ORIGINAL RESEARCH PAPER

Microbiology

PURIFICATION AND CHARACTERIZATION OF ESTERASE ENZYME ISOLATED FROM CONTAMINATED SOIL SAMPLE

KEY WORDS: Carbon, Nitrogen, Esterase, Purification.

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ABSTRACT

Isolation and partial purification of esterase from contaminated soil sample was the main aim of this study. The production medium for organism was optimized by using different pH, Temperature, Carbon and Nitrogen sources. The esterase enzyme was highly active and stable from pH 5.0 to 9.0 with an optimum at pH 9. Its optimum temperature was 35°C. The best carbon and nitrogen sources were mannitol and yeast extract. Esterase was partially purified by ammonium sulphate precipitation, dialysis. The specific activity of partially purified esterase obtained from ammonium sulphate fractionation is found to be 8.6485U/mg and the fractionation is 5.4 fold purified from the crude enzyme preparation yielding 17.5U/mg from the crude protein. This result showed that Bacillus subtilis under study is a good producer of extra cellular esterase, which can be beneficial for industries.

1. INTRODUCTION

Microorganisms are the most important sources for enzyme production and serve as preferred source because of their rapid growth, the limited space required for their cultivation. Many of the organisms produce more than one kind of esterase enzyme. Esterase execute a large variety of functions and have important biotechnological applications. Esterases represent one of the three largest groups of industrial enzymes and find application in detergents, leather industry, food industry, pharmaceutical industry and bioremediation processes (Anwar and Saleemuddin, 1998;Gupta , 2002). Esterases are widespread in nature, microbes serve as a preferred source of these enzymes because of their rapid growth, the limited space required for their cultivation and the ease with which they can be genetically manipulated to generate new enzymes with altered properties that are desirable for their various applications (Anwar and Saleemuddin, 1998; Beg and Gupta, 2003). Probably the largest application of esterases is in laundry detergents, where they help removing protein based stains from clothing (Banerjee , 1999; Banik and Prakash, 2004). *Bacillus subtilis* is one of the most widely used bacteria for the production of industrially important esterase enzymes. These strains are specific producers of extracellular esterase and can be cultivated under extreme temperature and pH conditions to give rise to products that are, in turn, stable in a wide range of harsh environments.

In the paper we aim to isolate and purify esterase producer *Bacillus subtilis* collected from soil sample of karur district and then focus on optimizing the production of extracellular protease by testing various environmental and nutritional factors.

2. MATERIALS AND METHOD

MICROORGANISM ISOLATION

Soil samples were taken from Karur district), and used for the isolation of bacterial cultures on nutrient agar. A total of 10 bacterial isolates were isolated. The isolates were purified by streaking on agar plates and picking single colonies. The isolates were examined for various morphological and biochemical characteristics as per Bergey's Manual of determinative Bacteriology. The culture was maintained on nutrient agar medium at 4°C.

DETERMINATION OF THE ESTEROLYTIC ACTIVITY - GELATIN AGAR PLATE

About 2g of agar was weighed and dissolved in 100ml of distilled water. About 1g of gelatin was weighed and dissolved in 100ml of distilled water. The solutions 1&2 were autoclaved separately and cooled. Both were mixed aseptically and about 20ml of media was poured in to sterile petriplate and allowed to solidify. With the help of a sterile cork borer wells were cut and the culture. Supernatant was added into the wells with a positive and negative control under aseptic conditions and incubated at room temperature for 24 hours. After 24 hours the plate were developed with 15% mercuric chloride in 20% HCL solutions and the mean diameters were recorded .(Ammar ,1991).

EFFECT OF PH

The effect of pH was studied with various pH namely 4.0, 5.0, 6.0, 7.0, 8.0, 9.0, 10.0, 11.0 and 12. The optimum pH for maximum activity was selected by varying the pH of medium and medium from pH 4 to 12 for the culture isolated from the soil.

EFFECT OF TEMPERATURE

The effect of temperature was studied with various temperatures namely 25°C, 30°C, 35°C, 40°C, 45°C and 50°C. The optimum temperature for maximum activity was selected by varying the temperature of medium (pH- 9.0) from 25°C to 50°C for the culture isolated from the soil. All other parameters were kept unaltered.

EFFECT OF CARBON SOURCE

The effect of carbon source was studied with different carbon sources like Glucose, Sucrose, Mannitol, Coconut Oil, Olive Oil, Glycerol, Tween 80 and Palm Oil. The optimum carbon source for maximum activity was selected by varying the carbon sources of medium (pH-9.0) at 35° C for the culture isolated from the soil. All other parameters were kept unaltered.

EFFECT OF NITROGEN SOURCE

The effect of Nitrogen source was studied with different nitrogen sources like Peptone, Ammonium Nitrate, Potassium Nitrate, Yeast Extract, Tryptone, Beef Extract and Urea .The optimum nitrogen source for maximum activity was selected by varying the nitrogen sources medium (pH 9.0) with mannitol as carbon source medium (pH 8.0) with at 35°C for the culture isolated from soil. All other parameters were kept unaltered.

PURIFICATION OF ESTERASE ENZYME BY AMMO NIUM SULPHATE FRACTIONATION:

Ammonium sulphate (Qualigens, India) required to precipitate the esterase enzymes were optimized by adding

Submitted : 18th July,2019 | Accepted : 25th September,2019 | Publication : 15th November, 2019

varying concentrations of ammonium sulphate (20%, 40%, 60%,80% and 100%) to the crude extract as detailed below.

