# EFFECTS OF CARBON AND NITROGEN SOURCES ON LIPASE PRODUCTION BY *SARCODON ASPARATUS* *

YONG Qiang[1], KANG Jin-ha[2], YU Shi-yuan[1]

(1. *College of Chemical Engineering, Nanjing Forestry University, Nanjing* 210037, *China*;
2. *College of Agriculture, Chonbuk National University of Korea, Chonju* 561-765, *Korea*)

YONG Q

**Abstract:** Carbon sources and nitrogen sources affecting lipase production by *Sarcodon asparatus* were investigated. Lipase activity was the highest(0.65 U/mL) for olive oil compared with other carbon sources. Lipase production was stimulated by peptone(0.65 U/mL), urea(0.67 U/mL) and ammonium chloride(0.62 U/mL), and repressed by ammonium sulfate with a low lipase activity of 0.08 U/mL. C/N ratio was a significant factor for lipase production and cell growth. When C/N ratio was raised from 2 to 10, lipase activity decreased from 0.65 to 0.14 U/mL, and biomass increased from 3.08 to 4.58 mg/mL. The optimal culture conditions of *S. asparatus* were pH value 5.5 and 28 ℃, and lipase activity was 0.65 U/mL with a biomass concentration of 3.08 mg/mL.

**Key words:** lipase; carbon source; nitrogen source; white rot fungi; *Sarcodon asparatus*

**CLC number:** Q556 **Document code:** A **Article ID:** 0253-2417(2004)04-0001-06

# 碳源和氮源对白腐菌 *Sarcodon asparatus* 合成脂肪酶的影响

勇 强[1], 姜镇河[2], 余世袁[1]

(1. 南京林业大学 化学工程学院, 江苏 南京 210037;
2. 韩国全北大学校 农科大学, 全州 561-765)

摘 要: 研究了碳源和氮源对白腐菌 *Sarcodon asparatus* 合成脂肪酶的影响。橄榄油是脂肪酶合成的最佳碳源,脂肪酶活力达 0.65 U/mL。蛋白胨、尿素和氯化铵对脂肪酶的合成有促进作用,它们的酶活力分别为 0.65、0.67 和 0.62 U/mL,而硫酸铵抑制脂肪酶的合成,其酶活力仅为 0.08 U/mL。C/N 比对细胞生长和酶的合成影响很大,当 C/N 比从 2 增加到 10 时,脂肪酶活力从 0.65 下降到 0.14 U/mL,而细胞浓度从 3.08 升高到4.58 mg/mL。在培养基初始 pH 值 5.5、28 ℃下培养, *S. asparatus* 合成的脂肪酶活力最高,脂肪酶活力和细胞浓度分别为 0.65 U/mL 和 3.08 mg/mL。

关键词: 脂肪酶; 碳源; 氮源; 白腐菌; *Sarcodon asparatus*

Lipase(EC 3.1.1.3), which catalyzes the hydrolysis of triglycerides to diglycerides, monoglycerides, glycerol and fatty acids at an oil-water interface, has been obtaining more interest over the last two decades

* **Received date:** 2004-05-25
**Foundation item:** 国家自然科学基金资助项目(30100142)
**Biography:** 勇 强(1968- ), 男,江苏宜兴人, 副教授, 博士, 主要从事植物资源生物转化的研究;
**E-mail:** njyq@21cn.com

due to its potential application in industries. Since lipase could not only hydrolyze ester bonds, transesterify triglycerides and resolve racemic mixture, but also synthesize ester bonds in non-aqueous media, it has been found potential applications in fat and oil modification, pharmaceuticals, detergents, food and chemical industry[1]. In recent years, a promising application of lipase is for biological deinking in waste paper recycling process. The main drawbacks of conventional deinking technology with chemicals are the reduced dewatering property of recovered paper and the serious impact on environment. Biological deinking, which employs cellulase or lipase as catalyst, is an alternative process to overcome the disadvantages of chemical methods[2-3]. Since fiber length and repulping yield will drop during enzymatic treatment with cellulase due to cellulose degradation, the application of cellulase in deinking is very limited. However, the mechanism of lipase deinking is that the ink, which consists of oil or other polyesters as pigment carrier, is hydrolyzed into small particles and released from the fiber surface, followed by removing with floatation or washing, therefore the fiber will not be attacked during lipase treatment.

