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Research article

THE EFFECT OF SALINITY AND CULTURE MEDIUM COMPOSITION ON THE EXTRACELLULAR LIPASE ACTIVITY OF *MARINOCOCCUS HALOPHILUS* JCM 2472

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This paper deals with the effects of salinity and culture medium composition on the growth and extracellular lipase activity of the strain *Marinococcus halophilus* JCM 2472. This way, the strain was cultivated on two culture media, namely MH and R-agar containing either 5% or 10% NaCl. The lipase activity was recorded in the presence of 1M, 2M, 3M NaCl and in the absence of NaCl from reaction mixture. The data recorded revealed that the investigated strain is able to grow on all tested media but the best growth was recorded on MH media containing 5% NaCl. The best values of lipase activity were recorded at 22 hours and 40 hours of bacterial strain cultivation and in the presence of 1M NaCl representing an optimum for the enzymatic activity. The results from this paper revealed the halotolerant characteristics of the extracellular lipase of *Marinococcus halophilus*

Key words: Lipase, halophilic microorganisms, *Marinococcus halophilus*, salt.

INTRODUCTION

Lipases (EC 3.1.1.3 – triacylglycerol acyl hydrolases) represent an important enzyme class able to transform fatty acids, mono-, di- and triglycerides[4] in aqueous conditions and having also esterolytic activity[8]. These enzymes able to degrade those substrates are currently highly investigated and under attention for their huge biotechnological potential[1,6]. The lipases represent important biocatalysts in various biotechnologies and are considered the third largest class from commercial importance point of view, after carbohydrases and proteases[6]. Between these biotechnologies the production of biodiesel is also included[6]. During this process, a lot of waste glycerol result as main secondary product and because its contamination with various salts appeared the necessity to transform it in other valuable chemicals[7]. Such kind of biocatalytic processes in many cases claim for enzymes stable in hostile conditions and also for new solvents[5] in the reaction media. The production of lipases appears to be a general feature of moderately halophilic microorganisms[8].

These bacteria are able to grow in media with wide range of salt concentrations being considered a very euryhaline group of microorganisms[8]. Moderately halophilic bacteria harbour optimum growth between 5%–15% NaCl (10% being the predominant optimum concentration) and belong to various genera which include both halophilic and non-halophilic members[10]. Thus, the extracellular enzymes produced by these microorganisms are stable and biologically active in media characterized by high ionic strength[2]. Due to this potential to have activity in hard conditions and being also stable in the presence of various solvents[3,11], the enzymes obtained from halophilic microorganisms could be a solution for modern biocatalytic solvents[5]. This paper deals with investigations of the effect of salinity and culture medium composition for the obtaining of extracellular, considering that this enzymes have potential in biocatalytic conversion of waste glycerol from biodiesel industry[9] to various value added products for several areas in industry, agriculture and biotechnologies.

MATERIALS AND METHODS

Culture media used in this work were MH and R-agar supplemented with 50 (5%) and 100 (10%) g/L NaCl. The chemical composition of the MH medium is (g/L): yeast extract 10, proteose peptone

5, glucose 1, $MgCl_2 \times 6H_2O$ 7, $MgSO_4 \times 7H_2O$ 9.6, $CaCl_2 \times 2H_2O$ 0.36, KCl 2, $NaHCO_3$ 0.06, NaBr 0.026. The chemical composition of the R-agar medium is (g/L): bacto peptone 10, yeast extract 5, malt extract 5, casamino acids 5, beef extract 2, glycerol 2, Tween80 0.05, $MgSO_4 \times 7H_2O$ 1. The culture volume was 50 mL. In order to stimulate the synthesis of the extracellular lipase, the culture media were supplemented with olive oil 1%. The cultivation has been made for 96 hours at 37°C and shaking at 120 rpm. At different time periods, namely 16, 22, 40 and 96 hours, 10 mL of the culture were centrifuged and the resulted supernatant was used for enzymatic activity determination.

The growth of the culture was determined spectrophotometrically at 660 nm using BMG LABTECH FLUOstar Omega microplate's reader.

