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Research Article

Effect of medium constituents on the growth and lipase production in *Pseudomonas aeruginosa* 2036

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Abstract

Lipases are particularly important due to the fact that they specifically hydrolyze acyl-glycerol, oils and greases, which is of great interest for different industrial applications. In this study, Lipase producing bacteria, *Pseudomonas aeruginosa 2036* was procured from NCL, Pune. Lipase producing ability of the strain was checked in media containing different chemical constituent like starch, Olive oil and glucose as substrate for maximum enzyme production. Growth of the bacteria in all these media was checked with Lipase Activity. The growth of *Pseudomonas aeruginosa 2*036 was dependent on the constituents of the media as well as the shaking or non shaking condition of the media. Different substituent has the ability to work as suppressor or stimulator for producing maximum enzyme. They can also work in synergistically to enhance the production. This phenomenon has show in this study. The Presence of Starch, Olive oil and glucose has pronounced effect on Lipase activity. Based on the findings of present study, *P. aeruginosa 2036* is a potential candidate for higher lipase Production by modifying the medium condition and good candidate for industrial applications such as detergent, leather and fine chemical industries.

Keywords: Lipase, growth cruve, *Pseudomonas aeruginosa*, synergistic effect.

Introduction

Lipases are enzymes that catalyze hydrolysis of fatty acid ester bond in triglycerol (TAG) thus releasing free fatty acids (FFA). Note the reaction is reversible so the enzyme can catalyze esterification of glycerol to form mono-di and triglyceride (Gupata et.al., 2004). Lipase is produced by plants, animals, bacteria and moulds. Plant enzymes are not used commercially while animal, bacterial and mould enzymes are used extensively. Most important sources are cattle, sheep, and pigs i.e. pancreatic lipases and hog pancreatic lipase is most widely used. Lipases catalyze the hydrolysis of oils and fats (Van Laack et.al., 1992). Main use of lipases has appeared in pharmaceuticals as digestives and partly in dairy industries for flavor production. This means that a factor of "safety", which is needed in the fields of edible oils and fats, has already been confirmed in the enzymatic method (Kanwar and Goswami, 2002). Lipases constitute an important class of industrial enzymes and are currently used in variety of applications i.e. Resolution of racemic mixtures, regioselectivity acylation, bio-transformations of fats and oils, polymer synthesis, preparations of specialty esters for the food and cosmetic industry, and in cheese making (Kordel et.al., 1994). Advantages of lipase mediated processes over conventional chemical process are the high specificity for certain fatty acids or triglyceride positions and the possibility to allow reaction to proceed at much lower

temperatures (Kojima et.al., 1994). This can lead to saving in energy costs and most of all, to a reduction in unwanted side reactions, especially when the fatty acids involved are heat labile (Gilbert et.al., 1994). Increased interest in the chemistry and biotechnology of fats and oils has emerged in recent years. This trend can be mainly attributed to the fact that oleo-chemicals are derived from renewable sources (Stuer et.al., 1986).

Esterification reactions between polyhydric alcohols and free fatty acids are in essence, the reverse of the hydrolysis reaction of the corresponding glyceride. The equilibrium between the favoured and the reverse reactions is usually controlled by the water content of the reaction mixture (Zelles, 1999). Examples of high value chemical obtained via use of lipases include the synthesis of oleic acid esters of primary and secondary aliphatic and terpenic alcohols and the production of geranyl and menthyl esters from butyric acid and geranoil, or lauric acid and menthol respectively (Brondz, 2002).

The selection of media for growth of microorganisms is usually based on a combination of experimentation and logic, relying to a large extent on previous media that have been successful. When the amount of growth or growth rate obtained with a particular organism on a particular medium is believed to be less than optimal, recourse to experimentation is required (Prakash and Srivastava, 2005). The experiments are normally carried out by changing the concentration or nature of medium constituents, and comparing the growth rate or amount of growth obtained as a consequence of the change with that obtained on the basal medium (Lee et al., 1999). By a sequence of experiments using shake flasks, an improved medium, i.e. one that supports a greater cell concentration or a higher growth rate, can usually be obtained (Kokusho et al., 1982).

Lipases from *Pseudomonas* were probably the first studied. Many reports have shown the capacity of *Pseudomonas aeruginosa* for Lipase Production (Sankar, 2013; Zouaoui and Bouziane, 2011; Yossef and Neomi, 1992; Suryavanshi and Ghosh, 2010). Suryavanshi and Ghosh carried out a study to determine the optimum pH and temperature at which *Pseudomonas aeruginosa NCIM 2036* can produce lipase (Suryavanshi and Ghosh, 2010). The present study is carried out to maximize the production of lipase from *Pseudomonas aeruginosa* NCIM 2036 by using growth media of different composition and growth condition (Shaking and Static).