A widespread number of microorganisms, including fungi, bacteria and yeasts, have been found to produce lipase, especially the genera *Mucor*, *Aspergillus*, *Rhizopus* and *Penicillium* of fungi. Numerous literatures have been published about their lipase production and characteristics[4-5]. However, lipase production by white rot fungi was scarcely reported. The present work aims to investigate the effects of carbon sources, nitrogen sources and environmental factors on lipase production by *S. asparatus*.

## 1 Methods

### 1.1 Microorganism

*S. asparatus* obtained from Korea Forest Research Institute was maintained at 4 ℃ on peptone malt extract medium consisting of(g/L, deionized water): malt extract 3, peptone 5, yeast extract 3, glucose 10 and agar 20.

### 1.2 Inoculum preparation and fermentation

A loopful of mycelia from a 10-day-old agar slant was inoculated into a 250 mL flask containing 100 mL medium and incubated in a rotary shaker(100 r/min) at 30 ℃ for 2 d. The composition of the medium was(g/L, deionized water): glucose 25, malt extract 10, peptone 5, $KH_2PO_4$ 0.3 and $MgSO_4 \cdot 7H_2O$ 0.2. After inoculum preparation, 5 mL of the culture was transferred into 250 mL flasks containing 50 mL basal medium consisting of(g/L, deionized water): carbon source 5, peptone 20, yeast extract 1, $CaCl_2$ 0.4, $KH_2PO_4$ 2, $MgSO_4 \cdot 7H_2O$ 0.5, and cultivated at 28 ℃ for 10 d with shaker rotated at 100 r/min.

### 1.3 Biomass determination

50 mL sample was withdrawn at 2 d intervals and the mycelia were separated by centrifugation at 4 000 r/min for 10 min. The clarified supernatant was washed twice with acetone and water respectively, and dried at 105 ℃ for 24 h.

### 1.4 Lipase activity determination

25 mL olive oil was added in 75 mL polyvinyl alcohol and emulsified by stirring the mixture for 15 min with a homogenizer(15 000 r/min). To this emulsion(5 mL), 4 mL 0.1 mol/L citrate phosphate buffer(pH value 7.0) and enzyme solution(1 mL) were added and the mixture was incubated at 37 ℃ in a shaking bath(110 r/min) for 4 h. As a control, enzyme solution(1 mL) heated for 10 min in a boiling water bath was added to the substrate-containing emulsion(9 mL) and incubated in the same way as the assay mixture. The reaction was stopped by addition of 20 mL ethanol and acetone(1∶1), and the amount of liberated fatty acid was titrated with 0.05 mol/L NaOH against phenolphthalein as the indicator. One unit of lipase activity was defined as the amount of enzyme that liberated 1 μmol fatty acid equivalent per minute under the

given condition.

### 1.5 Lipid assay

The residual olive oil in the fermentation culture was extracted with a mixture of petroleum-ether and ethyl-ether(11). After separation, the organic solvent layer was transferred into a preweighed test tube; petroleum-ether and ethyl ether were evaporated using a rotary evaporator. The test tube was then placed in a 37 ℃ incubator for 24 h before weighing.

