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# EFFECTS OF TWEEN 80 ON PROTEASE PRODUCTION BY *Candida lipolytica*

*(Efectos del Tween 80 en la producción de proteasa por* ***Candida lipolytica****)*

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**Key Words:** *Candida lipolytica*; protease; growth; Tween 80

## SUMMARY

*The influence of Tween 80, a non ionic tensoactive agent on extracellular protease produced by* ***Candida lipolytica*** *was investigated. The microorganism was grown in a medium containing different surfactant concentrations added to culture at different intervals of growth. The addition of the surfactant on the* ***C. lipolytica*** *culture medium, resulted in a increase of intracellular and extacellular protease activity as well as in an increase in the growth of the fungi which could be related to Tween 80 concentration and addition time.*

## RESUMEN

*Se estudió la acción del Tween 80, agente tensoactivo no iónico, sobre la proteasa producida por* ***Candida lipolytica.*** *El microorganismo fue cultivado en medio de cultivo conteniendo diferentes concentraciones del agente, el cual fue introducido en el medio en diferentes intervalos de tiempo. La presencia del agente en el medio de cultivo incrementó la actividad de la proteasa extracelular e intracelular y el crecimiento del hongo. Los efectos del agente podrían estar relacionados con el tiempo de adición y las concentraciones empleadas en el medio de cultivo.*

## INTRODUCTION

***Candida lipolytica*** is known as a lipase-producing and hydrocarbon-assimilating yeast. Several investigators have reported that isolates of *C. lipolytica* are proteolytic but the extent and magnitude of proteolysis among strains of the specie have not been documented and preliminar data suggest that this proteolytic activity is inducible or repressed in the presence of glucose ( Ahear et al., 1968; Tobe et al., 1976; Meyers & Ahearn, 1977; Ogrydziak et al.,1977; Yamada & Ogrydziak, 1983 Lodder & Krieger van Rij, 1984 ).

Recently, interest in the pathogenesis of *Candida* infection has led to studies on extracellular proteases produced by some species of the genus, which are positive for protease activity. The protease activity is related to invasive properties and virulence of the *Candida* genus (Chakrabarti et al.,1991).

A group of suface-active agents, the Tweens (fatty acid esters of polyoxyethylene sorbitan) is currently being used to solubilize membrane proteins (Helenius & Simons, 1975; Tanford & Reynolds, 1974) and to promote cellular growth and enzyme productions when included in culture media of several microorganisms supplying saturated and unsaturated fatty acids to organisms (Reese & Maguire, 1969; Jagger et al., 1985; Asther & Corrieu, 1987; Espinosa et al., 1990; Okeke & Okolo, 1990).

The present studies were undertaken to investigate in vitro of Tween 80 can influence the extracellular and intracellular protease production by ***Candida lipolytica.***

## MATERIALS AND METHODS

### Organism and Cultural Conditions

*Candida lipolytica* IA 1055 was grown in Yeast Mold medium described by Cirigliano & Carman (1983),

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over 96 h at 27° C on a reciprocal shaker (120 Hz). The surfactant, Tween 80 (Sigma, St. Louis, Mo), in concentrations of 0,01%, 0,05%, 0,2%, 0,5% and 1% was added to the medium at intervals of 0, 8,16 and 24 h during culture. These intervals corresponded to beginning of culture, beginning of exponential growth phase, intermediate growth phase, and end of exponential phase/ begining os stationary growth phase, respectively.

**Growth Estimation**

The cellular growth was determined by viable cell counts which were measured after a serial dilution in pH 7,0 PBS buffer of culture samples collected at intervals of 0, 8, 16, 24, 48, 72, 96 h by plating on Yeast Mold Agar (YMA)medium (Cirigliano & Carman,1983). The plate were prepared in triplicate and were incubated for 24 h at 27° C.

**Enzyme Assay**

The protease activity of *Candida lipolytica* was determined by the method of Hankin & Anagnostakis (1975) modified by using YMA medium. The protease activity was tested by two methods.

**Extracellular Protease Activity**

Samples of 3 ml of *C. lipolytica* cultures, both controlled and treated, were collected at intervals of 0, 24, 48, 72 and 96 h of growth, and centrifugated at 2000 rpm for 10 min. The culture supernatants were tested for enzyme activity. Twenty microliters of samples were loaded in 2 mm diameter well in YMA-Gellatin plates. The plates were incubated for 24 h at 27° C. After this period, the precipitation zone around wells were measured. The plates were prepared in triplicate.

