Scientific Bulletin. Series F. Biotechnologies, Vol. XIX, 2015
ISSN 2285-1364, CD-ROM ISSN 2285-5521, ISSN Online 2285-1372, ISSN-L 2285-1364

SCREENING OF OLEAGINOUS MICROORGANISMS FOR LIPID PRODUCTION

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Abstract

Among hundreds species of yeasts and molds, only few are able to accumulate more than 25% lipids. The aim of our experiments was to select microorganisms with high potential of lipids producing ("oleaginous" microorganisms). Several strains of yeasts and molds were tested in order to determine their ability to produce and accumulate lipids. The strains were cultivated in Petri dishes on specific media. Evaluation of lipid accumulation was achieved by microscopic observations, through Sudan Black B dyeing technique. Preliminary tests in order to determine the capacity of lipid production were made by Soxhlet extraction, after batch fermentations on liquid media. The results showed that five strains of yeasts and molds, Yarrowia lipolytica, Hansenula anomala, Sacharomyces cerevisiae, Aspergillus niger and Trichoderma viridae, were the best lipid producers, which accumulated up to 40% of lipids in dry biomass. One of the key factors in achieving an economically attractive bioprocess is the conversion yield of the substrate into lipids. In this case, yeasts are superior to fungi with a conversion yield over 20%, in comparison with less than 18% for molds. Given these considerations, additional experiments will be conducted to optimize the conditions of lipid production with Yarrowia lipolytica.

Key words*: biomass, fermentation, lipids, oleaginous microorganism.*

INTRODUCTION

Like all living cells, microorganisms contain lipids for the essential functioning of membranes and membranous structures, but not all microorganisms can be considered as abundant sources of fats and oils. Those microorganisms that do produce a high content of lipids may be termed "oleaginous" (Ratledge, 1994). From 600 different species of yeast, only 25 or so are able to accumulate more than 20% lipid; of the 60,000 fungal species fewer than 50 accumulate more than 25% lipid. The lipids that are accumulated by the oleaginous microorganisms are mainly triacylglycerols (Ratledge, 1993). Generally, oleaginous microorganisms are eukaryotes and thus representative species include algae, yeasts, and molds. Bacteria do not usually accumulate significant amounts of triacylglycerols, but many do accumulate waxes and polyesters (Ratledge, Cohen, 2008).
Oleaginous microorganisms, such as microalgae, yeasts, molds and bacteria can accumulate high levels of lipids and do not require arable land, so that they do not compete with food production (Gouda et al., 2008).
Microbial lipophilic compounds, called single cell oils (SCO), are of potential industrial interest due to their specific characteristics (Rattray et al., 1974). The aim of our experiments was to select microorganisms with high potential of producing lipids.

MATERIALS AND METHODS

Microorganisms and media

Ten strains including molds and yeasts from the Collection of Microorganisms of the Faculty of Biotechnology were tested in order to determine their ability to produce and accumulate lipids: *Trichoderma viridae, Aspergillus niger, Aspergillus oryzae, Rhizopus nigricans, Yarrowia lipolytica, Candida utilis, Saccharomyces cerevisiae, Saccharomyces ellipsoideus, Hansenula anomala, Saccharomyces carlsbergensis.*

The strains were cultivated in Petri dishes on specific media. The yeasts were cultivated on YPG medium (yeast extract, peptone and glucose), meanwhile the molds were cultivated on PDA (potato infusion, glucose) medium (Ma et al., 2009).

Maintenance of strains and shake flask cultivation conditions

The yeasts were maintained on YPG-agar medium at 28^0C for 48-72 hours and then stored in a freezer at 4^0C.
The molds were cultivated on PDA-agar medium at 35^0C for 5 days and then stored at 4^0C (Ratledge et al., 2008).
From the culture stored at 4^0C, a loop of cell mass was transferred aseptically to 20 ml liquid media (YPG/PDB) and then incubated in a rotary shaker incubator at 240 rpm, 28^0C for 24 hours for yeasts, and 220 rpm, 35^0C for 48 hours for molds. Next, this culture was used to inoculate 200 ml of liquid media (Erlenmeyer flasks of 500 ml) using a 10% (v/v) inoculum (Qier, 2013).

Biomass assessment

Samples were collected at 8 hour intervals for the determination of wet cell weight, dry cell weight, cell concentration and microscopy examination. Biomass was assessed by absorbance for yeasts and by weighing wet biomass for molds.
For absorbance measurement of yeast media, 0,5 ml of culture medium were taken at 8 hour intervals during fermentation, properly diluted in water (1:25) and measured with a spectrophotometer, at 570 nm (Subramaniam et al., 2010).
For wet biomass preparation, 5 ml of culture medium were taken during fermentation, transferred to pre-weighted tubes and centrifuged. The supernatants were removed and the tubes containing the cellular sediments were weighted (wet cell weight).
Calibration curves were performed using absorbance measurements for yeasts and wet cell biomass for molds (Liu et al., 2010).

Evaluation of lipid biosynthesis

Preliminary evaluation of lipid accumulation was achieved through Sudan Black B staining method, monitoring lipid production by microscopic observation of the cells cultivated on solid media (Liu et al., 2010; Thakur et al., 1988).
The strains were screened for their capacity of producing lipids in shake flask cultures.
The strain that showed the greatest potential ability to produce lipids was selected for further investigation.

Lipid extraction and determination

Wet biomass was transferred in a thermo-balance were it was dried at 115^0C, to constant weight.
The lipids were extracted processing the dried biomass with a Soxhlet extractor, using chloroform:methanol 2:1 (Ratledge et al., 2008).
Quantitative assay of lipids was done by solvent evaporation and weighing the remaining product (Ratledge, 1993).