## 2 Results and discussion

### 2.1 Effect of carbon source on lipase production

An effective and inexpensive medium is necessary for lipase production economically by microorganism. Carbon and nitrogen source selection would be important not only for its impact on lipase activity and productivity, but also for its impact on the cost of the production. On the other hand, the selection of carbon source also affects the role of nitrogen in the culture. Like most extracellular enzymes, lipase is an inducible enzyme. Many microbial lipases are only biosynthesized in the presence of inducers, which may be sugars, triglycerides, fatty acids and other lipids. A large number of carbon and nitrogen sources have been investigated on the lipase production by a variety of microorganisms such as fungi, bacteria and yeasts. The results revealed that different carbon and nitrogen sources have different effects on lipase production, even if the same carbon and nitrogen source are used, the impact of the carbon and nitrogen source are different among tested microorganisms. In this study, the effects of monosaccharides(glucose and xylose), disaccharides(sucrose, lactose and maltose), polysaccharides(starch, cellulose and xylan), esters with short fatty acid residues (myristic acid methyl ester, myristic acid ethyl ester and tributyrin), esters with long fatty acid residues(triolein), and oils(olive oil, soy oil, corn oil and sesame oil) on cell growth and lipase production by *S. asparatus* have been investigated. The results are shown in Table 1.

**Table 1　Effects of carbon sources on growth and lipase production by *S. asparatus***

| carbon sources /0.5% | final pH value | biomass concn. /(mg·mL$^{-1}$) | lipase activity /(U·mL$^{-1}$) | yield /(U·mg$^{-1}$) |
|---|---|---|---|---|
| basal medium | 6.34 | 1.34 | 0.22 | 0.16 |
| glucose | 5.76 | 1.71 | 0.42 | 0.25 |
| xylose | 6.07 | 1.25 | 0.40 | 0.32 |
| sucrose | 6.35 | 1.16 | 0.39 | 0.34 |
| lactose | 5.19 | 1.75 | 0.37 | 0.21 |
| maltose | 5.00 | 1.83 | 0.39 | 0.21 |
| starch | 5.91 | 2.43 | 0.49 | 0.20 |
| cellulose | 6.30 | 3.09 | 0.44 | 0.14 |
| xylan | 5.52 | 2.53 | 0.44 | 0.17 |
| myristic acid methyl ester | 6.32 | 1.58 | 0.40 | 0.25 |
| myristic acid ethyl ester | 5.43 | 1.19 | 0.39 | 0.33 |
| tributyrin | 4.36 | 0.85 | 0.43 | 0.51 |
| triolein | 5.77 | 3.28 | 0.45 | 0.14 |
| olive oil | 5.22 | 3.08 | 0.65 | 0.21 |
| soybean oil | 5.14 | 2.33 | 0.55 | 0.24 |
| corn oil | 5.52 | 2.46 | 0.52 | 0.21 |
| sesame oil | 4.95 | 3.14 | 0.55 | 0.18 |

All the carbon sources tested induced lipase production. The highest lipase activities were obtained with the media containing oils (0.52 – 0.65 U/mL), followed by the medium employing triolein as carbon source (0.45 U/mL). The inducement of polysaccharides was slightly higher than that of esters, which have short fatty acid residues. Since polysaccharides are too large in molecular size and insoluble, they have to be hydrolyzed to smaller and soluble fragments at first by polysaccharide hydrolytic enzymes such as cellulase and xylanase, which are also synthesized by *S. asparatus* (data not shown). The ability of disaccharides to induce lipase production was the lowest among the tested carbon sources, the lipase activity being 0.37– 0.39 U/mL, only 57% – 75% of that when oils were used as the carbon sources. On the basis of biomass concentration, trolein was proved to be beneficial for cell growth, followed by oils and polysaccha-

rides.

Among oils tested, olive oil was the best inducer for lipase production by *S. asparatus* with a lipase activity of 0.65 U/mL, compare to soybean oil(0.55 U/mL), sesame oil(0.55 U/mL) and corn oil(0.52 U/mL). Usually, lipids are effective inducers for lipase production because they are natural substrate of lipase. However, the best inducers are different depending on microorganisms employed. Denise *et al*[6] and Lin[7] reported that the highest lipase activity occurred in the medium containing olive oil by *Penicillum restrictum* and *Pseudomonas pseudoalcaligenes* F-111, respectively. Macris *et al*[8] found that *Aspergillus niger* could produce the highest extracellular lipase when corn oil was used as the substrate, while Maia *et al*[9] reported that sesame oil containing medium resulted in the highest lipase activity and specific activity by *Fusarium solani* compare to other carbon sources.