The lipase activity has been determined following the method in which a mixture of 0.5 ml olive oil, 0.4 ml sodium deoxycholate 2%, 0.2 ml BSA 0.05%, 0.4 ml enzymatic extract and 0.5 ml NaCl (0M, 1M, 2M, 3M and 4M) was incubated 30 min at 37°C. The reaction was stopped by adding 2 ml of ethanol 96%. The acids resulted as a consequence of lipase activity were titrated with NaOH 0.05N using phenolphthalein as indicator. A blank sample was prepared in similar conditions excepting the stopping of reaction at time zero.

RESULTS AND DISCUSSIONS

The results showed in Figure 1 revealed that bacterial strain *Marinococcus halophilus* is able to grow on culture media containing either 5% or 10% NaCl. The best growth was recorded on the media MH containing 5% NaCl, but there was not a significant difference between the growth profiles in the presence of 10% NaCl. On the other hand, on media R-agar the growth profile is different if compared with MH media. The obtained optical density values were low and revealed that in the first 22 hours the growth was very slow. On this media, the presence of 10% NaCl appeared to be a positive factor for growth (Figure 1).

The extracellular lipase activity showed various profiles and apparently was not influenced by the composition of culture media. After 16 hours of cultivation the best values were recorded on MH media with 5% NaCl and in the absence of NaCl in reaction mixture (Figure 2).

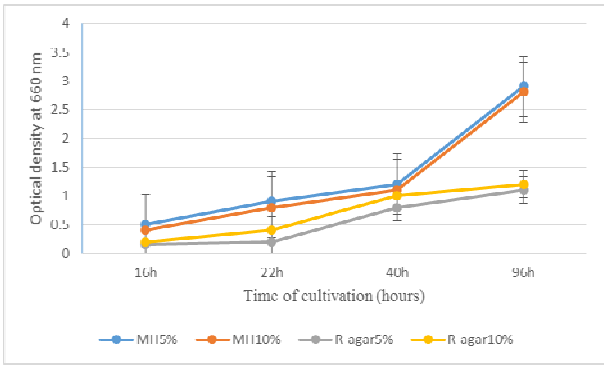

Fig. 1. The growth profile of *Marionococcus halophilus* JCM 2472 on the culture media MH and R-agar containing 5% and 10% NaCl.

The value recorded in similar conditions from supernatant on MH media having 10% NaCl was half from the previous one. On R-agar media with either 5% or 10% NaCl, the lipase activity showed similar values, 1.5 and 1.3 µmol/ml/min respectively (Figure 2). When the enzymatic activity was determined in the presence of 1M NaCl, excepting supernatant from MH media with 5% NaCl, the values were similar for all cultivation conditions (Figure 2).

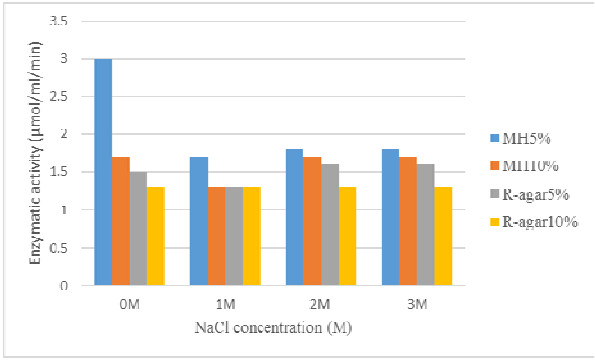

Fig. 2. The effect of NaCl on the lipase activity at 16 hours of cultivation.

In the presence of 2M or 3M NaCl the enzymatic activity appeared to increase but not significantly. There was observed that in case of supernatant from R-agar 10% media, the enzymatic activity was similar at all tested NaCl concentrations.

