International Journal of Biological Macromolecules 105 (2017) 873–878

Contents lists available at ScienceDirect

International Journal of Biological Macromolecules

journal homepage: www.elsevier.com/locate/ijbiomac

Lipolytic bacteria use as bio-decontaminating natural in the water purification stations

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ARTICLE INFO

Article history:
Received 15 June 2017
Received in revised form 9 July 2017
Accepted 16 July 2017
Available online 18 July 2017

Keywords:
Bacteria
Lipase enzyme
Production
Fermentation
Purification

ABSTRACT

Different samples were collected from polluted waters by fat materials in the Mascara region for the isolation of bacterial strains capable of degrading fat materials and used in the production of lipase by the fermentation method. A total of two strains (*Pseudemonas* sp. et *Streptococcus* sp.) were isolated at 37 °C from the sample water rich slaughterhouses fat materials. Production of extracellular bacterial lipase was studied as a function of several inductors of lipid nature by the fermentation method. The enzyme activity reached a maximum value, in the presence of olive oil as inducer. The enzyme was purified by precipitation with ammonium sulfate in a yield of 63.73% and 50% in *Streptocoques* sp. and *Pseudemonas* sp. respectively. The lipase produced by these two bacteria is resistant to 50 °C and is strongly inhibited in the presence of 1 mmol Zn^{+2} and Mg^{+2}.

Contents

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http://dx.doi.org/10.1016/j.ijbiomac.2017.07.106
0141-8130/

1. Introduction

Industrial waste and fat derived from plants cause irreversible environmental disaster (pollution of water, soil and air). Biological methods use microorganisms to degrade as biodepolluants fat ecological way [1]. Currently available solutions for the elimination of these fats are far from satisfactory. In recent decades, interest in the production of microbial lipases increases. There is a strong utility of the molecular structure and catalytic properties of these enzymes with potential application in various industries such as food, wastewater treatment, cosmetics, oleochemicals, and pharmaceuticals detergents [2]. In the fuel sector, which applies lipase as a catalyst for the ester synthesis and transesterification of oil for biodiesel production. Microbial lipases have gained special industrial attention due to their stability, selectivity and broad substrate specificity [3].

The work was to monitor the degradation of greasy residues by bacterial lipase enzyme from sewage water slaughterhouses.

2. Materials and methods

2.1. Biological materials

The two bacterial strains was used in this study were isolated from sewage water from slaughterhouses.

2.2. Isolation, purification and identification of lipolytic bacteria

According to Sierra method, after the isolation of two bacterial strains from samples of sewage water, mass ensemencement was realized on the Sierra agar medium. After 4 h, a central hole of 0.5 cm in diameter was made in each box. Then, after the use of the tween 80, it was followed by incubation over 4 days at 37 °C [4].

The presence of a halo around the central point characterized the presence of lipolytic strains and the strength of the lipolytic activity was proportional with the diameter of the halo. The identification of lipolytic bacteria was provided by a physiological and biochemical characterization.

2.3. Production of lipase enzyme (fermentation)

2.3.1. The preculture

The medium of the preculture (Fig. 1) was composed of 20 g/l glucose, 10 g/l yeast extract, 10 g/l of peptone and 5 g/l of sodium chloride. The medium pH was adjusted to 7.2. Mayer in 250 ml Erlenmeyer flasks containing 100 ml of medium were inoculated with the isolated lipolytic strains. The flasks were incubated at 30 °C with shaking at 125 rpm for 24 h [5].

2.3.2. Culture

Enzyme production was sought in the first place according to several sources carbone. Six medium types with various compositions were studied (Fig. 2). A production medium composed of 3 g/l yeast extract, 1 g/l peptone, 10 ml of olive oil as a carbon source and inducer and 0.7 g/l dipotassium phosphate, 0.3 g/l of potassium hydrogen phosphate, 0.5 g/l magnesium sulfate, 0.1 chloride manganese, 0.25 g/l of ammonium sulfate and 0.1 g/l calcium chloride. Medium B was 10 ml of Tween 80 as a carbon source and inducer, which replaced the olive oil in medium A. Medium C had 10 ml of oleic acid as carbon source and inducer, which replaced oil to olive medium A medium D was 2% added glucose milieu A. The medium E was 2% glucose added to the medium B. The medium F was 2% glucose added to the medium C, and culture medium without inducers as control [6].

Fig. 1. The preculture (*Psedemonas* sp. and *Streptococcus* sp.).

Fig. 2. Fermentation (production of lipase enzyme).

2.4. Determination of biomass

According to Beer-lambert formula, a volume of culture medium (cell suspenssion) was used for the measuring of the optic density (DO) at 600 nm, and then retrieves the biomass by centrifugation, then dried at 105 °C to constant mass [7].

The determination of biomass g/l is done every 24 **($C = DO/\varepsilon$)**.