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ORIGINAL RESEARCH PAPER

PRELIMINARY STUDY REGARDING THE USE OF SOME *Yarrowia lipolytica* STRAINS FOR SOLID STATE HYDROLYSIS OF CRUDE COCONUT FAT

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Abstract: Coconut fat could be an important source of bioactive compounds with a large applicability in industry. In this study it was realized the enzymatic hydrolysis of coconut fat with different yeast strains of *Yarrowia lipolytica.* The aim of this study was to optimize the hydrolysis conditions and determination of *Yarrowia lipolytica* specificity on coconut fat as substrate. The hydrolysis was performed by yeast strains cultivated on stationary solid state conditions on spirit blue agar medium supplemented with 3% coconut fat, at 25°C and 4°C, and at values of water activity of 0.98, 0.96 and 0.93. The substrate hydrolysis index was recorded every 24 hours, during of 240 hours of stationary cultivation. The most active lipolytic yeast was strain coded S5 which produce a high level of extracellular lipase with high coconut hydrolyze activity at 25°C and two water activities 0.98 and 0.96, in the shortest time (24 – 72 hours). In the presence of 3% NaCl in media, the strains coded S9, S5 and S4 were the most active from all tested strains at 25°C and 4°C. At low temperatures and in presence of 10.1% NaCl all tested yeasts had no lipolytic activity.

Keywords: *coconut fat, fatty acids, food biopreservation, hydrolysis, Yarrowia lipolytica*

INTRODUCTION

Coconut tree take a part into the palm trees family and is strongly cultivated for its comestible fruits. The coconut it was marketed for the first time in 1860 by South Asia traders. The applications of those products are in the ice-cream industry and topping of ice-cream, making of pop-corn. Coconut oil has direct applicability in food industry or by industrialization for obtaining cosmetic products. It is always used for soap and cosmetics producing. Coconut fat is present in solid phase, white colour, without taste or odour. Coconut oil is the most stable fat; it has a low oxidation point and the rancidity begin after other oils (up to 2 years) because of the higher content in saturated fats. Fat is rich in medium chain fatty acids (MCFA) and exhibits good digestibility [1].
Many studies have shown that the coconut fat is an instant source of saturated fatty acids with antimicrobial activity. This fat contains 50% lauric acid, 20% myristic acid, 10% capric acid, 9% caprylic acid. In many studies it was followed the way that obtain these fatty acids after the enzymatic hydrolysis with active microbial lipases.
Lipases are one of the most important classes of industrial enzymes. They hydrolyse esters preferentially at the interface between lipid and water in heterogeneous systems. L Lipases are used in the production of detergents [2], cosmetics [3], pharmaceuticals [4], flavour enhancers [5, 6] and foods [7]. Promising fields include the biodegradation of plastics such as polyhydroxyalcanoate and polycaprolactone [8]. Due to their biotechnological interest, many of these enzymes have been identified cloned and characterized. Nevertheless, the demand for the production of highly active preparations of lipolytic enzymes has led to research on lipase producing microorganisms and culture strategies [9]. Among these microorganisms, yeast *Yarrowia lipolytica* is one of a great interest, being able to biosynthesis several enzymes, including lipase, depending on the growth conditions [10].
Few reports concerning the lipolytic activity of *Yarrowia lipolytica* are present in the literature [11, 12]. The aim of this study is the identification of the most active and adapted *Yarrowia lipolytica* strains able to hydrolysis crude coconut fat in order to obtain a high yield of fatty acids with antimicrobial potential. In that way were tested nine strains of *Yarrowia lipolytica* for hydrolysis of crude coconut fat at 4°C and 25°C and at different water activity levels, i.e.0.93, 0.96 and 0.98.

MATERIALS AND METHODS

Materials and microorganisms

The crude coconut fat, obtained by traditional extraction method, was purchased from Cameroun, Africa. The media used for yeasts cultivation was Sabouraud agar from Merck KgaA Darmstad, Germany and Spirit Blue Agar (SBA) from DIFCO Laboratoires Detroit Mi, USA. The SBA was prepared at different water activities (0.98, 0.96, and 0.93) by addition of NaCl (Merck KgaA Darmstad, Germany).
The nine strains of *Yarrowia lipolytica* coded S1, S2 S3, S4, S5, S6, S7, S8 and S9 used in this study was obtained from Microorganism's Collection of University of Bologna, Facolta di Scienze degli Alimenti, Cesena, Italy.

