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Isolation and Screening of Lipase Producing Bacteria from Oil Spilled Soils of Bilaspur District (C. G.)

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Abstract: ***The present investigation focuses on the attempt of obtaining potential lipase-producing bacteria from oil spill soil samples of Bilaspur district. Primary screening of lipolytic activity on agar plates was done with certain substrates such as tributyrin. A total of the 52 bacterial Lipase producers were isolated from 06 different oil spilled soil samples of Bilaspur district. Out of 52 lipase producing bacteria, 10 isolates showed high lipase activity. One of the ten isolated strain exhibited a greater zone of clearance, indicating higher lipase activity. It was further characterized and identified morphologically and biochemically. Isolate S6C2(a) showed maximum zone of clearance (3cm) when plated on tributyrin agar base, upon incubation period 24 hrs. and gave positive test for catalase, cellulose, amylase, protease and urease while do not produce acid in glucose peptone broth therefore were MR negative.***
Key words: lipase-producing bacteria, Tributyrin agar base

I. INTRODUCTION

Lipase (triacylglycerol acylhydrolase) is an omnipresent enzyme with important physiological significance and industrial potential. Lipase (EC 3.1.1.3) is an ester hydrolase, which catalyze the hydrolysis of triglycerides to glycerol and free fatty acids. They are soluble in water and hydrolyze insoluble substrates to more polar lipolytic products. The first lipase was identified by Claude Bernard in 1856 and from then on, they have been identified in microorganisms, plants and animals.

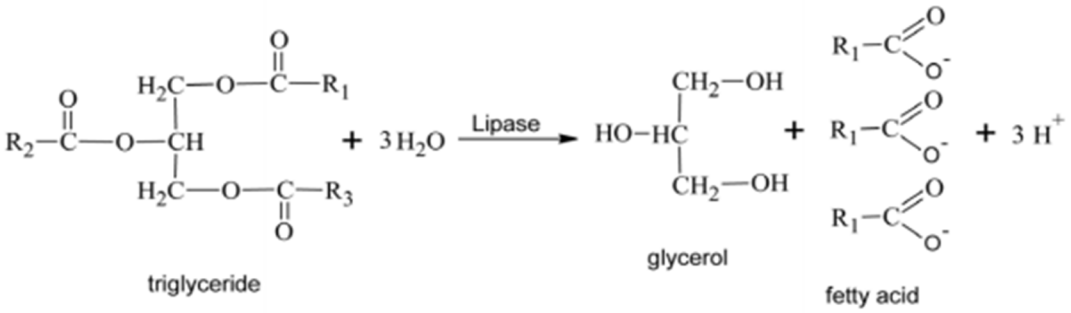


Fig: -Hydrolysis of triglycerides with the participation of lipase

Lipase-producing microorganisms have been found in diverse habitats such as industrial wastes, vegetable oil processing factories, diaries, soil contaminated with oil, etc. Microbial lipases have gained special industrial attention due to their stability, selectivity, and broad substrate specificity (Dutra et al.,2008; Griebeler et al.,2009). Many microorganisms are known as potential producers of extracellular lipases, including bacteria, yeast, and fungi (Abada et al., 2008; Joseph et al.,2008 and Thakur, 2012). Of all these, bacterial lipases are more economical and stable (Snellman et al.,2002; Sirisha et al.,2010). *Bacillus* sp., *Staphylococcus* sp., *Lactobacillus* sp., *Streptococcus* sp., *Micrococcus* sp., *Propionibacterium* sp., *Burkholderia* sp., are reported to be the lipase producing Gram positive bacteria. Whereas *Psuedomonas* sp. *Chromobacterium* sp., *Acinetobacter* sp., *and aeromonas* sp., are such Gram-negative bacteria. (Gunasekaran and Das, 2005). Different genera of bacteria including *Streptomyces* spp. are known to produce lipase but among them *Achromobacter* sp., *Alcaligenes* sp, *Arthrobacter* sp, *Pseudomonas* sp and *Chromobacterium* sp have been well exploited for lipase production (Ghosh et al.,1996). *Staphylococci* is another genera showed the potential of lipase production. Staphylococcal lipases are classified as true lipases (Rosenstein and Götz, 2000). In most instances lipase production ability of Staphylococci has been related to their pathogenecity.

