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Effects of Carbon and Nitrogen Sources on Lipase Production by *Candida rugosa*

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Abstract

The production of lipase by *Candida rugosa* growing on media with various carbon and nitrogen sources was studied. While high yields of enzyme activity (5.58 U mL^{-1}) were obtained with yeast extract and proteose-peptone in the medium with olive oil, the minimum lipase activity (2.81 U mL^{-1}) was observed with tryptone and lactose. In the absence of olive oil, the media with proteose peptone and glucose gave the maximum enzyme activity (2.21 U mL^{-1}). The best results in the production of lipase were obtained with the use of olive oil as the carbon source in the presence of nitrogen sources. Biomass concentration was also high in the presence of olive oil. Biomass concentration ranged from 1.76 to 2.05 mg mL^{-1} and 0.09 to 0.23 mg mL^{-1} in the medium with and without olive oil, respectively. Both lipase activity and lipase specific activity were higher in bran than in soyflour and cheese whey. Among the industrial by-products, bran was determined to be an efficient medium for the production of lipase.

Key words: *Candida rugosa,* Lipase activity, Biomass

Introduction

Lipases (EC 3.1.1.3) comprise a group of enzymes which catalyses the hydrolysis of triacylglycerols (Macrae and Hammond, 1985; Brockerhoff and Jensen, 1974). Interest in lipase has grown significantly in recent years. The development of technologies using lipases for the synthesis of novel compounds will result in their expansion into new areas and increase the number of industrial applications (Björkling *et al.*, 1991). The demand for the production of highly active preparations of lipolytic enzymes has led to research on lipase producing microorganisms and on culture strategies (Lechner *et al.*, 1988; Suzuki *et al.*, 1988). Different studies have been published on the selection of lipase producers (Yamuguchi *et al.*, 1973; Kordel *et al.*, 1991; Lee and Rhee, 1993; Taipa *et al.*, 1994), but there is less information available on the fermentation process.

Candida rugosa is a well-known lipase producing yeast. Its extracellular lipase has been reported to be non-specific with respect to the glycerol position. Moreover, lipase from *C. rugosa* has been found to be a highly stereospecific catalyst suitable for the preparative resolution of racemic acids and alcohols (Brockerhoft and Jensen, 1974). The present study was therefore undertaken to investigate the effect of different growth media (with and without olive oil) supplemented with various nitrogen (yeast extract, tryptone and proteose-peptone) and carbon sources (glucose and fructose) on lipase production by *C. rugosa.* Different industrial by-products were also used as carbon and nitrogen sources in the production of lipase.

Materials and Methods

Microorganism and media preparation: The yeast species *Candida rugosa* CBS 6330 was used. The slant culture of *C. rugosa* on glucose peptone

yeast extract agar was obtained from Central Bureau Voor Schimmelcultures (Baarn, Holland). The culture was activated in the broth medium (medium-A) consisting of 5 g L^{-1} glucose, 5 g L^{-1} proteose peptone, 1 g L^{-1} yeast extract and 1.25 g L^{-1} KH_2PO_4, and maintained on agar slants (medium-B). Medium-B was prepared by adding 15 g L^{-1} agar into medium-A. The inoculation medium (medium-C) was prepared as described for medium-A except glucose and proteose-peptone. The compositions of fermentation media used in the production of lipase by *C. rugosa* are given in Table 1.

Inoculum and cultural conditions: The pathway for the activation of *C. rugosa* and inoculation into fermentation media in the production of lipase is given in Figure 1. Five millilitres of culture medium-C was also inoculated into 45 ml of solution of wheat mill bran and soybean flour (50 g/L), and white cheese whey. At the end of the incubation period, the media were centrifuged at 1500xg for 30 min to remove pseudomycelium and were assayed for lipase activity and biomass content.