Methods

Inoculum preparation

For the preparation of inoculum, *Yarrowia lipolytica* strains were first cultivated in Petri dishes on Sabourand agar medium. These were incubated at 28°C for 72 hours. A small quantity of activated biomass was then inoculated in glass tubes with 8 mL Sabouraud broth. The tubes were then incubated at 28°C for 72 hours. The preparation of inoculum was performed by transferring of every 1 mL of preinoculum from tubes in other new tubes with Sabourand broth. Tubes were incubated at 28°C for 72 hours. The number of viable cells was established by plate count method, except where viable cell numbers were less than 10^3 CFU/mL, when the MPN (Most Probable Number) technique was used.

Medium supplemented with crude coconut fat preparation

35 g of Spirit Blue Agar (SBA) powder of commercial medium was suspended in 1 L of purified water. Different concentrations of NaCl (3, 6 and 10.1%) were added in order to obtain a water activity (WA) of 0.98, 0.96 and 0.93 respectively. Then it was heated with frequent agitation and boiled for 1 minute for a complete solubilization. The media was autoclaved at 121°C, for 15 minutes and after that was cooled to 50 – 55°C. 30 mL aseptically coconut fat was added and mixed thoroughly. The media was poured into Petri dishes. After that, plates were dried [13].

Yeast cultivation and coconut fat hydrolysis evaluation

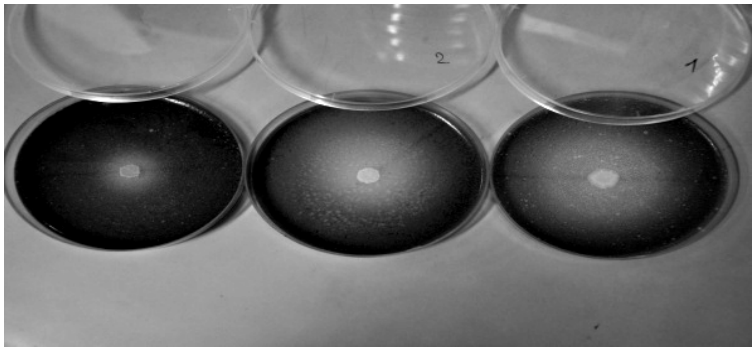
After drying the SBA in plates with coconut oil it was added in centre of the plate 30 µL of every yeast inoculum (10^6 CFU/mL). The cultivation took place at 25ºC for 240 hours. It was measured the yeast colony diameter and the diameter of the hydrolysis zone. The evaluation of the lipolytic potential was established by determination of the substrate hydrolysis index (SHI) as ratio of these two parameters. The evaluation was recorded every 24 hours. Also there were realized control samples. Samples were done in duplicate. The incubation was performed during 240 hours for every temperature.

RESULTS AND DISCUSIONS

Hydrolysis of crude coconut fat at 25°C and different water activity levels

It was observed that at 0.98 WA and 25°C the most effective yeasts that hydrolyzed the coconut fat were strains coded S5, S4, S6, S9 and S1. In the first 24 hours of cultivation the most effective strains were S4, S8 and S9. It was observed that yeasts coded S5 and S4 have a hydrolysis zone only on the first 72 hours up to the 2.42 mm and 2.32 mm respectively; from that point it remain unchanged until the final time of cultivation of 240 hours (Figure 1).

After 72 hours, in the same conditions of temperature and water activity, it was observed an increase of the diameters of hydrolysis for strains coded S5, S4 and S1. These were the most effective to hydrolyse of the coconut fat. The other strains showed smaller hydrolysis diameters. The yeast coded S8 hasn't showed any hydrolysis zone in the first 72 hours of action.



72 h 144 h 240 h

Figure 1. *Hydrolysis potential of crude coconut fat by Yarrowia lypolitica S5 strain at 25°C and WA 0.98*

