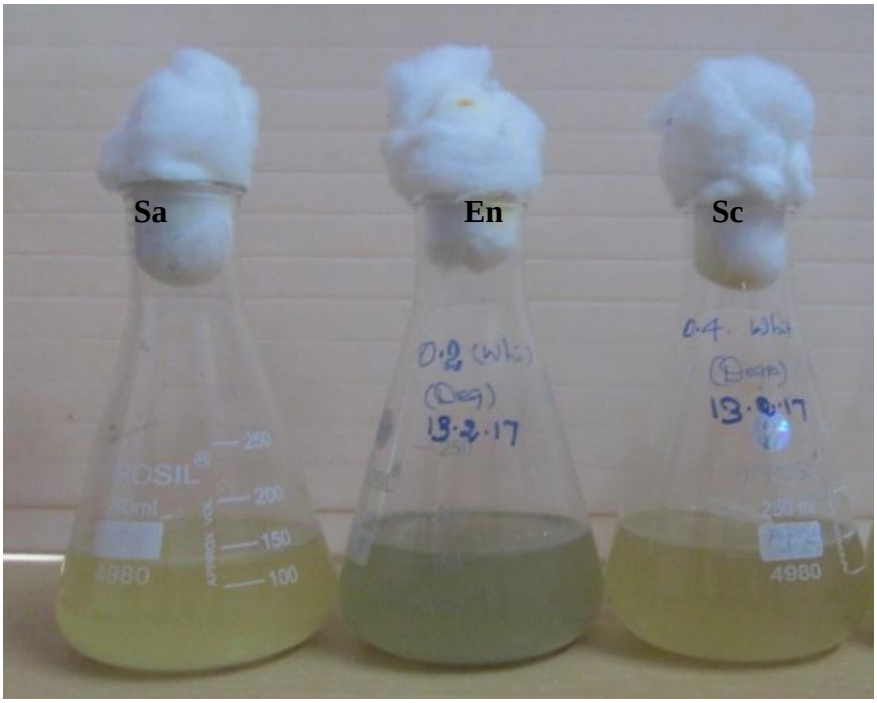
PLATE VI: PRODUCTION OF L-ASPARAGINASE ENZYME BY YEASTSTRAIN



Sa- Saccharomycese sp., En- Endomycosis sp., Sc- S. cerevisiae

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FIG. 2: PRODUCTION OF L-ASPARAGINASE ENZYME BY YEAST