## 2.2 Effect of nitrogen source on lipase production

Various organic and inorganic nitrogen sources were selected to evaluate the effects of *S. asparatus* lipase production. The medium contained 0.5% olive oil as the carbon source and the C/N ratio was kept constant, therefore the concentration of different nitrogen sources was varied depending on the N content of each nitrogen source. The peak values of biomass and lipase activity for various nitrogen sources are presented in Table 2.

**Table 2 Effects of nitrogen source on growth and lipase production by *S. asparatus***

| nitrogen source | final pH value | biomass concn. /(mg·mL$^{-1}$) | lipase activity /(U·mL$^{-1}$) | yield /(U·mg$^{-1}$) |
|---|---|---|---|---|
| malt extract(1.0%) + peptone(0.5%) | 4.47 | 4.60 | 0.13 | 0.03 |
| peptone(2.0%) | 5.22 | 3.08 | 0.65 | 0.21 |
| casein(2.0%) | 7.51 | 2.82 | 0.41 | 0.15 |
| urea(0.434%) | 5.18 | 3.19 | 0.67 | 0.21 |
| $(NH_4)_2SO_4$(0.943%) | 4.30 | 2.92 | 0.08 | 0.03 |
| $NH_4Cl$(0.765%) | 3.95 | 2.02 | 0.62 | 0.31 |

The lipase production by *S. asparatus* was stimulated by peptone, urea and ammonium chloride, and the lipase activities were 0.65, 0.67 and 0.62 U/mL, respectively. Lipase production was repressed by ammonium sulfate with a low lipase activity of 0.08 U/mL. Although casein is also reportedly a good nitrogen source, its inducible capability was apparently lower than that of peptone, the lipase activity was 0.41 U/mL when casein was used compare to 0.65 U/mL when peptone was employed as nitrogen source. Even though malt extract was poor in lipase production with a lipase activity of 0.13 U/mL, it was very beneficial for cell growth, and the biomass concentration increased to 4.60 mg/mL, the highest among all the nitrogen sources tested.

Peptone is a good nitrogen source for almost all microorganisms, such as *Yarrowia lipolytica*, *P. pseudoalcali* genes F-111, *Streptomyces* sp., and *Penicillum*. However, peptone is an expensive substrate, this would result in a high cost of lipase production. It is necessary to employ cheaper inorganic and organic compounds, such as urea, ammonium chloride, ammonium sulfate and ammonium nitrate, for lipase production cost-effectively in large scale. The utilization of inorganic and organic nitrogen sources by microorganisms is strain depending. *P. restrictum* was unable to grow in media containing only inorganic nitrogen nutrition[6]. Kamini *et al*[10] reported that addition of inorganic and organic nitrogen sources such as urea, ammonium nitrate and ammonium dihydrogen orthophosphate to the culture of *Aspergillus niger* did not increase the lipase production. Urea was the best nitrogen source for lipase production by *Y. lipolytica*[11], but it inhibited lipase production by *P. pseudoalcali* genes F-11[7]. Miranda *et al*[12] also reported that the inducible capability of ammonium sulfate was much higher than that of urea for lipase biosynthesis by *Penicillum citrinum* using an industrial residual as the carbon source.

## 2.3 Effect of C/N ratio on lipase production

The ratio of C to N in the medium is also a critical factor, which influences microbial growth and enzyme production. Lipase production with supplementation of 0.5% olive oil was carried out to assess the effect of C/N ratio on cell growth and lipase production by *S. asparatus*. The peptone concentrations in the medium were 20, 10, 6.3, 4.5 and 3.3 mg/mL, corresponding to C/N ratio of 2, 4, 6, 8 and 10, respectively. The final pH value of the fermentation broth and the highest value of biomass, lipase activity and yield are presented in Fig. 1.