RESULTS AND DISCUSSIONS

Strain screening

Ten strains of molds and yeasts were screened through Sudan Black B staining method, in order to highlight the capacity to accumulate lipids.
The results showed that three strains of molds exhibit lipid bodies in their hyphae, when examined with optical microscope.
The analysis of yeasts showed that all of them have the potential to accumulate lipids.
Therefore, these nine strains were further tested for their ability to produce lipids in shake flask culture.

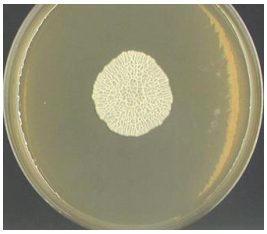

Figure 1. Culture of *Yarrowia lipolytica*

Figure 2. Culture of *Saccharomyces cerevisiae*

Shake flask cultivation and biomass assessment

The nine strains were studied in liquid cultures in order to evaluate the cell growth and lipid accumulation.
Table 1 shows biomass yield of the tested nine strains.

Table 1. Dry biomass yield of tested strains

Strain	Dry biomass (g/l)	Biomass productivity (g/l·h)
Trichoderma viridae	13,3	0,111
Aspergillus niger	14,9	0,124
Aspergillus oryzae	11,8	0,098
Yarrowia lipolytica	15,3	0,213
Candida utilis	11,1	0,154
Saccharomyces cerevisiae	9,1	0,190
Saccharomyces ellipsoideus	10,1	0,210
Hansenula anomala	11,4	0,238
Saccharomyces carlsbergensis	11,6	0,161

Growth curve determination

All yeasts and molds were cultivated in 500 ml shake flasks with 200 ml medium. Optical density/wet biomass were measured every 8 hours. The measurements were used for drawing a growth curve to identify which time points are suitable for harvesting the microorganisms culture.
According to the test results, the best harvesting times were determined to be around 72 hours for *Yarrowia lipolytica,* 48 hours for *Candida utilis*, 48 hours for *Saccharomyces cerevisiae,* 48 hours for *Saccharomyces ellipsoideus,* 48 hours for *Hansenula anomala,* 72 hours for *Saccharomyces carlsbergensis,* 120 hours for *Trichoderma viridae,* 112 hours for *Aspergillus niger,* 120 hours for *Aspergillus oryzae.*

Lipid production

Lipids were extracted from the dry biomass in chloroform:methanol 2:1, with a Soxhlet extractor.
Yarrowia lipolytica displayed the greatest potential ability in accumulating microbial lipids, the lipid content reaching 39.9% of dry cell weights.

The tested molds showed a much lower potential of accumulating lipids then yeasts, of 18.1-21.8% (g lipids /g dry biomass). Table 2 shows lipid yield of the tested yeasts and molds.

Table 2. Lipid yield of tested strains

Strain	Lipid fraction (%)	Lipid productivity (g/l·h)
Trichoderma viridae	21,8	0,024
Aspergillus niger	18,1	0,022
Aspergillus oryzae	18,9	0,019
Yarrowia lipolytica	39,9	0,085
Candida utilis	28,5	0,044
Saccharomyces cerevisiae	38,4	0,073
Saccharomyces ellipsoideus	30,2	0,064
Hansenula anomala	22,6	0,054
Saccharomyces carlsbergensis	20,5	0,033

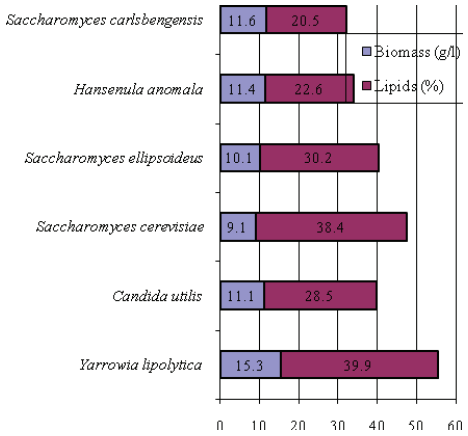

Figure 3. Biomass and lipid content of yeasts

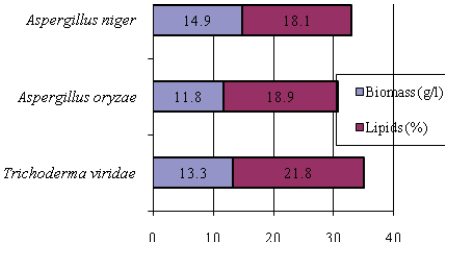

Figure 4. Lipid content of molds

CONCLUSIONS

- Screening of the ten strains of yeasts and molds pointed out a varied potential of lipid biosynthesis.
- The strains characterized by a higher potential of lipid biosynthesis were yeasts, with lipid productivities of: 0,085 g/l·h *Yarrowia lipolytica*, 0,073g/l·h *Saccharomyces cerevisiae* and 0,064g/l·h *Saccharomyces ellipsoideus*.
- In the case of molds, the results were lower than those mentioned in the literature. The best results were obtained from *Trichoderma viridae*, with a lipid productivity of 0,024 g/l·h.
- The strain *Yarrowia lipolytica* showed the greatest potential ability in producing lipids and it was selected for further investigations.

ACKNOWLEDGEMENTS TNR 12

This research was carried out with the support of the program "Invest in people!", project co-financed by the European Social Fund POSDRU2007-2013,
POSDRU/159/1.5/S/132765

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