学校编码：10384　　分类号＿＿＿＿＿密级＿＿＿＿
学号：20620091151290　　UDC＿＿＿＿＿

厦门大学

硕　士　学　位　论　文

海洋高产油脂真菌的选育及其发酵条件的优化

Screening of High Lipid-Producing Fungus from Marine Environment and Studies on the Optimization of Fermentation Conditions

马占军

指导教师姓名：郑天凌　教　授

专　业　名　称：生　物　化　工

研　究　方　向：应用微生物学

论文提交日期：2012 年 05 月

论文答辩时间：2012 年 06 月

学位授予日期：2012 年　　月

答辩委员会主席：＿＿＿＿＿＿＿＿

评　　阅　　人：＿＿＿＿＿＿＿＿

2012 年 05 月

厦门大学学位论文原创性声明

本人呈交的学位论文是本人在导师指导下,独立完成的研究成果。本人在论文写作中参考其它个人或集体已经发表的研究成果，均在文中以适当方式明确标明，并符合法律规范和《厦门大学研究生学术活动规范（试行）》。

另外，该学位论文为（　　）课题（组）的研究成果，获得（　　）课题（组）经费或实验室的资助，在（　　）实验室完成。（请在以上括号内填写课题或课题组负责人或实验室名称，未有此项声明内容的，可以不作特别声明。）

声明人（签名）：

年　　月　　日

厦门大学学位论文著作权使用声明

本人同意厦门大学根据《中华人民共和国学位条例暂行实施办法》等规定保留和使用此学位论文，并向主管部门或其指定机构送交学位论文（包括纸质版和电子版），允许学位论文进入厦门大学图书馆及其数据库被查阅、借阅。本人同意厦门大学将学位论文加入全国博士、硕士学位论文共建单位数据库进行检索，将学位论文的标题和摘要汇编出版，采用影印、缩印或者其它方式合理复制学位论文。

本学位论文属于：

（　　）1.经厦门大学保密委员会审查核定的保密学位论文，于2013年12 月 30日解密，解密后适用上述授权。

（　　）2.不保密，适用上述授权。

（请在以上相应括号内打“√”或填上相应内容。保密学位论文应是已经厦门大学保密委员会审定过的学位论文，未经厦门大学保密委员会审定的学位论文均为公开学位论文。此声明栏不填写的，默认为公开学位论文，均适用上述授权。）

声明人（签名）：

年　　月　　日

目 录

Contents

摘 要

微生物油脂(microbial oils)又称单细胞油脂(single cell oil，SCO)，即微生物以碳水化合物、碳氢化合物和普通油脂为碳源、氮源、辅以无机盐生产的油脂和另一些有商业价值的脂质。随着人口的增长、社会的进步使油脂需求量与自然资源短缺的矛盾日益加剧，环境污染日趋严重，开发新型油脂资源及清洁能源是当务之急。微生物生产油脂具有原料来源丰富、可再生、成本低、周期短、环境友好等优点，已引起人们的高度关注。开展微生物油脂的研究对解决人类面临的资源短缺、环境恶化等现实问题具有重要意义。

本论文从产油脂菌株的筛选开始，对高产菌株进行理化诱变以获得高产突变株，对菌株产油脂发酵工艺条件进行了系统研究，并对油脂成分进行分析，为微生物油脂工业化生产提供理论依据，主要研究结果如下：

1、以苏丹黑染色观察脂肪粒大小为指标，从 10 份土样中初步分离筛选到 38 株产油脂真菌。经摇瓶初筛、复筛，获得一支油脂得率较高的酵母菌株 MD2，油脂含量达 27.96%(m/m)。通过细胞形态特征观察及 18S rRNA 基因序列同源比对分析，菌株 MD2 初步鉴定为粘红酵母菌。

2、为了进一步提高菌体的油脂含量，以粘红酵母 *Rhodotorula* sp.MD2 为出发菌株，采用紫外诱变、亚硝基胍诱变和紫外- 亚硝基胍复合诱变等方法，对其进行处理。经过初筛、复筛，获得一株油脂高产菌株 MD2-1，其油脂产量达到 5.32 g/L，比出发菌株提高了 13.44 %， 连续传代多次，其产量性状无显著变化，说明该菌株遗传稳定性良好。

3、采用酸热法提取粘红酵母 *Rhodotorula* sp. MD2-1 的菌体油脂，测定了其产脂能力，并采用气相色谱－质谱联用法(GC－MS) 测定了其脂肪酸组成。结果表明，粘红酵母 MD2-1 的产脂能力较强，油脂含量达菌体干重的 41.40%，脂肪酸组成较为特殊，10－十八碳烯酸占脂肪酸总量的 71.53%。

