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Strain Improvement of *Pseudomonas* sp for the Production of Lipase

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ABSTRACT: *Pseudomonas* sp. capable of extracellular lipase production was isolated from rhizosphere soil of paddy fields in Tamilnadu, India. The isolated strain was mutated using ultra-violet rays and chemical agents like Sodium Azide and EMS. The study indicated that the lipase activity of the mutant strain using chemical mutagen was 2-fold higher than the wild strain.

Key words: *Pseudomonas*; Lipase; Mutagenesis; UV; Sodium Azide; EMS

Abbreviation: SA: Sodium azide, EMS: Ethyl methane sulphonate, UV: Ultra Violet, PSsa: Sodium azide treated *Pseudomonas* sp., PSems: Ethyl methane sulphonate treated *Pseudomonas* sp., PSuv: Ultra Violet treated *Pseudomonas* sp.

Introduction

Lipase enzyme plays a vital role in many food, dairy, leather, paper, pharmaceutical, detergent, textile and cosmetic industries. Lipases are secreted by microorganisms like bacteria, yeasts, molds and a few protozoa. The production of lipase by microorganisms depends largely on the species, strains and culture conditions. Microbial lipases are diverse in their enzymatic properties, substrate specificity and are usually more thermo stable than animal or plant lipases (Vanitha, 2002). Research on lipase production has intensified in recent years due to its potential application in various industrial processes. There are several reports on isolation of lipase producing microorganisms and the effect of nutritional factors on their growth and lipase production (Kamini *et al.*, 1997; Gisela *et al.*, 1991; Sangilyandi and Gunasekaran, 1996). Jette and Ziomek (1994) determined the lipase activity by a Rhodamine-Triglyceride-Agarose assay. Several species of *Pseudomonas* have been reported to produce this enzyme (Suziki *et al.,* 1988; Narasaki *et al.,* 1968; Sugiura *et al.,* 1977 and Sugiura and Oikawa, 1977). However, no report is available on lipase production by mutated *Pseudomonas* species. Therefore, the present study has been undertaken to study the efficacy of lipase production after inducing mutation in *Pseudomonas* strains isolated from rhizophere soil of the paddy fields.

Materials and Methods

Microorganism

Pseudomonas strain isolated from rhizosphere soils of paddy fields in Mannachanallur, one of the largest paddy producing area located in Trichy district of Tamil Nadu, India. The strain was sub-cultured on nutrient agar slant and incubated at 37ºC for 48hrs. This strain was stored at 4ºC and used as the parent (Wild strain) culture.

Mutagenesis by UV

The 24 hr old nutrient broth of the parent culture was diluted with phosphate buffer (pH 7.0) to contain 10^8 cells/ml. The culture was spread plated on nutrient agar plates. Then the plates were exposed for various time intervals (1 to 10 min) to UV light (2600 A^o) at a distance of 15cm (Kumar and Dhruv, 1990). After the stipulated time interval, each set of plates were covered with black paper to avoid photo reactivation and incubated at 37ºC for 2 days. The unexposed plate containing the wild strain served as positive control. On incubation, the resistant strains were selected based on colony development in nutrient agar plates and given code names as PSuv 1, PSuv 2, PSuv 3 and PSuv 4 .These strains were transferred to nutrient agar slants and further screened for lipase activity.

Mutagenesis by Chemical

Sodium azide and ethyl methane sulphonate (EMS) were the chemical mutagens used for mutagenesis of the parent culture, *Pseudomonas* sp. The bacterial suspension was diluted in similar way as that for U.V mutagenesis. To nine ml of bacterial suspension, one ml of sterile solution of sodium azide (50 $\mu g\ ml^{-1}$ in phosphate buffer) was added. Similar method was adopted for ethyl methane sulphonate (50$\mu g\ ml^{-1}$ in phosphate buffer). The reaction was allowed to proceed. Samples were withdrawn from the reaction mixture at intervals of 30, 60, 90, 120, 150 and 180 min and immediately centrifuged for 10 min at 5000 rpm. The pellet was washed three times with sterile phosphate buffer and resuspended in 10 ml of the same buffer (pH 7.0). The samples were serially diluted using the same buffer and plated on nutrient agar. The plates were incubated at 37ºC for two days. The selected sodium azide treated mutants were designated as PSsa30, PSsa60, PSsa90 and PSsa120, whereas ethyl methane sulphonate treated mutants were designated as PSems60, PSems90, PSems120 and PSems150.

Submerged fermentation

18 hr old culture broth of wild type and mutant strains of *Pseudomonas* sp. was used for this study. 0.5 ml of each strain (O.D = 1.0) was inoculated into 100ml Czapek-Dox broth containing 1% olive oil as inducer. Sucrose and 10% olive oil as the carbon source. The flasks were incubated at 37°C for 96hrs. Uninoculated Czapekdox broth served as control. Each experiment was done in triplicates.

