2013 2nd International Conference on Environment, Energy and Biotechnology
IPCBEE vol.51 (2013) © (2013) IACSIT Press, Singapore
DOI: 10.7763/IPCBEE. 2013. V51. 22

Screening Six Potential *Yarrowia Lipolytica* Strains for Best Lipid, Citric Acid, Biosurfactant and Lipase Production

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Abstract. *Y. lipolytica* is an industrially important microorganism for production of biodiesel, citric acid, biosurfactant, lipase etc. The aim of the present work was to study the ability of the six different *Y. lypolitica* strains (IMUFRJ 50682, ATCC 18943, SO678, S2301, W29, Po1g) to produce citric acid, biosurfactant, lipase and lipid in a complex medium with glucose as carbon source and yeast extract and ammonium sulfate as nitrogen sources. Among six strains of *Y. lypolitica* studied, ATCC 18943 was found to be best biosurfactant producer, SO678 could accumulate high amount of lipid, whereas IMUFRJ 50682 yielded both citric acid and lipase at highest level.

Keywords: *Yarrowia lypolitica*, Lipid, Citric acid, Biosurfactant, Lipase

1. Introduction

Yarrowia lypolitica, a strictly aerobic and a dimorphic yeast in Dipodascaceae family, attracts researchers for its capability to produce several important metabolites, which are useful in industry, in molecular biology and in genetic studies. It is considered as non-pathogenic and several processes based on this microorganism are "generally regarded as safe" (GRAS) by the Food and Drug Administration (FDA, USA) [1]. A set of multigene family in *Y. lipolytica* are dedicated to uptake and utilization of a variety of hydrophobic substrates, which makes them able to store lipid more than 50% of their dry biomass-weight [2].

Y. lipolytica is known to secrete several industrially important proteins at very high level. It secretes lipase to digest oil in culture medium. Lipase is an enzyme that attracts the interest of scientists and industrial researchers because it can be utilised for several applications in detergent, food, pharmaceutical and environmental industries [3].

For bioremediation applications *Yarrowia lypolitica* has been proven useful. Many species of *Y. lypolitica* have the ability to degrade a variety of organic compounds, including aliphatic and aromatic hydrocarbons by producing biosurfactants. These molecules are mainly glycolipids, but other types have also been reported [4], [5].

Y. lypolitica is also known for secreting high amount of citric acid in culture media [6], [7], which makes its competence in citric acid industry rewarding considering other intracellular and extracellular products altogether. However no work has been reported in literature regarding screening of *Y. lipolytica* strains to find a master strain that can produce all or many of these products at very high level to make it a winner in biotechnology industry. Hence the aim of the present study was to investigate the ability of six *Y. lypolitica* strains to produce four such valuable metabolites namely lipase, lipid, biosurfactant and citric acid in a complex medium.

2. Materials and Methods

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2.1. Microorganisms and Culture Conditions

Six slants of six strains of *Y. lipolytica* (IMUFRJ 50682, ATCC 18943, SO678, S2301, W29, Po1g) were prepared in YPD-agar medium (1% yeast extract, 1% peptone, 2% dextrose, 2% agar) and were incubated at 28 ℃ overnight. Inoculums were prepared by inoculating cells from the overnight grown fresh slants into a complex medium The production medium used contained (g/l): Glucose 30, $(NH_4)_2SO_4$ 0.5, yeast extract (Fluka, nitrogen content: 7% (w/w) 0.5, KH_2PO_4 7,Na_2HPO_4 2.5, $MgSO_4.7H_2O$ 1.5, $CaCl_2$ 0.15, $FeCl_3.6H_2O$ 0.15, $ZnSO_4.7H_2O$ 0.02, $MnSO_4.H_2O$ 0.06 [8] and were cultivated at 28 °C in a rotary shaker at 200 rpm speed for 24h. 10% inoculums were added to same sterile medium [8] and were cultivated at 28 °C and 200 rpm shaking. All experiments were performed in triplicate and average values of product yield have been used.

2.2. Analytical Methods

Samples were withdrawn every 24 hours for determination of biomass concentration, residual glucose, citric acid, lipase, biosurfactant, lipid content and pH. Samples from all strains were observed under phase-contrast microscope (Nikon Eclipse E200, Japan) with 100×10 magnification. For quantification of citric acid, biosurfactant and lipase cell-free supernatant was obtained by sample centrifugation at 1000g for 10 minutes. Glucose was determined by phenol-sulphuric method [9]. Concentration of citric acid was determined by HPLC using a method described elsewhere [6]-[7]. Lipase activity was determined by tributyrin method with the cell-free supernatant using a method described by [10]. Lipid droplet (LD) measurement was performed by fluorescence based method as reported by [11]. Lipid extraction from biomass was performed by a method described by [12].