Materials and Methods

Microorganism and Media: The microorganism, *Pseudomonas aeruginosa 2036*, used in this study was procured from NCL, Pune (India). Different composition of the media used in this study is shown in table 1.

Table 1: Composition of various medium used for the study

Medium	2% Soluble starch	1% Olive Oil	0.5% Glucose	2% Glucose	0.5% $caco_3$	2% Polypeptone	0.2% K_2HPO_4	0.1 Urea	0.1% $MgSO_4.7H_2O$
1	✓	✓			✓	✓	✓	✓	✓
2	✓				✓	✓	✓	✓	✓
3		✓	✓		✓	✓	✓	✓	✓
4			✓		✓	✓	✓	✓	✓
5		✓		✓	✓	✓	✓	✓	✓
6				✓	✓	✓	✓	✓	✓
7		✓			✓	✓	✓	✓	✓

Growth study of bacteria

All the above media were prepared and sterilized by autoclaving at 120°C, 15 Lbs pressure for 15 minutes in 50 ml of side arm flask. The flasks were inoculated with 0.2ml of bacterial culture (With 90% transmittance at 660 nm) and aerobically grown at 37°C (±2°C) temperature with shaking (at 100 rpm) and with out shaking condition. The colorimeter readings were taken at different time intervals of 4 hours up to 96 to 120 hours.

Enzyme Preparation

All the above media were prepared and dispensed in 50ml volumes in100ml conical flasks and sterilized at 121°C for 25mins at 15 lbs pressure. Media were inoculated with 0.2ml of bacterial suspension with initial transmittance of 90% at 660 nm. Flasks were incubated under stable and static conditions at 37°C (±2°C) temperature. 5ml of culture broth was taken out from the stock at day 1, 2, 3, 4, 5, 6 and centrifuged at 15,000 rpm for 20 min at 4°C in Remi K-24 centrifuge. The fluid supernatant was used as enzyme source (Kojima and Shimizu, 2003).

Assay for Lipase activity

The substrate was prepared by mixing 22.9 ml of olive oil with 75ml of 2% polyvinyl alcohol solution at 0oC. The substrate was mixed twice with a Waring blender at 17,000 rpm for three min each. The reaction mixture, consisting of 2 ml of olive oil emulsion, 1.6ml of 0.1N Potassium phosphate buffer (pH 7.0) and 0. 4ml of crude enzyme solution was incubated at 37oC for 20 min. The reaction was stopped by the addition of 8 ml of an acetone-ethanol (1:1) mixture. The free fatty acids liberated were titrated with 0.05N NaOH. One unit of enzyme is defined as the amount which liberates 1µmol of fatty acid per minute per ml of enzyme under these conditions. The crude enzyme solution was prepared by sonicating 10ml of the culture for three min at a maximal output in an ice bath using a sonicator (Lin et. al., 1995).

Results

Growth curve of *P. aeruginosa* in different growth condition and different growth medium:

To compare the effect of different carbon sources (2% soluble starch, 0.5% glucose, 2.0% glucose and 1% olive oil) and growth condition on lipase production by the *P. aeruginosa 2036*, bacterial growth was monitored by at specific time points from day 1 up to day 5.

As depicted in Fig. 1 and 2, the overall growth curves were similar for both the growth condition (static and shaking) tested. The growth of *P. aeruginosa* in medium 6 and 4 (supplied with 2.0% and 0.5 % glucose respectively) were superior compared to others. In both, static and shaking growth condition, the medium 2 supplied with 2.0% starch have shown delayed in growth of *P. aeruginosa* compared to medium supplied with 2.0% and 0.5 % glucose. Very less growth were observed in medium 7 containing 1.0% olive oil as a sole source of carbon. Growth pattern of *P. aeruginosa* in medium 5 and medium 3 supplied with 2.0% glucose + 1% olive oil and 0.5 % glucose + 1% olive oil respectively had shown delayed in on set of log phase, but had shown comparatively better growth then the medium 7. Also medium 1 and medium 2 with 2% sucrose +1% olive oil and 2% sucrose respectively have shown little delay in on set of log phase.

Lipase production by *P. aeruginosa* in different growth condition and different growth medium:

Result presented in fig 3 and 4 demonstrate the effects of carbon sources on the production of lipase by *P. aeruginosa*. Over all lipase productions were higher in static growth condition compared to shaking condition. In both the growth conditions the lipase production by *P. aeruginosa* in 2nd and 3rd day of growth in all the different medias were higher, this may be due to higher growth of this organism in this time of the growth curve.

