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# Effect of Sodium Dodecyl Sulfate on Lipase of *Candida lipolytica*

A. E. NASCIMENTO[1] AND G. M. CAMPOS-TAKAKI[1,2,*]

[1]*Laboratório de Imunopatologia Keizo Asami; and* [2]*Departamento de Antibióticos, Universidade Federal de Pernambuco, Campus Universitário, Cidade Universitária, Recife, Pernambuco, Brazil*

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## ABSTRACT

The effects of sodium dodecyl sulfate on extracellular lipase produced by *Candida lipolytica* have been studied. The microorganism was grown in culture medium containing different sodium dodecyl sulfate concentrations added to the culture at different intervals of growth. The extracellular lipase activity was not detected when the treated culture supernatants were directly tested in Yeast Mold Agar-Triolein-Rhodamine plates, regardless of surfactant addition time and concentrations. However, after ammonium sulfate precipitation and dialysis, the extracellular lipase activity could be recovered. Therefore, the surfactant, under the experimental conditions used here, does not seem to be able to inhibit lipase production, but it does inhibit the enzyme activity because of its presence in the mixture of the reaction.

**Index Entries:** Lipase; sodium dodecyl sulfate; *Candida lipolytica;* growth.

## INTRODUCTION

Lipases are enzymes that hydrolyze triglycerides in mono- and diglycerides, glycerol, and fatty acids, and act specifically at oil/water interfaces. Lipase activities have been found in molds, yeasts, and bacteria (*1–4*).

*Author to whom all correspondence and reprint requests should be addressed.

Substances that can alter the nature of the oil/water interface can markedly influence the hydrolysis of triglycerides by lipases. In order to maintain the maximum rate of hydrolysis in triglycerides by lipase, many attempts have been made to provide an adequate interfacial area of substrate using surface-active agents. However, it was reported that the effect of surface-active agents on the lipase reaction was complex and dependent on the chemical nature of the agents employed (*1,5,6*).

Many researchers have attempted to improve enzyme production by using surface-active agents directly in the culture medium. Some surfactants proved to enhance the growth of microorganisms and, consequently, enzyme production, specifically, the nonionic surfactants. Ionic surfactants, even in low concentrations, inhibited the growth and enzyme production (*7–11*). The present studies were undertaken to investigate the effects of sodium dodecyl sulfate on lipase activity of *Candida lipolytica* when the surfactant was added to the culture medium in different growth phases.

## MATERIALS AND METHODS

### Microorganism, Medium, and Growth

*Candida lipolytica* IA 1055 was used for these experiments. The microorganism was grown in Cald Yeast Mold medium described by Cirigliano and Carman (*12*) over 96 h at 27°C on a reciprocal shaker (120 Hz). The surfactant, sodium dodecyl sulfate (SDS—Sigma, St. Louis, MO), in concentrations of 0.07, 0.09, and 0.11%, was added to the culture at intervals of 0, 8, 16, and 24 h during culture. These intervals corresponded to the beginning of the culture, beginning of the exponential growth phase, intermediary exponential growth phase, and end of exponential growth phase/beginning of stationary phase, respectively.

### Measurement of Growth

The cell growth was determined by viable cell counts. Cell counts were measured after serial dilution in pH 7.0 PBS buffer of culture samples collected in interval of 0, 8, 16, 24, 48, 72, and 96 h by plating on solid medium YMA. The plates were prepared in triplicate and were incubated for 24 h at 27°C.

### Detection of Extracellular Lipase Activity

The extracellular lipase activity was determined by the method of Kouker and Jaeger (*13*) modified by using YMA medium and triolein as substrate. Samples of 3 mL of *Candida lipolytica* cultures both control and treated, previously collected at intervals of 0, 24, 48, 72, and 96 h of culture, were centrifugated at 2000 rpm for 10 min. The culture supernatants were tested for lipase activity by two methods.

*Direct Assay*

Twenty microliters of samples were loaded in wells of 2 mm diameter made in YMA-Triolein-Rhodamine plates. The plates were incubated for 24 h at 37°C. After this period, the halo formation was analyzed.