Emulsification activity (E_{24}) as an indicative of biosurfactant acivity in culture was measured by a method described by [13]. The emulsification index (E_{24}) was then calculated using the formula:

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where h_1 = height of emulsion layer, h_2=height of the total mixture.

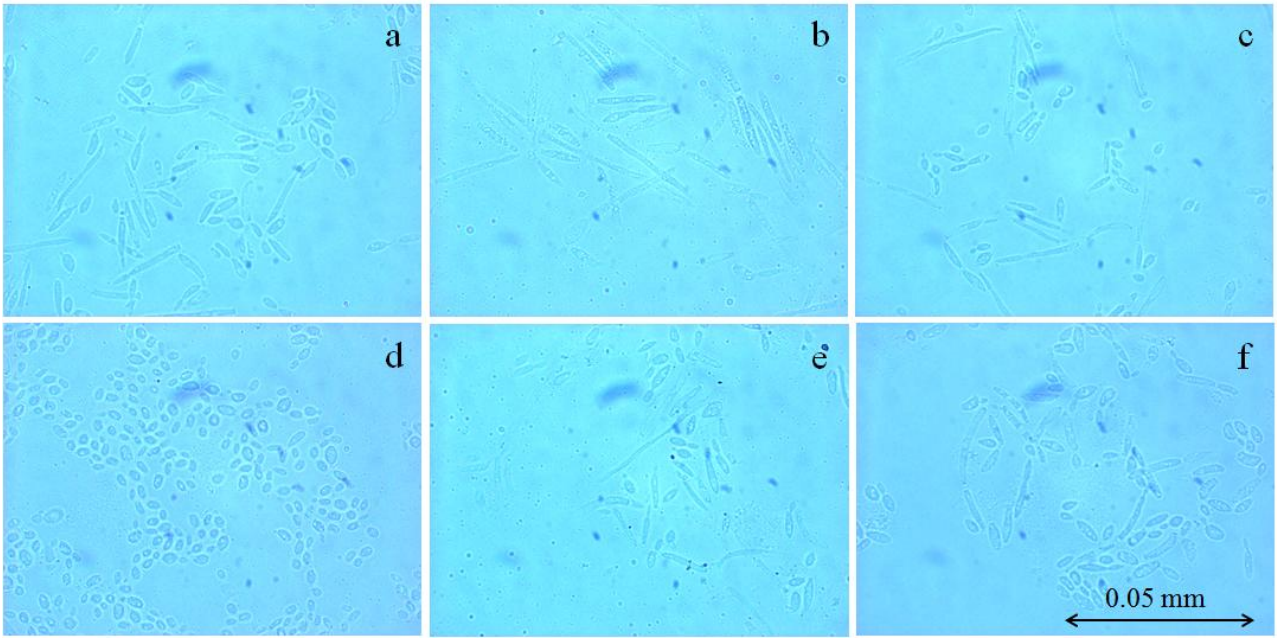

Fig. 1: Phase contrast micrographs of 24 h old cultures of *Y. lipolytica* (a) IMUFRJ 50682, (b) ATCC 18943, (c) SO678, (d) S2301, (e) W29, (f) Po1g.

3. Results and Discussion

Phase contrast microscopic photographs of 24h cultures revealed similarities and dissimilarities in shapes and sizes of the six strains of *Y. lipolytica* (IMUFRJ 50682, ATCC 18943, SO678, S2301, W29, Po1g) as in Fig 1. While S2301 appeared to be oval shaped (Fig. 1d), other 5 strains appeared as long lanceolate (Fig 1). However cells of ATCC 18943 were even more thin and elongated (Fig 1b). *Y. lipolytica* IMUFRJ 50682 (Fig 1a), SO678 (Fig 1c), W29 (Fig 1e), Po1g (Fig 1f) had similarity in that while younger dividing cells

were long and thin and arranged in linear fashion, older cells appeared to be smaller oval shaped individual entities. Older cells could be identified with presence of lipid (visible as white patches in the cells).