Lipase activity: Lipase activity was determined in an emulsifier free system using olive oil as substrate (Fadıloğlu and Söylemez, 1997). Reactions were carried out in 100 ml conical plastic-stoppered flasks at 40°C by immersion in a water bath and shaking at 120 oscillations per min. The reaction mixture, consisting of 2 ml of 0.1 M potassium phosphate buffer, pH 7.0, 1 ml of olive oil and 1 ml of culture supernatant, was incubated at 40°C for 30 min. The enzyme reaction was terminated by the addition of 5 ml of 96% ethanol and followed by titration (titration system, E415 Metrohm Herisau, Switzerland) with 0.05 N KOH solution using phenolphthalein as the indicator. One unit of lipase activity was defined as the amount which liberated 1 μmole of fatty acid min^{-1} at 40°C.

Biomass content: The fungal biomass was separated from the culture filtrate by collection on filter paper discs (Whatman GF/C) which were dried to constant weight (Seaby *et al.*, 1988). The cells were dried at 105°C to constant weight and biomass concentration was determined by the gravimetric method (Fadıloğlu and Erkmen, 1999).

Statistical analysis: Analysis of variance was performed on the data by means of the Statgraphics program (Stse, Inc., Rockville, MD).

Results and Discussion

Table 2 gives the changes in biomass concentration, lipase activity and lipase specific activity of *C. rugosa* depending on various carbon and nitrogen sources. Fermentation was monitored for up to 72 h, and carried out with and without olive oil to evaluate the effect of lipid material on the production of lipase. Both lipase activity and biomass concentration were higher in the media with olive oil (MWO) than in the media without olive oil (MWHO). Dalmau *et al.* (2000) obtained the highest yield of lipase by *C. rugosa* with lipids or fatty acids as carbon sources. Vegetable oils may be considered economically viable substrates for lipase production on an industrial scale. The organism grew on glucose, but the amount of lipase secreted was significantly lower than that in vegetable oils (Lakshmi *et al.*, 1999). There was a significant difference ($P < 0.05$) in the biomass concentration and lipase activity between the media with and without olive oil. Biomass concentration ranged from 1.76 to 2.05 mg ml^{-1} for MWO and

Table 1. Types of Fermentation Media Used in the Production of Lipase

Basic medium*	Type of medium	Nitrogen source (70 g L^{-1})	Carbon source (10 g L^{-1})
MWO (3% olive oil)**	1	Yeast extract	-
	2	Yeast extract	Glucose
	3	Yeast extract	Lactose
	4	Tryptone	-
	5	Tryptone	Glucose
	6	Tryptone	Lactose
	7	Proteose-peptone	-
	8	Proteose-peptone	Glucose
	9	Proteose-peptone	Lactose

*Basic medium composed of 1 g L^{-1} $NaNO_3$, 1 g L^{-1} KH_2PO_4, 0.5 g L^{-1} $MgSO_4.7H_2O$.