Medium 3 (0.5 % glucose + 1.0% olive oil) have shown highest production of lipase. Medium 1 (2.0 % sucrose + 1.0% olive oil) had shown second highest production of lipase next to medium 3. Even though medium 7 (1.0% olive oil) had shown least growth of organism but it has shown the third highest lipase production. Very interesting result of this study was that the medium 5 (2.0 % glucose + 1.0% olive oil) had shown better growth of the organism but the lipase production was less compared to medium 3 (0.5 % glucose + 1.0% olive oil), this may be because the lipase production by *P. aeruginosa* was inhibited by higher concentration of glucose.

Figure 1: Effect of different medium on the growth curve of *P. aeruginosa* 2036 in static condition

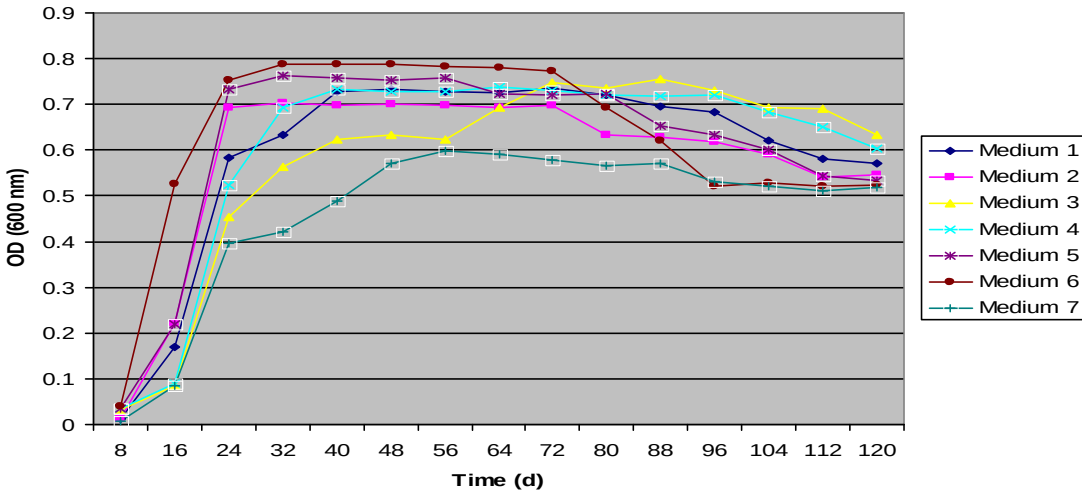


Figure 2: Effect of different medium on the growth curve of *P. aeruginosa* 2036 in shaking condition

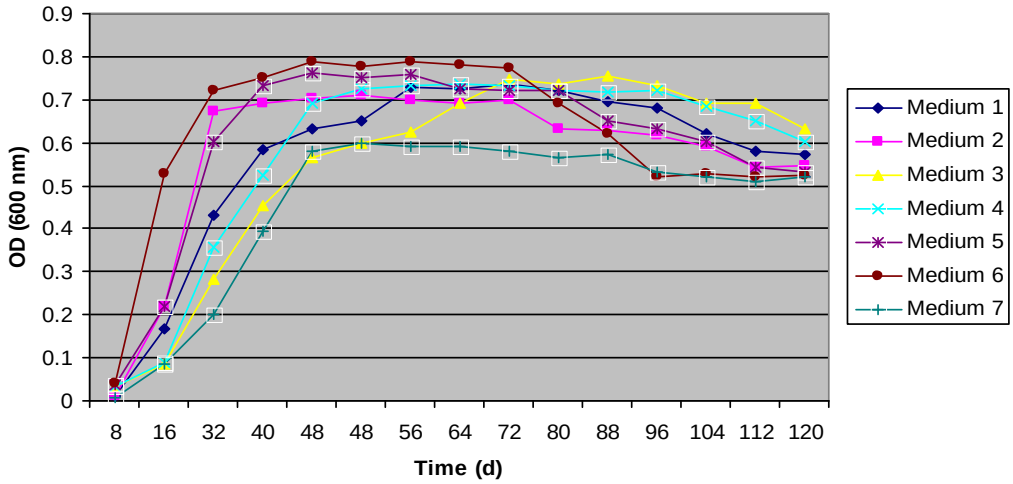


Figure 3: Effect of different medium on Lipase production by *P. aeruginosa* in static condition