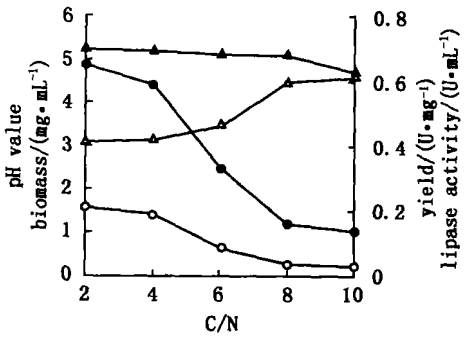


**Fig. 1　Effects of C/N ratio on cell growth and lipase production**

It was found that the ratio of C to N had a significant effect on lipase production by *S. asparatus*. The highest value of enzyme activity(0.65 U/mL) was obtained when C/N ratio was 2, meanwhile the lowest biomass concentration, 3.08 mg/mL, was detected. It was obvious that the enrichment of nitrogen source was beneficial for lipase production by *S. asparatus*. The lipase activities decreased with an increase in the ratio of C/N, while the opposite results were obtained with biomass concentrations. As shown in Fig. 1, when the ratio of C/N was raised from 2 to 10, lipase activity decreased from 0.65 to 0.14 U/mL, while biomass concentration increased from 3.08 to 4.58 mg/mL, resulted in a dramatic drop in yield from 0.21 to 0.03 U/mg dry weight of biomass. This ***result was in agree with the study of Denise et al*** [6], who reported that increasing C/N ratio would trigger a metabolic shift in *P. restrictum* towards cell growth with a significant detriment of enzyme production.

## 2.4　Effect of olive oil concentration on lipase production

The highest data of biomass concentration and lipase accumulation for the evaluation of olive oil concentration on lipase production by *S. asparatua* are presented in Fig. 2. When olive oil concentration in the medium increased from 0 to 0.5%, lipase activity improved from 0.28 to 0.64 U/mL, and biomass concentration improved from 1.34 to 2.48 mg/mL. As olive oil concentration in the medium increased from 0.5% to 1.0%, lipase activity leveled off, while biomass concentration continued to increase. When olive oil concentration further increased to 1.5%, no significant changes could be observed both for lipase and biomass production, and this could be due to a decrease of C/N ratio.

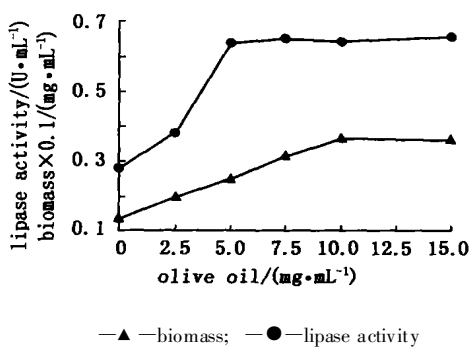


**Fig. 2　Effects of the olive oil on growth and lipase production**

## 2.5　Typical time course of lipase production by *S. asparatus*

A typical time course of lipase production by *S. asparatus* with supplementation of 0.5% olive oil as carbon source is shown in Fig. 3. After 10 d cultivation under optimal conditions, lipase activity and biomass concentration of the culture increased to 0.65 and 3.08 mg/mL, respectively, which were coincident with the consumption of olive oil, the concentration of olive oil dropped from 5 to 0.21 mg/mL. The pattern of lipase production by *S. asparatus* was shown to be growth-associated, and this phenomenon was corresponded to other microorganisms such

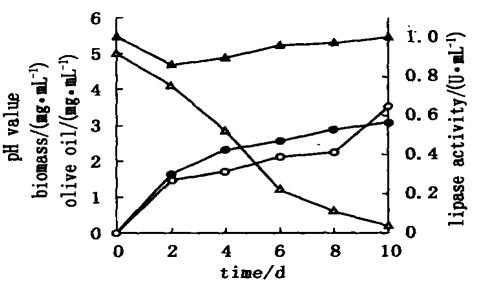


**Fig. 3　Time course of lipase production by *S. asparatus***

as *Pseudomonas fluorescenes*[13], *P. restrictum*[6] and *Candida rugosa*[14]. The pH value of the culture dropped slightly from 5.5 to 4.7 during the first 2 d, and then increased slowly in the next cultivation period before reaching maximum value of 5.46.