The proteins were precipitated by adding ammonium sulphate slowly, initially at 20% saturation to the crude extract while keeping in ice with gentle stirring. After complete dissolution of ammonium sulphate, the solution was kept at 4°C for overnight precipitation. The precipitated protein was collected by centrifugation at 10,000 rpm for 15 minutes at 4°C. To another batch of culture supernatant, required ammonium sulphate for next level of saturation was added and procedure mentioned above was repeated for 40%, 60%, 80% and the precipitation was continued for up to 100%.Followed by dialysis procedure.

Dialysed ammonium sulphate fraction was loaded on a DEAE –Cellulose column (2cm x 25cm),pre -equilibrated with 50mM phosphate buffer pH 7.2 and then the column was eluted with same buffer .Preparation of the gel column and the fractionation procedure was carried out as mentioned by Abdel-Raouf (1990). Forty fractions were collected (each of 5ml) and sharp peaks of fraction obtained were collected and the esterase enzyme activity as well as the protein content was performed for each separate fraction.

RESULT AND DISCUSSION

In the present study ten bacterial isolates were obtained from soil samples, of which one isolates were identified as esterolytic *Bacillus* species based on gram staining and some biochemical tests such as indole, catalase, oxidase and nitrate reduction in which all the species were positive in these tests and only one bacterial isolate no (S8) was identified as *Bacillus subtilis* (Shereen Gul, , Rahman, ,. Achazai & Kamin 2008**).** (Table1.).The esterase activity was observed as clear zone of inhibition around the gelatin agar plate .(Fig.1)

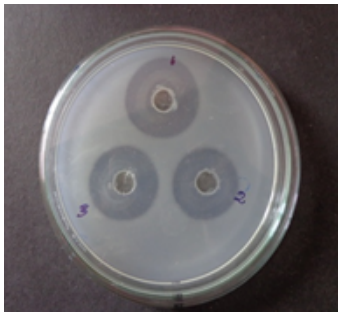

Fig 1. Zone of inhibition shows Esterase Activity

Table 1: Biochemical characters of isolated organisms

Identification Tests	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10
Grams staining	+	+	-	+	+	+	_	+	+	+
Motility	+	+	+	+	+	+	+	+	+	+
Indole test	+	+	-	-	-	-	+	-	+	+
Methyl red test	+	-	-	+	+	-	+	-	_	+
Vogas proskauer	_	_	_	_	+	+	+	+	_	+
Catalasetest	-	-	-	-	+	+	+	+	-	+
Oxidase test	+	+	-	+	-	+	-	+	+	+
Citrate utilization	+	+	-	-	+	+	+	+	-	-
Nitrate reduction	+	+	-	-	+	+	-	+	-	+
Casein hydrolysis	+	+	+	+	+	+	+	+	+	-
Starch hydrolysis	+	+	+	+	-	+	-	+	+	+

EFFECT OF PH AND TEMPERATURE

The study of the effect of different pH reveals that the enzyme secretion is greatly influenced by varying pH of the environment. In bacterial isolate no.S8 the maximum enzyme was at pH 5 and the maximum temperature was 35°C.

EFFECT OF CARBON AND NITROGEN SOURCES ON PROTEASE PRODUCTION

Among various media tested, mannitol and yeast extract medium was found most suitable. Mannitol was the best source for esterase production .(Fig 2). Results obtained showed that the best nitrogen source for esterase production was yeast extract (Fig 3). Maximum enzyme production were yeast extract> beef extract>peptone > tryptone >urea>ammonium nitrate>potassium nitrate (Abidi , Limam & Nejib 2008).

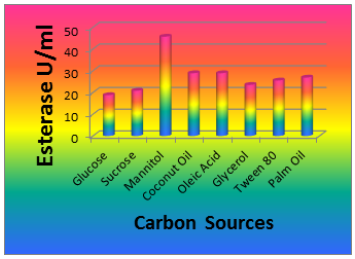

Fig 2: Effect of Carbon source on Esterase Activity in isolate no.S8

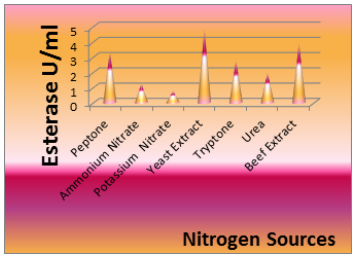

Fig 3 : Effect of Nitrogen source on Esterase Activity in isolate no.S8

PURIFICATION AND CHARACTERIZATION OF ESTERASE

In bacterial isolate noS8, the specific activity of crude esterase enzyme was 1.616 U/mg of protein which increased during ammonium sulphate fractionation (8.6485 U/mg) with 5.4 fold increase in purification which further increased to 29.5 fold increase in purification after column chroma tography with specific activity of 24.4146 U /mg.(Table3).

Table 3: Purification of Esterase enzyme by ammonium sulphate fractionation & column chromatography

S.no	Fraction	Volume (ml)	Esterase Activity (U/m L)	Total Activity (IU)	Protein (mg/ml)	Total Protein (mg)	Specific Activity (U/mg)	Yield (%)	Purification fold
1	Crude	100	9.81	989.1	6.12	612	1.616	100	1
2	80% (NH4)2SO4	50	3.4594	172.82	0.400	20	8.6485	17.5	5.4
3	Column Chromotograph	10	31.4533	314.533	1.288	12.883	24.4146	31.8	15.1

CONCLUSION

In the present study, we could isolate *Bacillus subtilis* and esterase enzyme. Many studies such as those showed that researches will continue to isolate alternative strains for production of enzymes as well as esterase. The Esterase had an optimum pH of around 5, The optimum temperature was 35°C.The best esterase producing Carbon and Nitrogen substrates were mannitol and yeast extract. The present study

achieved by reducing the production cost, increasing the products quantity and overall profit. Esterase applications ranged from industrial, bioremediation process to high quality in case of products involved pharmaceutical medicines.

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