In a similar way with the enzymatic activity observed at 16 hours of cultivation, the relatively high values were recorded from the supernatant obtained from the MH culture media, most probably due to the best growth of the bacterial strain on this media. At this time (22 hours of growth) the higher values of the lipase activity were recorded for the

supernatant from MH culture medium having 10% NaCl. In the case of R-agar 10%, if at 16 hours of growth were observed similar values of enzymatic activity at all NaCl concentration tested in reaction mixture, at 22 hours the lipase activity was influenced by the NaCl concentration and the best values were recorded at 1M and 3M NaCl (Figure 3).

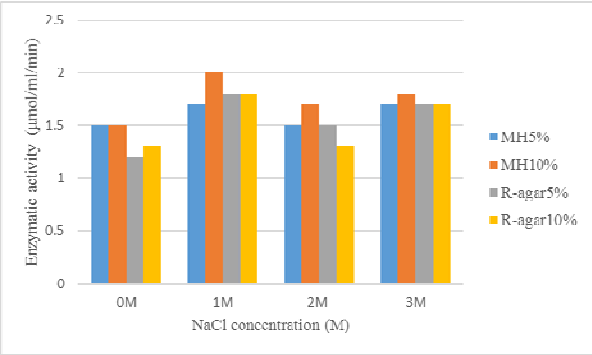

Fig. 3. The effect of NaCl on the lipase activity at 22 hours of cultivation.

After 40 hours of growth (Figure 4) the profile of lipase activity was not significantly different from previous one (at 22 hours) in terms of units of activity.

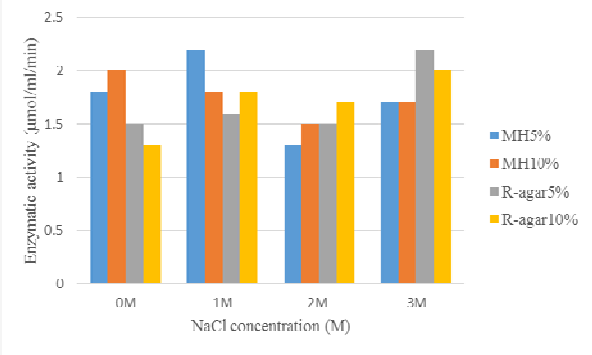

Fig. 4. The effect of NaCl on the lipase activity at 40 hours of cultivation.

There, the best values were recorded at 1M and 3M NaCl. At this incubation time there was observed a good activity in the case of supernatant from R-agar media, either 5% or 10% NaCl (Figure 4) in the presence of 3M NaCl in the reaction mixture, despite the slow growth rate of the bacterial strain on this media (Figure 1).

The results from Figure 5 revealed that the best lipase activity (2.7 µmol/ml/min) was obtained in the case of the supernatant from MH medium with 5% NaCl, in the presence of 3M NaCl in the reaction mixture. In the presence of 2M NaCl and in the absence of NaCl the lipase activity profiles were similar with the exception of the supernatant from the medium MH 5%. In the presence of 1M NaCl the values of lipase activity from the supernatant of the media R-agar 5% or 10% were similar, 2.2 µmol/ml/min respectively.

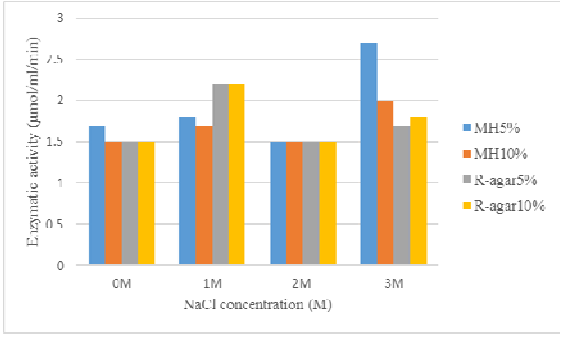

Fig. 5. The effect of NaCl on the lipase activity at 96 hours of cultivation.

The data from this study revealed that good values of the enzymatic activity were recorded at 22 hours and 40 hours of growth and the presence of 1M NaCl appeared to be optimum for the activity. The behaviour of enzymatic activity at investigated NaCl concentrations showed halotolerant character of the enzyme, taking into account that in some cases the values recorded in the absence of NaCl in the reaction mixture were similar with the values observed at 3M NaCl.

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