Lipase assay

Culture broth was withdrawn aseptically at three days interval from each flask and centrifuged at 3000 rpm for 15 minutes and the supernatant was collected and used for lipase activity (Ray *et al.*, 1999). Lipase activity in culture broth was determined by titrimetry using olive oil as substrate (Vanitha, 2002).

Results and discussion

Lipase activity of *Pseudomonas* sp treated by ultraviolet rays, sodium azide and ethyl methane sulphonate are presented in the Table 1, 2 and 3 respectively. The results indicated that lipase activity was more in sucrose medium than in olive oil medium after 96 hrs of UV Sodium azide and EMS treatments. The enzyme activity was found to be higher in mutant strains than the wild type (Table 1, 2 and 3). Efficacy of lipase activity by the mutant strains was observed in the descending order of EMS mutants > SA mutants > UV mutants. In the first part of the study, among the UV irradiated bacterial strains, PSuv3 was found as the predominant lipase producing strain followed by PSuv4. In the second part of the study involving chemical mutagenesis, PSems120 showed the highest lipase activity followed by PSems150.

The lipase activity considerably increased depending on nutritional conditions. Similar observations were made by Lawrence *et al.* (1967) in *Pseudomonas* (Suzuki *et al.,* 1988). Ray *et al.* (1999) reported the lipase activity increased in isolated bacterial strains by chemical mutagens. Ellaiah *et al.* (2002) reported that isolated fungal strain produced lipase with physical and chemical mutagens and the mutant has higher lipase activity than the wild strain. The increase in the production of lipase by the mutagens both physical and chemical may be due to the alternation of genotype of the micro organisms. Moreover, they may alter the gene sequence

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(Freifelder, 1990; Radman, 1999). The azide ion alters the structure of cytosine such that it forms hydrogen bonds with adenine, rather than guanine. This produces a cytosine to thymine transition. Ethyl methane sulphonate is a strong mutagenic agent. It alkylates N7 of Guanine and severely alters the base pairing. The present study also indicated that the strain of *Pseudomonas* sp treated with EMS produced 2-fold (PSems120) higher amount of lipase compared to wild strain.

Table 1. Lipase activity of *Pseudomonas* sp treated by ultraviolet rays

UV treated *Pseudomonas* sp strains	Lipase activity after 96 hrs(Unit/g of substrate)*		Increased % of Lipase activity	
	Sucrose medium	Olive oil medium	Sucrose medium	Olive oil medium
PSuv1	2.22±0.01	0.23±0.02	5.71	29.31
PSuv2	2.19±0.09	0.33±0.08	4.28	91.95
PSuv3	2.49±0.07	0.38±0.03	18.57	118.39
PSuv4	2.29±0.02	0.34±0.05	9.04	92.52
Wild type[a]	2.10±0.08	0.17±0.06		

*Values are expressed as mean ± standard deviation of triplicates
[a]Standard for calculation of increased %

Table 2. Lipase activity of *Pseudomonas* sp treated by sodium azide

Sodium azide treated *Pseudomonas* sp strains	Lipase activity after 96 hrs(Unit/g of substrate)*		Increased % of lipase activity	
	Sucrose medium	Olive oil medium	Sucrose medium	Olive oil medium
PSsa30	2.28±0.04	0.28±0.11	8.57	58.05
PSsa60	2.93±0.03	0.35±0.02	39.52	103.45
PSsa90	3.23±0.05	0.39±0.08	53.80	124.14
PSsa120	3.04±0.09	0.39±0.04	44.76	121.26
Wild type[a]	2.10±0.06	0.17±0.06		

*Values are expressed as mean ± standard deviation of triplicates
[a]Standard for calculation of increased %

Table 3. Lipase activity of *Pseudomonas* sp treated by EMS

EMS treated *Pseudomonas* sp strains	Lipase activity after 96 hrs(Unit/g of substrate)*		Increased % of lipase activity	
	Sucrose medium	Olive oil medium	Sucrose medium	Olive oil medium
PSems60	2.42±0.10	0.34±0.06	9.50	54.55
PSems90	3.53±0.05	0.47±0.09	59.72	113.64
PSems120	4.45±0.07	0.61±0.05	101.36	177.27
PSems150	4.12±0.04	0.52±0.05	86.43	136.36
Wild type[a]	2.21±0.09	0.22±0.12		

*Values are expressed as mean ± standard deviation of triplicates
[a]Standard for calculation of increased %

Lipases are enzymes that catalyze the hydrolysis of triglycerides to glycerol and fatty acids Microbial lipases are relatively stable and are capable of catalyzing a variety of reactions: they are potentially of importance for divorce industrial applications. The present study concluded the addition of chemical agents in bacterial strains may produce the higher quantity of lipase enzyme.

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