4、采用单因素试验和均匀设计试验对菌株 MD2-1 的发酵产油脂培养基和培养条件进行了优化，确定了最佳产油脂发酵培养基：葡萄糖 80 g/L，硫酸铵用量 0.5 g/L，硫酸镁用量 1.0 g/L；确定了较优培养条件为：起始 pH 5.0 培养温度 28 ℃，

接种量 10% (m/v)，转速 150 rpm，装液量 80 mL/ 250 mL，发酵时间 144 h。菌株 MD2 在最佳培养基和较优培养条件下培养，油脂得率达 5.587 g/L，菌体生物量 16.725 g/L。产脂条件优化后菌株 MD2-1 的油脂产量比优化前提高 28.11%，且性能稳定。

本课题筛选获得了一支油脂得率高、性能稳定的粘红酵母菌 MD2，对其进行理化诱变得高产突变株 MD2-1，利用酸热法提取突变株 MD2-1 油脂，并对菌株 MD2-1 的发酵产油条件进行了优化，为进一步的研究奠定了基础。

关键词：海洋真菌；诱变；脂肪酸；组成分析；发酵工艺

Abstract

Microbial oils, referring to oils produced by microorganisms with carbohydrate source(carbohydrates, hydrocarbons and ordinary fats), nitrogen sources and inorganic chemicals, were called single cell oil as well. With the population growth and social progress, the contradiction between demand of oil and shortage of natural resources is increasingly intensifying. On the other hand, the environmental pollution becomes more and more serious. So we need to found new types of cleaning energy resources. Vast diversity of raw materials, short fermentation period, being renewable, low costs and environmental friendliness made microbial oils the focus of researchers. It was of vital importance to relieve the shortage supply of resources, to terminate the environment deteriorating by exploration of microbial oils.

In this paper, we carried out a series of experiments, including screening, mutating,identifying, optimizing fementation process and analyzing its fatty acids, providing a theoretical basis For microbial oil production. The main results are as follows:

1. 38 oleaginous fungus were isolated from 10 soil samples by staining the fat particle of the cells with Sudan Black B. Strain MD2 was selected by investigating the glucose and oil production rate by shaking flask fermentation, oil content of which was up to 27.96%(m/m) and the oil gave off aroma compounds. Morphological properties as well as the 18S rRNA sequence analysis identified the strain as *Rhodotorula* species, preliminarily.

2. In order to improve oil yield of *Rhodotorula* sp. MD2, some mutation methods, such as UV mutation, nitrosoguanidine (NTG) mutation and their combination, were used to screen high-yield oil strains. Finally, one strain MD2-1 was obtained through interview screening and senior screening. The oil yield of mutation was reached 5.32 g/L and improved by 13.44 % compared with original strain. It's production characteristic had no significant change through successive transfer of

culture. And it was found that genetic characteristics of MD2-1 were rather stable.

3. The lipid was extracted from the strain *Rhodotorula* sp. MD2-1 by Thermal–acid method，and its fatty acids was analyzed by GC－MS. The results showed that *Rhodotorula* sp. MD2-1 exhibited strong ability to produce lipid，and the total amount of lipid represented an average of 41.40% of the dry strain weight. The fatty acid composition of the lipid from *Rhodotorula* sp. MD2-1 was special，and the 10－octadecenoic acid accounted for 71.53% of the total fatty acids.

4. The conditions of fermentation were optimized by single factor and orthogonal experiments. The optimal fermentation medium compositions were as follows:80 g/L glucose, 0.5 g/L $(NH_4)_2SO_4$, 1.0 g/L $MgSO_4$;The optimum culture conditions were initial pH5.0, temperature 28 ℃, Inoculation amount 10%(V/V), rotation speed of rocking bed 150 rpm, broths volume in shake flask 80 mL/500 mL and culture time 144 h. oil yield and biomass under the optimum conditions were 5.587 g/L, 16.725 g/L, oil production improved 28.11% of the value of the initial.

Rhodotorula sp. MD2 with stable performance, higher production oil and strongly aroma was screened in the thesis. mutagenized to obtain the mutant MD2-1.Thermal–acid method to determine the content was adopted and fermentation conditions were also optimized. This study laid foundation for future research of microbial oils.

Key words: Marine fungus; mutagenesis; fatty acid; composition analysis; fermentation process