**Fermentation media were also prepared without olive oil.

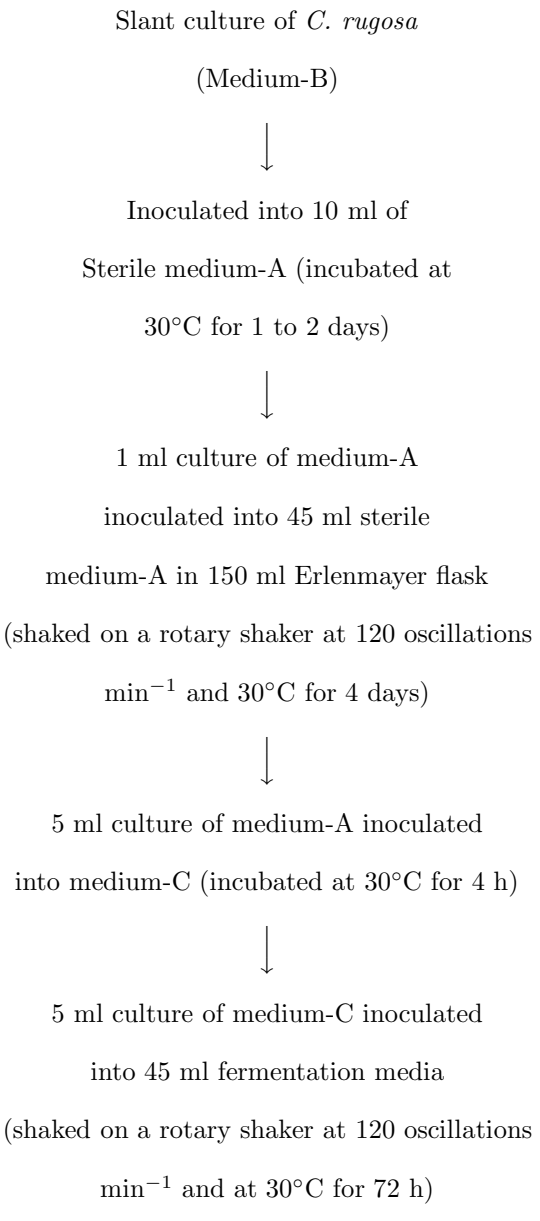

Figure 1. The pathway for the activation and inoculation of *C. rugosa* into fermentation medium for the production of lipase.

from 0.09 to 0.23 mg ml^{-1} for MWHO. The highest lipase activity was observed in MWO supplemented with yeast extract and proteose- peptone (5.58 U ml^{-1}), while the lowest lipase activity was obtained with tryptone + lactose (2.81 U ml^{-1}). It has been reported that some micro-organisms showed higher activities when grown in medium containing glucose (Banerjee *et al.*, 1985). Higher oxidized carbon sources had a significant stimulating effect on the synthesis of esterases; the most efficient being citrate with *C. curvata* and *Y. lipolytica* 3, and succinate with *Y. lipolytica* 8. A similar effect of citrate was observed by Kokusho *et al.* (1982) with the bacterium *Alcaligene*. On the other hand, the activity of lipases in *C. deformans* and *Y. lipolytica* decreased in the presence of the higher oxidized carbon sources. Novotny *et al.* (1988) found that olive oil in combination with glucose increased lipase activity, and, in most cases, the presence of olive oil, together with

Table 2. Effect of Various Carbon and Nitrogen Compounds on the Production of Lipase by *Candida rugosa*

	MWO*			MWHO		
Substrate added to base medium	Lipase activity [U (ml medium)$^{-1}$]	Lipase specific activity [U (mg mycelium)$^{-1}$]	Biomass (mg ml^{-1})	Lipase activity [U (ml medium)$^{-1}$]	Lipase specific activity [U (mg mycelium)$^{-1}$]	Biomass (mg ml^{-1})
Yeast extract	5.58	2.72	2.05	0.86	9.56	0.09
Yeast extract + Glucose	3.60	1.88	1.92	2.03	10.15	0.20
Yeast extract + Lactose	3.56	1.90	1.88	1.20	8.00	0.15
Tryptone	3.26	1.69	1.93	0.80	8.89	0.09
Tryptone + Glucose	2.86	1.54	1.85	1.51	9.44	0.16
Tryptone + Lactose	2.81	1.60	1.76	0.91	7.58	0.12
Proteose-peptone	5.58	2.80	1.99	0.95	8.64	0.11
Proteose-peptone+ Glucose	3.78	2.01	1.88	2.21	9.61	0.23
Proteose-peptone + Lactose	3.28	1.84	1.78	0.89	6.84	0.13

*MWO = Medium with olive oil, MWHO = Medium without olive oil

Table 3. Production of Lipase by *Candida rugosa* in Wheat Mill Bran, Soybean Flour, and White Cheese Whey

Medium	Lipase activity [U (ml medium)$^{-1}$]	Lipase specific activity [U (mg mycelium)$^{-1}$]	Biomass (mg ml^{-1})
Wheat mill bran	3.38	6.15	0.55
Soybean flour	0.15	0.58	0.26
White cheese whey	0.10	0.45	0.22

glucose or glycerol in the medium, significantly decreased both lipase and esterase levels. If, however, olive oil was used as the only carbon source for growth, the enzyme activities of *C. guilliermondii* and *Y. lipolytica* 3 showed a four-to five-fold increase. The positive effect of olive oil on the synthesis of esterases was greater than that on the synthesis of lipases. As reported by Nakashima *et al.* (1988), the presence of olive oil, tea oil or oleic acid in growth medium greatly enhances lipase activity.