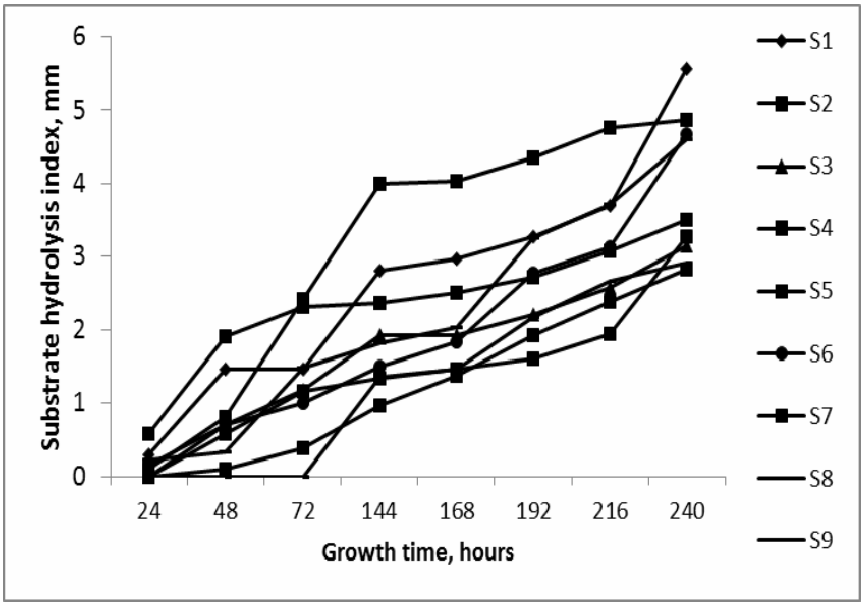


Figure 2. *Hydrolysis of crude coconut fat by Yarrowia lipolytica strains at 25°C and WA 0.98*

The dynamics of hydrolysis is presented in Figure 2. After 24 hours it was observed the appearance of hydrolysis zones for strains S4 and S1. These were the most active yeasts during the 24 and 48 hours of cultivation. After 48 hours, it was observed that hydrolysis activity of strain coded S1 remain constant up to 72 hours. After this time, the strain S5 had presented a large hydrolysis zone with a substrate hydrolysis index of 2.42 mm followed by strains coded S4 and S9 with diameters of 2.32 and 1.48 mm respectively. The strains S6, S7 and S8 haven't showed significant hydrolysis zones during of 72 hours. It was observed that during of 144 – 168 hours of yeasts cultivation the most active were strains S5, S1 and S4. These recorded good substrate hydrolysis zone as: 4.03 mm, 2.50 mm and 2.96 mm respectively.

Between 192 and 240 hours, strains S5, S9 and S1 have produced a very strong hydrolysis on the coconut fat. The yeast S9 did not show any diameters of hydrolysis for the first 72 hours, it showed a very good activity on the final time of this study with a substrate hydrolysis zone of 4.61 mm. After 144 hours the most active yeasts were

S1, S4, S5, S6 and S9. The substrate hydrolysis index of S1 was 5.58 mm, for S4 was 3.50 mm, for S5 was 4.86 mm, for S6 was 4.67 and for S9 was 4.61 mm.
Along all the time of the hydrolysis 24 – 240 h, the strongest hydrolyse potential was recorded for the strains S1 and S5. But the aim of this study was to find the strains that can hydrolyse the fat in the shortest time and with a strong hydrolysis action.
At WA 0.96 in the period of time 24 – 72 hours, the strongest hydrolysis potential was recorded for strains S4, S6, S3 and S1. These was considered to be the most effective yeasts able for hydrolysis of crude coconut fat for 72 hours with a substrate hydrolysis index of 2.40 mm, 1.73 mm, 1.75 mm and 1.75 mm respectively.
It was observed that along with increasing the salt concentration, the lipolytic potential is different. At 25°C it was identified small hydrolysis diameters produced by strains S4 and S9 after in 24 hours of yeast cultivation. After 48 hours, the strain S4 was most active for solid state crude coconut fat hydrolysis. It presented the biggest hydrolysis zone with a recorded substrate hydrolysis index of 2.40 mm. Up to 72 hours this yeast was recorded the biggest hydrolysis diameter (2.46 mm), followed by strains S1 (1.75 mm), S3 (1.75 mm) and S6 (1.73 mm). The strain S9 presented no hydrolysis zone after 72 hours of incubation. The strains S7 and S8 were not efficient in coconut fat hydrolysis at 25°C and WA 0.96. Also, on this WA value, strains S7 and S8 were inactive.
During 144 – 240 hours of cultivation, the most effective strains were S5, S1, S3 and S4, because all have recorded a substrate hydrolysis index greater than 3.30 mm (Figure 3). After 144 hours, the greatest hydrolysis diameters were recorded from species S5, S1 and S3 with more than 30 mm. Again yeast coded S5 showed that it is the most effective on coconut fat hydrolysis even if the water activity decrease from 0.98 to 0.96 at 25°C.