Biomass growth rate in the culture medium was observed to be different for six strains (Fig. 2). Except *Y. lipolitica* Po1g all five cultures had a biomass concentration above 10g/L at 144h (Fig. 2a-e), whereas Po1g produced only 3.2 g/L biomass (Fig. 2f). This indicated that the culture medium used was not suitable for Po1g, probably because the medium did not contain peptone, which has been described to be best organic nitrogen source for its growth [14].

Table 1: Biomass yield coefficient ($Y_{X/S}$), yield coefficients of lipid on glucose ($Y_{L/S}$) and lipid on biomass ($Y_{L/X}$) at 72h and biomass yield coefficient ($Y_{X/S}$), yield coefficients of citric acid on glucose ($Y_{P/S}$) and citric acid on biomass ($Y_{P/X}$) at 120h of cultures of *Y. lipolytica* (a) IMUFRJ 50682, (b) ATCC 18943, (c) SO678, (d) S2301, (e) W29, (f) Po1g

***Y. lipolytica* strains**	72h			120h		
	$Y_{X/S}$	$Y_{L/S}$ (lipid)	$Y_{L/X}$ (lipid)	$Y_{X/S}$	$Y_{P/S}$ (citric acid)	$Y_{P/X}$ (citric acid)
IMUFRJ50682	0.309	0.021	0.068	0.297	**0.271**	**0.912**
ATCC18943	**0.584**	0.059	0.101	**0.430**	0.012	0.027
S0678	0.461	0.063	**0.136**	0.349	0.143	0.410
S2301	0.556	**0.0703**	0.127	0.371	0.055	0.148
W29	0.271	0.021	0.076	0.306	0.186	0.606
Po1g	0.249	0.021	0.102	0.118	0.085	0.725

After 24h onwards citric acid was detected in media which kept on increasing till the end of study in general in all cultures, except for *Y. lipolytica* ATCC 18943 (Fig. 2). Citric acid production in the cultures was accompanied by lowering of pH (Fig 2). Since in case of ATCC 18943 strain where the production of citric acid was almost nil and initial pH of this culture media also did not change. Highest citric acid production was found in culture of IMUFRJ 50682 (8.64 g/L at 144 h, Figure 2a) although at day 5 (120h) yield coefficients of citric acid on biomass ($Y_{P/X}$=0.912) was highest in this culture (Table 1). High citric acid yield in IMUFRJ 50682 was followed by W29, Po1g, SO678, S2301 in decreasing order (Table 1).

In general biosurfactant activities of all six strains was found to be maximum at 4th (72h) day as emulsification indices were found high (Fig 2). In the succesive days E24 values decreased gradually in all the five strains except in case of ATCC 18943, where biosurfactant acivity seemed to have become steady. At 144h emulsification index of ATCC 18943 was still at 58.82 (Fig. 2). Biosurfactant activity of W29 and Po1g was very poor compared to other strains (Fig. 2). ATCC 18943 and IMUFRJ 50682 seemed to best biosurfactant producers among 6 cultures (Fig. 2).

The rate of lipid accumulation, degradation and yield varied significantly. LD Index value was found to increase after 24h in all cultures. The LD Indices of the six strains remain high on 3rd and 4th days (48h and 72h) followed by slow decrease, except for ATCC 18943. LD Index was found to be constantly increasing in case of ATCC 18943. However LD Index was found maximum in SO678 (15705.74) among all six strains at 48h. Highest lipid yield coefficient upon biomass, $Y_{L/X}$ of SO678 determined by chloroform-methanol lipid extraction method was 0.136 at 72h (Table1).

Lipase production seemed to start at day 3 (48h) in all the six culture media (Fig. 2). In IMUFRJ 50682 culture lipase production rate was elevated to maximum level at 96h as high as 9.33 TBU/ml followed by cultures of W29 (6 TBU/ml) and S2301 (5.33TBU/ml) at 120h (Fig. 2). It was interesting to note that increase in lipase production was accompanied with decrease in lipid content in cell simultaneously, probably indicating intracellular lipid catabolism and extracellular lipase synthesis are regulated concomitantly.

4. Conclusions

Among six strains of *Y. lypolitica* studied, ATCC 18943 and SO678 were best biosufactant and lipid producers respectively. IMUFRJ 50682 seemed to be best strain as it produced maximum citric acid and lipase than others and also biosurfactant and lipid at a very competitive level. The cultivation medium was not suitable for Po1g with respect to growth and product formation.