However, the addition of glucose or lactose to MWO had a negative effect on both lipase activity and biomass content. A similar inhibitory effect of glucose was reported by Montet *et al.* (1985) with *C. curvata* and by Muderhwa and Ratomahenina (1985) with *C. deformans*. Nahas (1988) reported that lipase activity was lower ($P < 0.05$) in olive oil medium containing 1% glucose or lactose. Olive oil in combination with nitrogen sources increased the lipase production but the presence of carbon sources in the olive oil significantly ($P < 0.01$) decreased both lipase activity and biomass content. This result may be due to the limited availability of these carbon sources to the fungus in the media supplemented with olive oil.

In the absence of olive oil, glucose seemed to be a better carbon source than lactose. Glucose increased both lipase activity and biomass content in combination with nitrogen sources in MWHO. There was a significant ($P < 0.05$) difference in lipase activity in MWHO supplemented with glucose and lactose. This resulted from a negative effect of higher oxidized carbon sources on lipase synthesis (Novotny *et al.*, 1988). Lipase activity increased to 2.03 and 2.21 U ml^{-1} in MWHO containing yeast extract + glucose and proteose-peptone + glucose, respectively, while they were 1.20 and 0.89 U ml^{-1} in the presence of lactose instead of glucose.

Organic nitrogen sources were found to increase lipase synthesis by *C. rugosa* grown in the presence of olive oil. Lipase production in MWO containing nitrogen sources was higher than in media containing both nitrogen and carbon sources. Nitrogen sources alone in MWO significantly ($P < 0.05$) enhanced lipase activity by *C. rugosa*. However, a similar effect was not observed in MWHO. It is evident that nitrogen sources stimulate lipase production in the presence of olive oil. In contrast, Montesinos *et al.* (1996) reported that no effect of nitrogen source on lipase production and biomass was observed and the specific productivity in continuous culture was slightly higher than in batch cultures

Lipase specific activity in MWO was higher when supplemented with only proteose-peptone but lower with tryptone + glucose. In MWHO supplemented with yeast extract + glucose, higher lipase specific activity was observed. The results showed that there was a considerable increase in biomass with MWO supplemented with only yeast extract (2.05 mg ml^{-1}) and MWHO supplemented with proteose-peptone+glucose (0.23 mg ml^{-1}). Olive oil increased growth and lipase activity nearly three- and 13-fold respectively but not lipase specific activity. Lipase specific activity was nearly four-fold higher in MWHO than in MWO.

Biomass, lipase activity and lipase specific activity by *C. rugosa* after 72 h in wheat mill bran, soybean flour and white cheese whey in shake flask studies are given in Table 3. Lipase activity and lipase specific activity in bran were significantly ($P < 0.05$) higher than in soybean flour and whey. The biomass concentration with bran was also two times higher than with soybean flour and whey. The wheat mill bran is rich in various minerals, pentosans, starch, total sugar, sucrose and reducing sugar (Ertürk *et al.*, 1988). The increase in lipase activity with wheat mill bran might be explained by its higher mineral, nitrogen and sugar composition.

Conclusion

The best results are obtained with olive oil as the carbon source in the presence of nitrogen sources. The presence of wheat mill bran in the medium with

olive oil promoted growth and lipase activity, while these effects were not observed with the soybean flour and white cheese whey. According to the results, *C. rugosa* has an ability to grow and produce lipase from industrial by-products. Further experiments are needed on detailed growth rates and the effect of temperature and pH on the production of lipase.

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