*Precipitation and Dialysis*

Samples of 2 mL of culture supernatants were precipitated initially with 50% ammonium sulfate in an ice bath for 90 min and centrifugated at 10,000 rpm for 15 min. The pellets were resuspended in 1 m*M* Tris-EDTA buffer, pH 8.1, with 20 m*M* MgCl. The dialysis was carried out for 48 h at 4°C in the same buffer. Samples of 20 μL were loaded in wells made in YMA-Triolein-Rhodamine plates as previously described, and the activity was analyzed. The supernatants obtained after precipitation reprecipitated with 80% ammonium sulfate as previously described, and the enzyme activity was determined as previously described.

## Cellular Lipase Activity

Samples of 0.1 mL of control and treated cultures, collected at intervals of 0, 24, 48, 72, and 96 h of growth, were diluted in PBS buffer pH 7.0 and plated on YMA-Triolein-Rhodamine plates. The plates, prepared in triplicate, were incubated at 27°C for 24 h, after which the halo formation around colonies was analyzed.

# RESULTS

Growth of *Candida lipolytica* treated cultures as determined by viable cell counts was affected according to addition time and concentration of SDS (Fig. 1). Cultures treated with SDS at all concentrations at 0 time showed a drastic decrease in cell viability as compared to the control (Fig. 1A). Cultures treated after 8 and 16 h of culture, when compared to previous treatment, showed an increase in cell viability. However, the cultures, when compared to controls, showed a reduction in viability that was concentration-dependent. Cultures treated with 0.07 and 0.09% of SDS after 16 h showed a similar cell viability (Fig. 1B and C, respectively). However, cultures treated with SDS after 24 h of cultivation did not show any difference in cell viability as compared to the controls (Fig. 1D).

The extracellular lipase activity was not detected in treated culture supernatants directly tested (Table 1). The SDS concentrations and addition time did not show any influence on the lipase activity. However, after ammonium sulfate precipitation and dialysis, we could recover the lipase activity in all supernatants independent of SDS addition time and concentration (Figs. 2 and 3). The highest lipase activity was detected in samples colleted after 24 h of culture in all supernatants, and the 50% ammonium sulfate fraction showed a higher activity compared to 80% fraction. The lipase activities in treated supernatants were lower than control

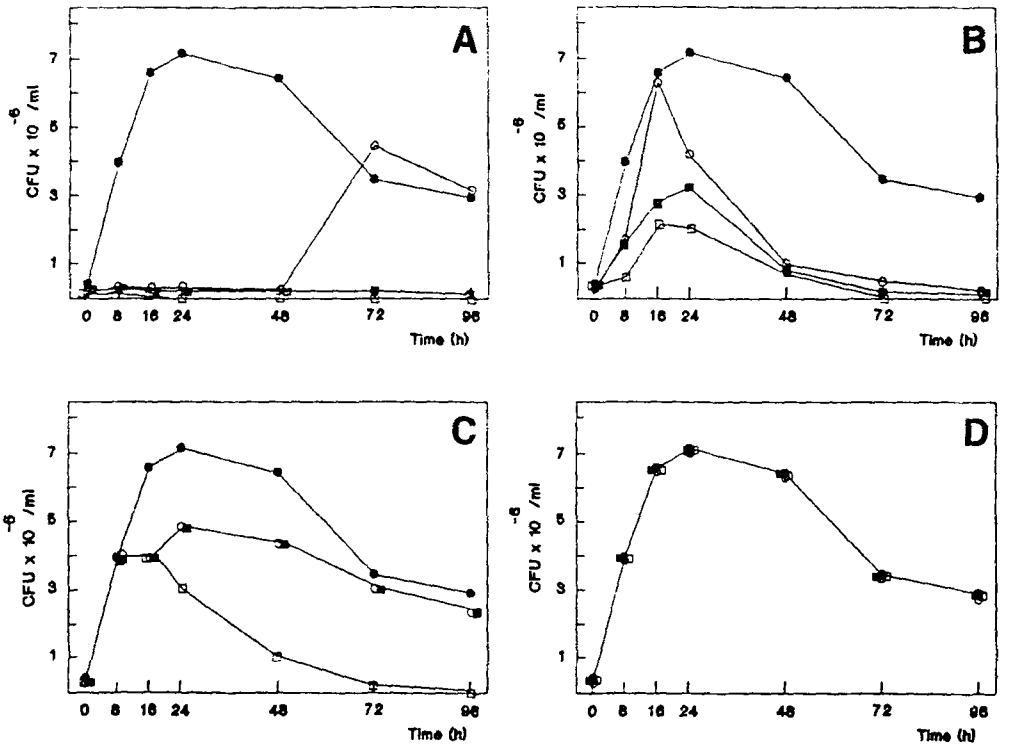


Fig. 1. Cell viability of *Candida lipolytica* cultures. Cultures treated in the beginning of growth—0 time (**A**) after 8 (**B**), 16 (**C**), and 24 (**D**) h of culture. SDS concentrations: 0.07% (○); 0.09% (■), and 0.11% (□). Control culture (●).