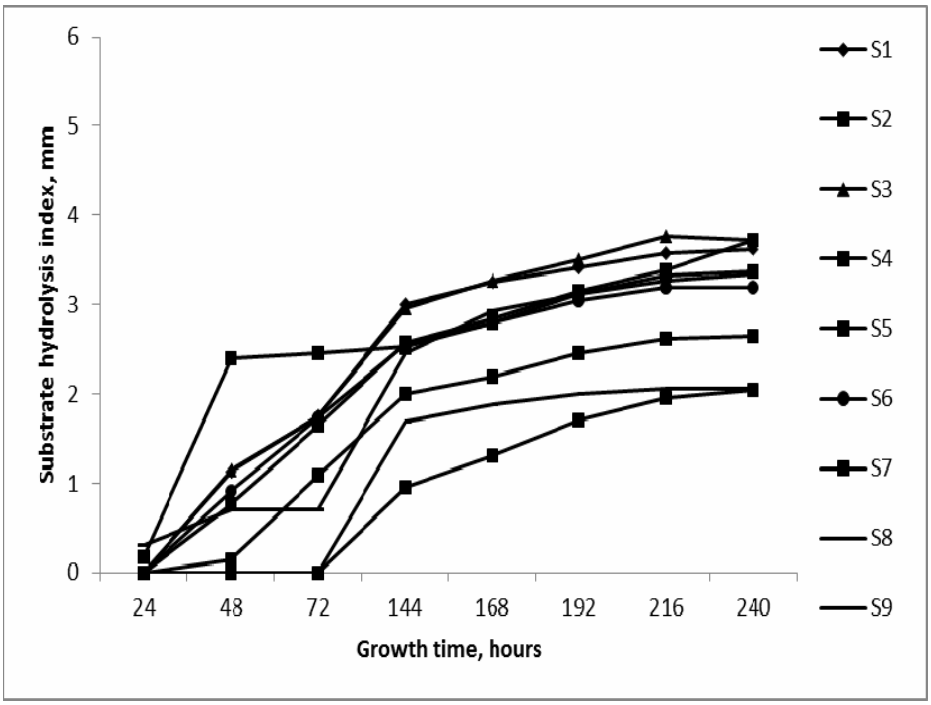


Figure 3. *Dynamic of solid state hydrolysis of coconut fat by Yarrowia lipolytica strains at 25°C and WA 0.96*

By increasing the quantity of NaCl in media (WA 0.93), more yeasts were inactivated. In this way the strain S1 that has a strong lipolytic activity on 0.98 and 0.96 WA, at 0.93 WA is significantly inactivated. In that way the strains S2, S1 and S8 were inactivated and they did not show any hydrolysis diameter between 24 and 240 hours. The other strains (S3, S4, S5, S6, S7, S9) did not record any diameter of hydrolyzation in the first 72 hours (Figure 4).

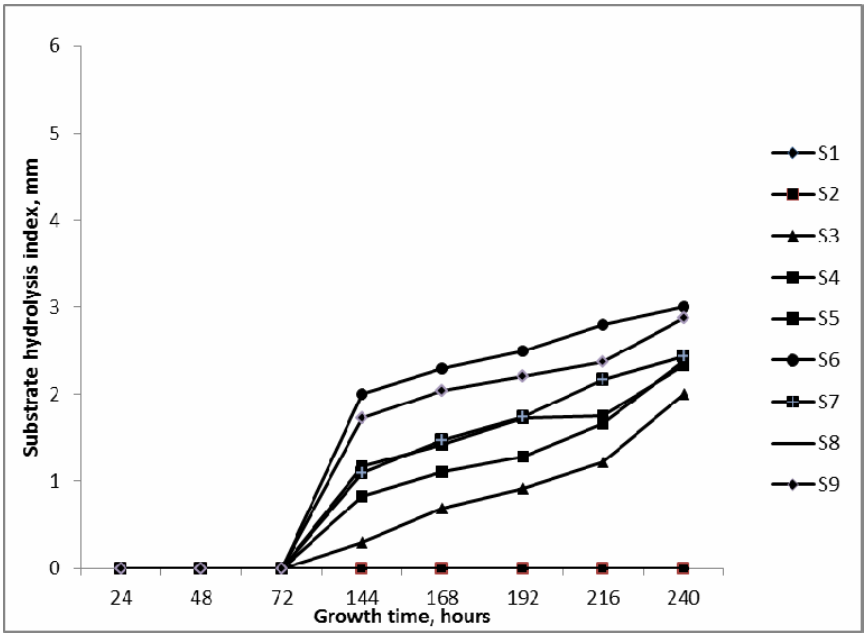


Figure 4. *Hydrolysis of coconut fat by Yarrowia lipolytica strains at 25°C and WA 0.93*

In Figure 4 was shown how strain S9 was the strongest of all yeasts tested. After 240 hours it recorded a large substrate hydrolysis index (2.88 mm) with WA 0.93. It is followed by strains S7 and S5 with substrate hydrolysis indexes of 2.43 mm, and 2.33 mm respectively.