## 3 Conclusion

A wide number of carbon sources including monosaccharides, disaccharides, polysaccharides, esters and oils have inducible capability for lipase production by *S. asparatus*. Among the tested carbon sources, olive oil was the best inducer, followed by soybean oil, sesame oil and corn oil. Lipase production by *S. asparatus* was stimulated by peptone, urea and ammonium chloride but inhibited by ammonium sulfate. Although malt extract was very beneficial for cell growth, its inducible ability for lipase production was low. The optimal conditions for cell growth and lipase production were pH value 5.5 and 28 ℃, and the lipase activity was 0.65 U/mL with a biomass concentration of 3.08 mg/mL. The production of lipase by *S. asparatus* was highly dependent on the ratio of C to N. Lipase activity decreased with increasing ratio of C/N, and the highest lipase production was obtained when the ratio of C/N was 2.

**References:**


[1] ABEL H, MARIE D, JONZO D, *et al*. Production, purification and characterization of an extracellular lipase from *Mucor hiemalis* F. *hiemalis*[J]. Enzyme Microb Technol, 1999, 25(2): 80-87.

[2] BAJPAI P. Application of enzyme in the pulp and paper industry[J]. Biotechnology Progress, 1999, 15(2): 147-157.

[3] VIESTURS M, LEITE M, EISIMONTE T, *et al*. Biological deinking technology for the recycling of office waste papers [J]. Bioresource Technology, 1999, 67(3): 255-265.

[4] JAEGER K E, RANSAC S, DIJKSTRA B W, *et al*. Bacterial lipases[J]. FEMS Microbial Rev, 1994, 15(1): 29-63.

[5] RAPP P, BACKHANS S. Formation of extracellular lipase by filamentous fungi, yeast and bacteria[J]. Enzyme Microb Technol, 1992, 14(11): 938-943.

[6] DENISE M, ELAINE M E T, ELBA P S B, *et al*. Lipase production by *Penicillum restrictum* in a bench-scale fermenter [J]. Appl Biochemistry and Biotechnology, 1997, 63(5): 409-421.

[7] LIN S. Production and stabilization of a solvent-tolerant alkaline lipase from *Pseudomonas pseudoalcaligenes* F-111[J]. J Ferment Bioeng, 1996, 82(5): 448-451.

[8] MACRIS J B, KOURENTIZI E. Studies on localization and regulation of lipase production by *Aspergillus niger*[J]. Process Biochemistry, 1996, 31(8): 807-812.

[9] MAIA M M D, HEASLEG A, CAMARGO M M, *et al*. Effect of culture conditions on lipase production by *Fusarium solani* in batch fermentation[J]. Bioresource Technology, 2001, 76(1): 23-27.

[10] KAMINI N R, MALA J G S, PUVANAKRISHAN R. Lipase production from *Aspergillus niger* by solid state fermentation using gingelly oil cake[J]. Process Biochemistry, 1998, 33(5): 505-511.

[11] GERARDO L, SERGIO R. Production and characteristics of the lipase from *Yarrowia lipolytica* 681[J]. Bioresource Technology, 1999, 70(2): 173-180.

[12] MIRANDA O A, SALGUEIRO A A, PIMENTEL M C B, *et al*. Lipase production by a Brazilian strain of *Penicillium citrinum* using an industrial residue[J]. Bioresource Technology, 1998, 69(2): 145-147.

[13] KIM S, KIM E, JWH S R. Effects of growth rate on the production of *Pseudomonas fluorescens* lipase during the fed-batch cultivation of *Escherichia coli*[J]. Biotechnol Prog, 1996, 12(5): 718-722.

[14] GORDILLO M A, SANZ A, SANCHEZ A, *et al*. Enhancement of *Candida rugosa* lipase production by using different control fed-batch operational strategies[J]. Biotechnol Bioeng, 1998, 60(2): 156-168.