Table 1
Extracellular Lipase Activity Detected by Halo Formation (mm) in Culture Supernatants of *Candida lipolytica*

| Samples, h | Control | SDS addition time, h | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 1 | 2 | 3 | 1 | 2 | 3 | 1 | 2 | 3 | 1 | 2 | 3 |
| | | 0 | | | 8 | | | 16 | | | 24 | | |
| 0 | 0 | | | | | | | | | | | | |
| 24 | 8.5 | | | | | | | | | | | | |
| 48 | 7.5 | ND | | | ND | | | ND | | | ND | | |
| 72 | 5.5 | | | | | | | | | | | | |
| 96 | 2.5 | | | | | | | | | | | | |

SDS concentrations: 1—0.07%; 2—0.09%; 3—0.11%.
ND—Not detected.

activities, and were influenced by surfactant concentration and addition time. The highest activities in supernatants of cultures treated in 0 time of cultivation compared to the other treatments could be related possibly to the liberation of intracellular lipase once the cells had been strongly damaged by the surfactant.

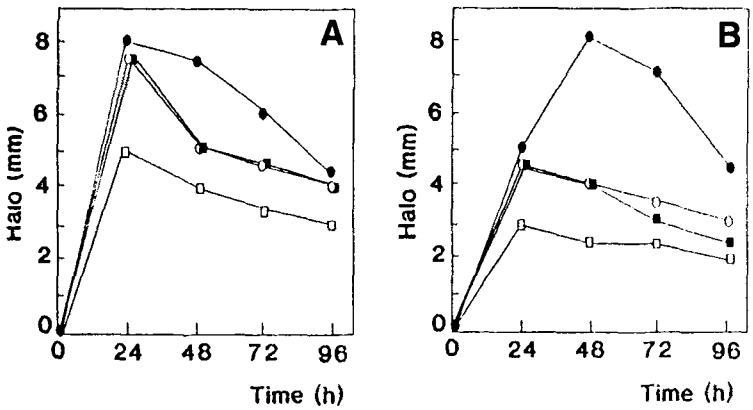


Fig. 2. Lipase activity of supernatants after 50% ammonium sulfate precipitation (**A**) and 80% (**B**) by halo formation (mm). Cultures treated with 0.07% (○), 0.09% (■), and 0.11% SDS (□) in the beginning of culture (0 time). Control culture (●).

The cellular lipase activity assayed shows that culture samples, where colony formation had occurred, had positive enzyme activity (Table 2), in spite of SDS addition time for 0.07 and 0.09% SDS treatment. However, SDS at concentration of 0.11% showed the higher inhibitor effect on the cell viability preventing the colony formation on cultures treated after 0, 8, and 16 h of growth.

## DISCUSSION

These experiments show that SDS can disturb the growth of *Candida lipolytica* as observed by viable cell counts depending on its addition time and concentration. Cultures treated at 0 time of culture showed the highest inhibition compared to other treatments. These results are confirmed by other authors (*9–11*). However, the study of SDS influence in different growth phases is reported here for the first time.

Many researchers reported that it was possible to recover the enzyme activities after a solubilization or treatment with surfactants, such as SDS, through dialysis, heating, or change of the physicochemical reaction conditions (*1,14–18*).

A research of the literature revealed that in studies where surfactants in the media were used at higher concentration, many researchers reported an inhibition of enzyme production by microorganisms. However, all the studies also revealed that the enzyme detection was made directly on the culture supernatant where the surfactant was present (*9–11*).