The only *Yarrowia lipolytica* strains that realized the coconut fat hydrolysis at 25 °C and WA 0.93 were S9, S7 and S5. The strain S9 was the most active after 144 hours. It was also demonstrated that along with salt concentration increase these extracelular lipases produced by *Yarrowia lipolytica* are still active but the power of hydrolysis decreases.

Hydrolysis of crude coconut fat at 4°C and different levels of water activity

At 4°C and water activity 0.98 the hydrolysis of coconut fat by *Yarrowia lipolytica* yeasts are inactive between 0-144 hours. In all cases the hydrolysis was produced by the strains S1 and S9 during 144-240 hours of solid state cultivation (Figure 5).

For samples that were kept at 4°C and with water activities of 0.96 and 0.93 the yeasts did not grew and did not produce extracellular lipase. It can be said that at concentrations higher than 6% NaCl in the media and temperatures around 4 °C the yeasts do not have the ability to grow and release extracellular lipases for fat hydrolysation.

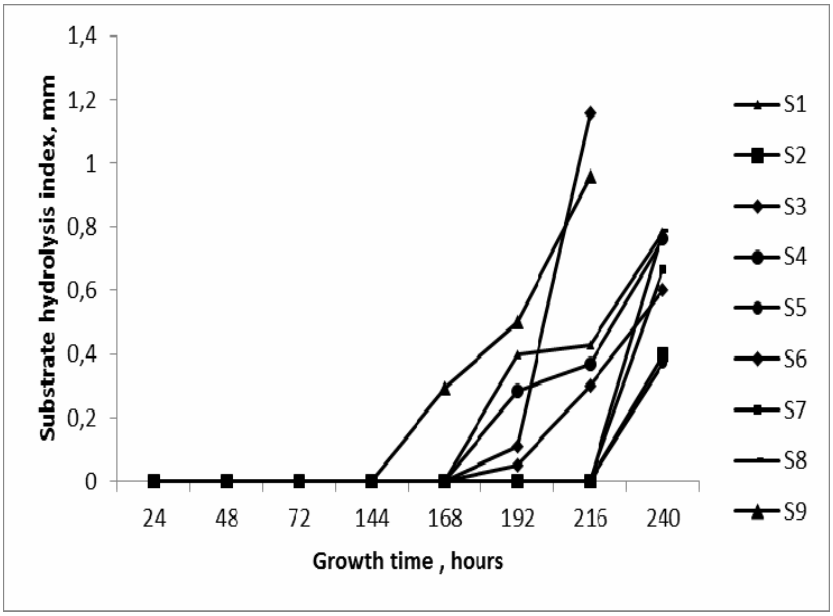


Figure 5. *Hydrolysis of coconut fat by Yarrowia lipolytica strains at 4°C and WA 0.98*

CONCLUSIONS

Yarrowia lipolytica strains are able to produce extracellular lipases at the temperature of 25°C at different water activities (0.98, 0.96 and 0.93) during 240 hours of cultivation on Spirit Blue Agar medium (SBA) supplemented with 3% coconut fat.

The most active *Yarrowia lipolytica* strains coded S5, S4 and S1 had recorded best results in coconut fat hydrolysed at 25°C, aw 0.98 and aw 0.96, during of 0-72 hours of solid state cultivation.

At low values of water activity (aw 0.93) and temperatures of 25°C it was demonstrated that the growth of *Yarrowia lipolytica* are seriously affected by the salt added in media. So, many of the strains were inhibited, and the others that growth, presented a small diameter of fat substrate hydrolysis index after much time of incubation (144 – 240 h).

At 4°C and 0.98 water activity the *Yarrowia lipolytica* strains that shows strong activity was: S9, S1, S6 and S7. These had produced enough extracellular lipase that performed a good hydrolysis of fat in 240 hours.

The best result of this study was obtained with *Yarrowia lipolytica* S5 strain, who shows the biggest hydrolysis potential of coconut fat in the short time of 0-72 hours at temperature of 25°C and aw 0.98 or 0.96.

This study demonstrated the ability of *Yarrowia lipolytica* selected strains to hydrolyse coconut fat in solid state cultivation system in optimised conditions in order to release some fatty acids with biopreservative potential in food industry.

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