**Cellular Protease Activity**

Samples of 0,1 ml of controlled and treated cultures, collected at intervals of 0, 24, 48, 72 and 96 h of culture, were diluted in PBS buffer pH 7,0 and plated on YMA-Gellatin plates. The plates prepared in triplicate were incubated at 27° C for 24 h, after which the precipitation zone was measured.

## RESULTS

The growth of *Candida lipolytica* treated cultures as determined by viable cell counts was influenced by Tween 80 according to its concentration and addition time (Figure 1). Cultures treated with Tween 80 at 0 time

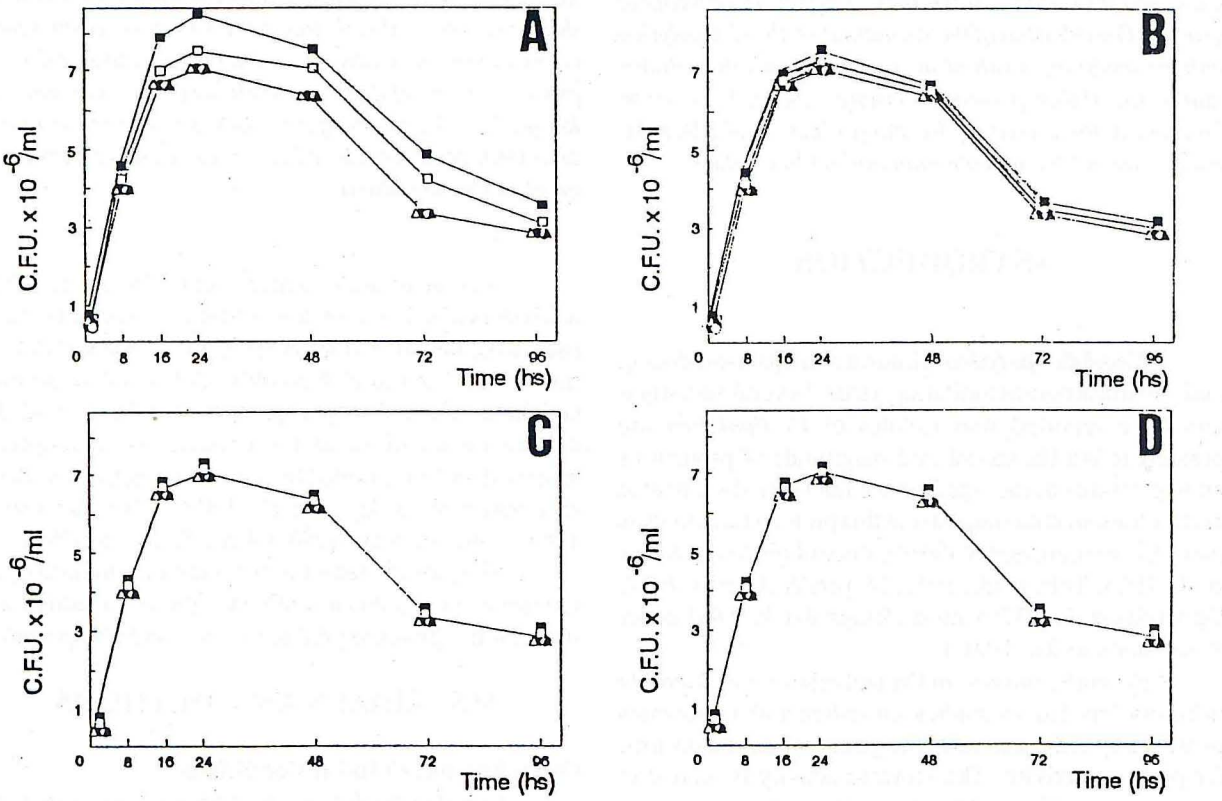


**Figure 1. Cell viability of *Candida lipolytica* cultures treated with 0.01% (△), 0,05% (▲), 0,2% (O), 0,5% (□) and 1% (■) Tween 80 at 0 time of culture (A); after 8 h (B); after 16 h (C) and 24 h (D). Control (●)**

showed the highest increase in cell viability as compared to control and to other treatments and the maximum stimulation was obtained with 1% (Figure 1A). Cultures treated after 8 h showed a cellular viability lower than previous treatment but, higher than controlled and cultures treated after 16 and 24 h culture. Again, the maximum stimulation was obtained with 1% Tween 80 (Figure 1B). cultures treated after 16 h and 24 h did not show a significant difference in cell viability as compared to control culture (Figures 1C and 1D, respectively).