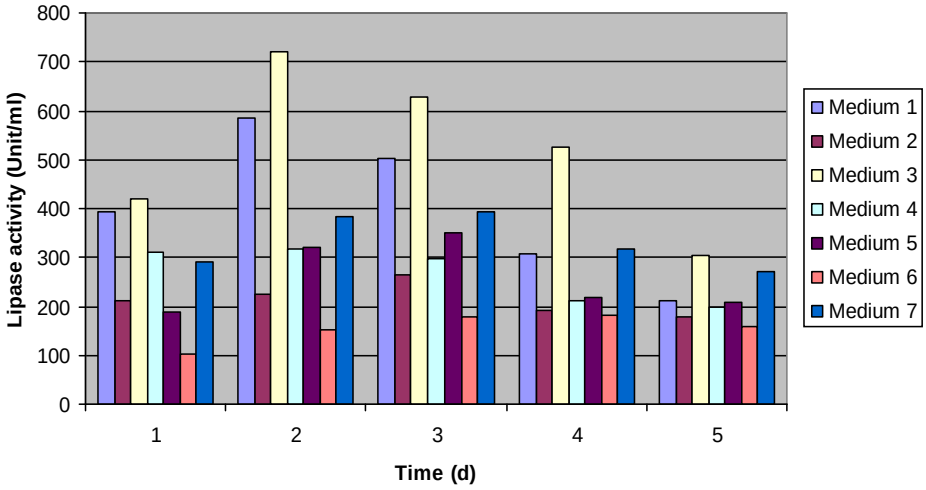
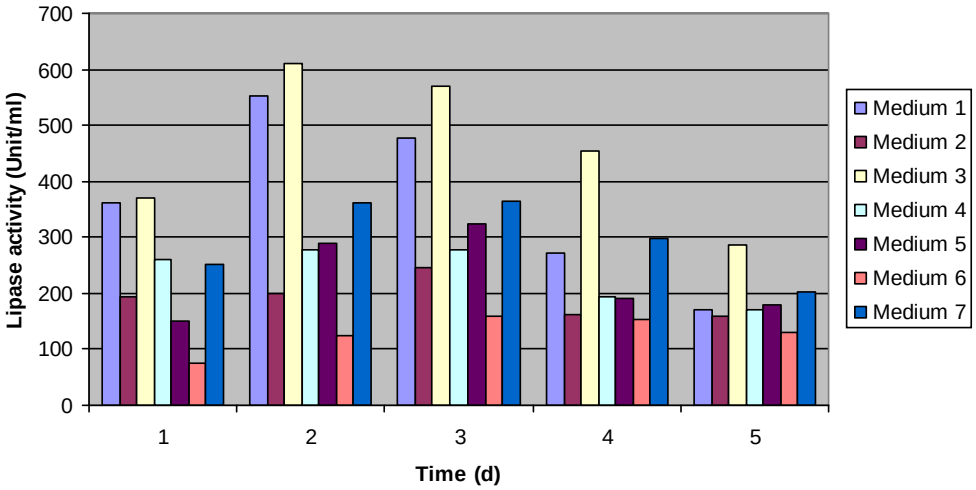


Figure 4: Effect of different medium on Lipase production by *P. aeruginosa* in shaking condition



Medium 2, medium 4, and medium 6 with 2% sucrose, 0.5% glucose and 2.0% glucose as carbon source respectively had shown less production of lipase compared with the medium containing additional 1.0% olive oil with these carbon sources.

Discussion

Carbon source is an important substrate for energy production in microorganisms. In order to investigate the effect of carbon source on lipase production in *Pseudomonas aeruginosa 2036*, different carbon sources – Starch, Glucose & Olive oil was selected for their efficiency to support lipase production. In our study we have observed similar trend of lipase production shown in the previous studies done by the research groups mentioned below.

In our study, in medium 3, presence of 0.5% glucose and 1% olive oil had shows maximum lipase production amongst all. Starch and olive oil also had shown good lipase production, this has proven the synergistic effect of carbohydrate and olive oil on the production of lipase. Medium 5 with 2% glucose and 1% olive oil had shows least lipase production amongst all medium. The addition of 2% glucose with olive in the medium had a negative effect on lipase production. A similar inhibitory effect of glucose was reported by Montet et al. (Montet et al. 1985) with *C. curvata* and by Muderhwa and Ratomahenina (Muderhwa and Ratomahenina, 1985) with *C. deformans*. Nahas reported that lipase activity was lower ($P < 0.05$) in olive oil medium containing greater than 1% glucose (Nahas, 1988). It can be concluded that presence of olive oil can be stimulator for lipase production by *P. aeruginosa* but it depend on the concentration of sugar and type of sugar.

The Presence of Starch, Olive oil and glucose has different effect on the growth and lipase production by *P. aeruginosa.* Olive oil along with the other carbon sources act as a stimulator for the lipase production by *P. aeruginosa.* Based on the findings of present study, *P. aeruginosa* 2036 is a potential candidate for higher lipase Production by modifying the medium condition and good candidate for industrial applications.

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