The results obtained in this study reveal that the SDS presence in the reaction mixture prevent the detection of lipase activity in supernatants of treated cultures. However, lipase is produced by cells as observed after

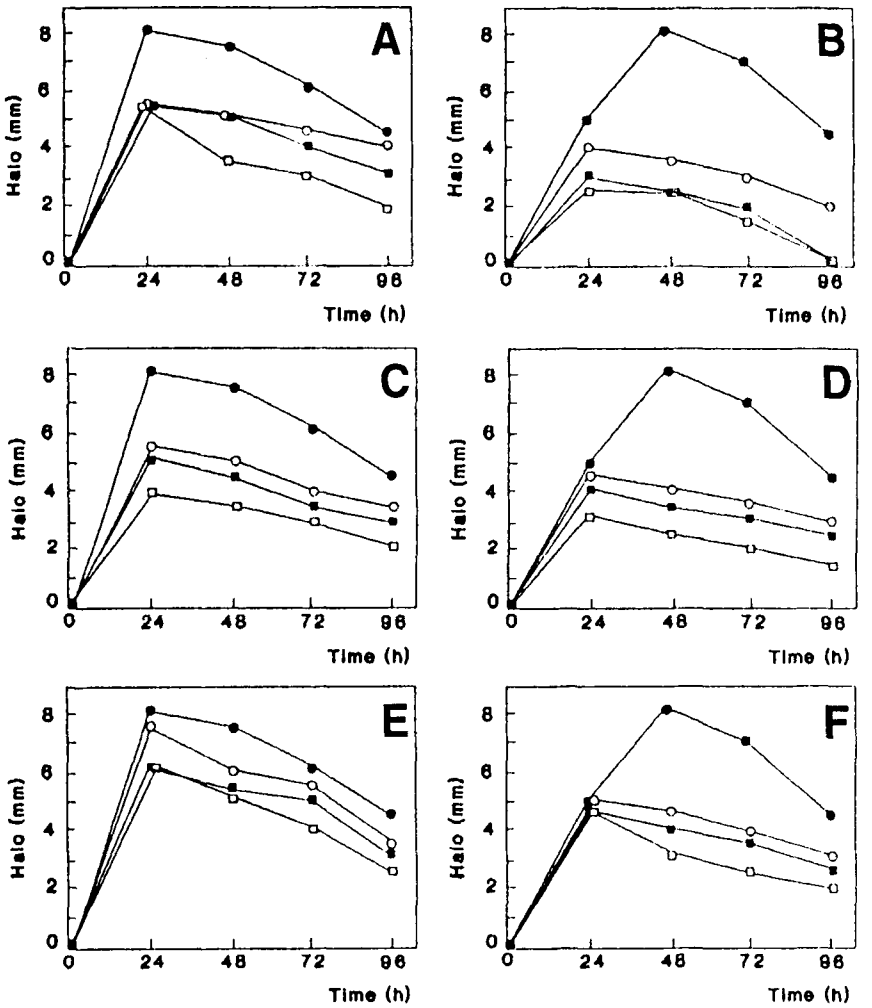


Fig. 3. Lipase activity detected by halo formation (mm) in culture supernatants after 50% ammonium sulfate precipitation (**A, C, E**) and 80% (**B, D, F**). 0.07% (○), 0.09% (■), and 0.11% SDS (□). Cultures treated after 8 (A,B), 16 (C,D), and 24 (E,F) h of cultivation. Control (●).

precipitation and dialysis. These results differ from others related to the inhibition of enzyme production by microorganisms grown in the presence of surfactants.

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Table 2
Identification of *Candida lipolytica* Cultures with Lipase Activity

| Samples, h | Control | SDS addition time, h | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 0 | | | 8 | | | 16 | | | 24 | | |
| | | 1 | 2 | 3 | 1 | 2 | 3 | 1 | 2 | 3 | 1 | 2 | 3 |
| 0 | + | + | + | + | + | + | + | + | + | + | + | + | + |
| 24 | + | + | + | ND | + | + | + | + | + | + | + | + | + |
| 48 | + | + | + | ND | + | + | + | + | + | + | + | + | + |
| 72 | + | + | + | ND | + | + | ND | + | + | ND | + | + | + |
| 96 | + | + | + | ND | + | + | ND | + | + | ND | + | + | + |

SDS concentrations: 1—0.07%; 2—0.09%; 3—0.11%.
+ Halo formation.
ND—Not detected.

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