The extracellular protease activity was detected in all culture supernatant tested (Table 1). The Tween 80 concentration and addition time show influence on the enzyme activity. The highest protease activity was detected in cultures treated at 0 time of growth and the maximum activity was detected by using 1% Tween 80. According to other 8 h (beginning of exponential growth phase) showed a reduction of enzyme activity as compared to previous treatment but, the protease activity was higher than cultures treated after 16 and 24 h and control. Culture treatments after 16 and 24 h did not show significant difference in protease activity as compared to control. The highest protease production was detected in samples collected after a 48 h culture in all supernatants tested.

The cellular protease activity assayed showed that all culture samples had positive enzyme activity which could be related to cell viability and was according to all Tween 80 concentrations and addition time.

## DISCUSSION

These experiments show that ***Candida lipolytica*** IA 1055, exhibit protease activity, which is influenced by Tween 80 concentrations and addition time. These results are confirmed by other authors, who showed that surfactants of Tween series have a beneficcial effect in increasing the yields of several extracellular enzymes when included in culture medium (Reese & Maguire, 1969; Jagger et al., 1985; Asther & Corrieu, 1987; Long & Knapp, 1991). The authors suggest that surfactant effect is on cell membrane permeability, but the basis for it effectiveness is not clear.

**Table 1 - Extracellular protease activity detected by halo formation (mm) in supernatants cultures of *Candida lipolytica***

| Samples (h) | Tween 80 addition time (h) | | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 0 | | | | | 8 | | | | | 16 | | | | | 24 | | | | |
| | Control | 1 | 2 | 3 | 4 | 5 | 1 | 2 | 3 | 4 | 5 | 1 | 2 | 3 | 4 | 5 | 1 | 2 | 3 | 4 | 5 |
| 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 24 | 11,0 | 13,0 | 11,9 | 11,6 | 11,4 | 11.0 | 12.0 | 11.8 | 11.5 | 11.2 | 11.0 | 11.8 | 11.3 | 11.0 | 11.0 | 11.0 | 11.3 | 11.1 | 11.1 | 11.0 | 11.0 |
| 48 | 13,0 | 14,5 | 13,5 | 13,4 | 13,2 | 13,0 | 14,0 | 13,6 | 13,5 | 13,0 | 13,0 | 13,2 | 13,1 | 13,0 | 13,0 | 13,0 | 13,0 | 13,0 | 13,0 | 13,0 | 13,0 |
| 72 | 9,5 | 11,0 | 10,0 | 9,7 | 9,5 | 9,5 | 10,5 | 10,0 | 9,6 | 9,5 | 9,5 | 9,7 | 9,6 | 9,5 | 9,5 | 9,5 | 9,7 | 9,5 | 9,5 | 9,5 | 9,5 |
| 96 | 7.0 | 9,0 | 8,0 | 7,7 | 7,5 | 7,0 | 7,5 | 7,3 | 7,0 | 7,0 | 7,0 | 7,6 | 7,4 | 7,1 | 7,0 | 7,0 | 7,3 | 7,1 | 7,0 | 7,0 | 6,9 |

Tween 80 concentrations: 1 - 1%; 2 - 0,5%; 3 - 0,2%; 4 - 0,05%; 5 - 0,01%.

Tween 20 and Tween 80 (lauric Acid and Oleic Acid, respectively) probably can supply fatty acids to the microorganisms cultures growth and could facilitates the uptake of nutrients into cells with the consequent stimulation to growth (Marvin, 1959; Massuco et al., 1981; Jagger et al., 1985). However, the study of Tween 80 different concentrations influence on growth and protease production by ***Candida lipolytica*** have not previously been reported.

The results showed that Tween 80 addition time and concentrations in culture medium influenced the enzyme production and growth of ***Candida lipolytica.*** Reese & Maguire (1969), demonstrated tha 0,01% Tween 80 added to culture medium gives the maximum stimulation of enzyme production when included at 0 time of incubation. These results are according to our experiments but, 1% Tween 80 was necessary for maximum enzyme and growth stimulation in ***C. lipolytica*** and later addition (8 h) still useful. This work showed that Tween 80 influence is according to its concentation and growth phase of the microorganisms.

It seems reasobable to suggest that this surfactant increase protease production by ***C. lipolytica*** and by other

microorganisms simply by increasing the extent of growth. It is necessary to verify if the enzyme activity in this specie could be related to infective power.

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