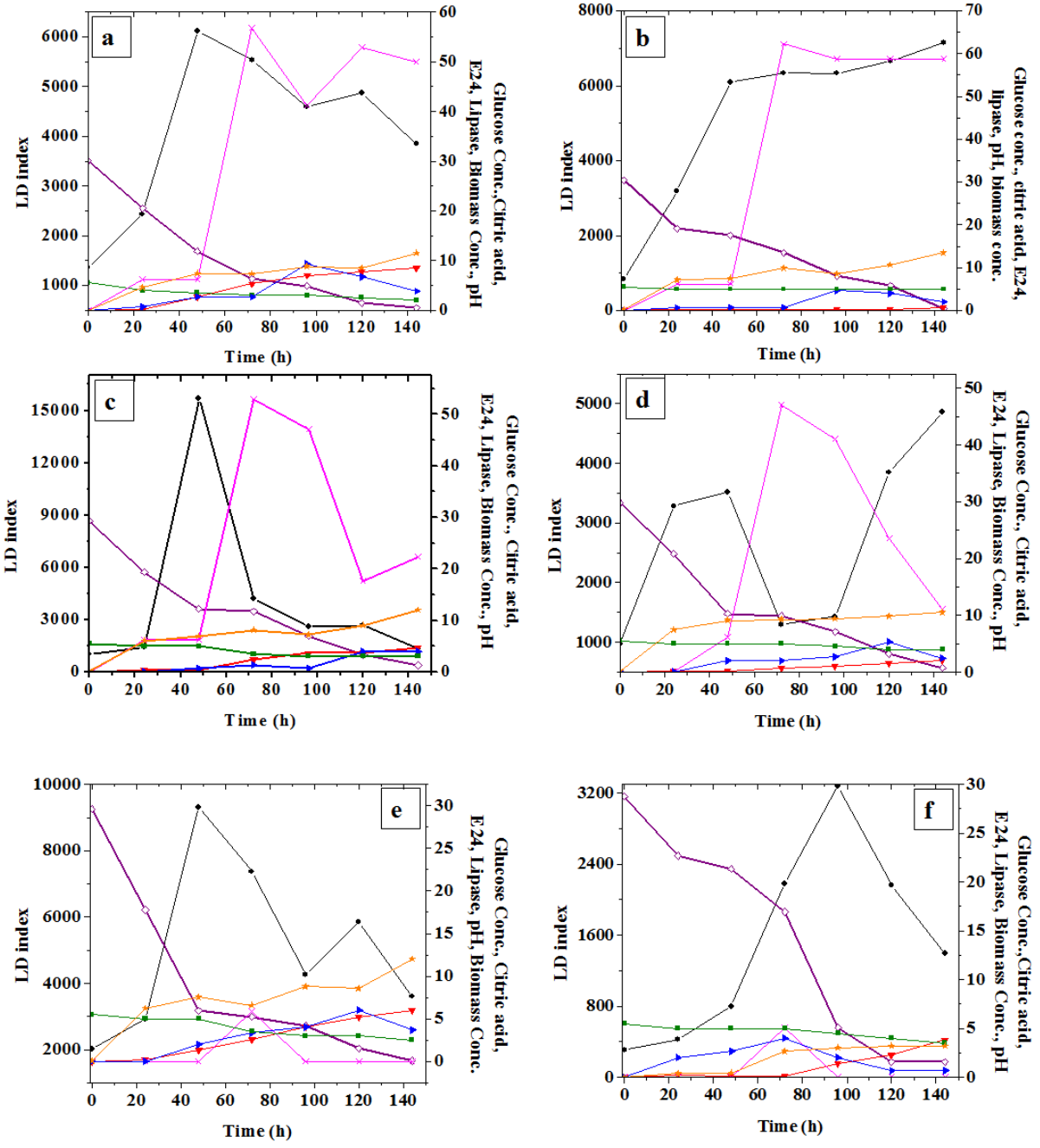

Fig. 2: Time course of LD index, residual glucose concentration (g/L), citric acid concentration (g/L), Lipase activity (TBU/ml), biosurfactant activity (E24), biomass concentration (g/L), pH of cultures of *Y. lipolytica* (a) IMUFRJ 50682, (b) ATCC 18943, (c) SO678, (d) S2301, (e) W29, (f) Po1g.

5. Acknowledgements

Jayeeta Sil and Subhasish Das acknowledge CNPq, Brazil for funding (Número do processo 382963/2012-1 e' número do processo: 503313/2011-4) and Bruno L. B- Morais for 96-well plate reader. The authors thank the financial support given by CNPq and FAPERJ.

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