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Greater part of bacterial lipases comes from Gram-negative bacteria and the most important Gram-negative genus is *Pseudomonas* which contains at least seven lipase producing species, that are *P.aeruginosa, P. alcaligenes, P. fragi, P. glumae, P.cepacia, P. fluorescens and P. putida* (Singh et al.,2007). Enzymes of *P. aeruginosa, P. cepacia* and *P. fluorescens* obtained in industrial conditions and are used in organic synthesis, including catalysis of reactions in aqueous solutions (Veerapagu et al.,2014). Some important lipase producing bacterial genera include *Bacillus*, *Pseudomonas* and *Burkholderia*etc (Gupta et al.,2007; Wang et al.,2009). In view of the above facts, there is a great demand to find novel lipases of industrial uses (Biswas *et al.*,2016). These lipase producing microorganisms have been isolated from various sources like Oil contaminated areas (Dharmsthiti and Luchai 1999), soil samples (Cardenas *et al.*,2001) contaminated water sample (Haba et al.,2000) Crude oil contaminated soil sample (Kanwar et al., 2002). Lipases are produced by animals, plants, and microorganisms. Microbial lipases have gained special industrial attention due to their stability, selectivity, and broad substrate specificity (Lomthaisong et al.,2012).

When screening bacteria for lipase production both culture pH and assay pH are important parameters. The stability depends upon the presence of substrate (Wood et al.,2001). Almost all microbial lipases can be regarded as acid lipases or neutral lipases if they are classified by their optimum pH value for the lipolytic activity (Baharum et al.,2003). The industrial demand for new sources of lipases with different catalytic characteristics stimulates the isolation and selection of new strains (Salihul et al.,2011).

Commercially useful lipases are usually obtained from microorganisms that produce a wide variety of extracellular lipases. Only about 2% of world's microorganisms have been tested as enzyme sources. Fungal Lipase is a specific enzyme that digests fat and is characterized by its ability to hydrolyze fat over a wide range of temperatures and pH. Bacteria produce different classes of lipolytic enzyme, including carboxyl esterase, which hydrolyze small ester containing molecules at least partly soluble in water, true lipases, (Sharma et al.,2001), which display maximal activity towards water insoluble long-chain triglycerides, and various types of phospholipids The fat decomposition for the microorganisms is a source of carbon and it begins through lipase enzyme acting on the fats and is accompanied by the formation of glycerol, fatty acids. (Veeranna et al.,2012). Microorganisms producing lipases have been isolated from diverse habitats such as industrial wastes, vegetable oil processing factories, dairies, soil contaminated with oil, etc. (Veeranna et al.,2012; Biswas et al.,2016). At present, microbial remediation of oil contaminated soil has been carried out and widely reported in the world (Daniel et al., 2007); Minai et al.,2009; Walworth et al.,2003; Zhang et al.,2010), which provides a reference and guidance for development and practical application of bioremediation technology (Yan et al.,2013).

Soil is a rich source of many types of microbial strains which can provide a particular group of microbial strains necessary for the degradation of different contaminants thrown in to the soil. Hence the soil samples can be used to isolate the novel strains that may be used as a part of the microbial pool to produce lipase at research labs and industries (Bhawani et al.,2012). Industries are still seeking strains of bacteria that produce a high yield of potent lipase with excellent properties using cost-effective methods. Therefore, the present study was conducted to isolate a novel lipase producing bacteria from oil spilled soil.

II. MATERIALS AND METHODS

A. *Sample Collection*

For the present study, soil samples were aseptically collected from oil and fat contaminated soil of dairy and oil refinery industries in a plastic bag, situated in and around Bilaspaur city, Chhattisgarh, India. Soil was collected randomly 5-10cm beneath the surface using spatula and were packed in sterile polybags and transferred to Microbiology laboratory, in Atal Bihari Vajpayee Vishwavidyalaya, Bilaspur (Mohan et al.,2008).

B. *Isolation of Lipolytic Bacteria*

Lipolytic microbes were isolated from the collected soil samples. Isolation of lipase producing bacteria were made by spread plate method using serial dilution on tributyrin agar medium containing 0.5 (w/v) peptone, 0.3 (w/v) yeast extract, 1% (v/v) Tributyrin and 2% agar, pH 7.0). For this, 1.0 g of soil was dissolved in 10ml of distilled water. Then it was serially diluted (10^{-1} to 10^{-9}) and one ml of 10^{-7}dilution from each sample was plated on tributyrin agar plates. The formation of halo zone around the colony on tributyrin agar was considered as the positive for lipase producers. Plates were incubated at 37^0C for two days. Pure culture of the isolates was maintained on nutrient agar slants (yeast extract, NaCl, peptone and agar 2%, pH 7.0) and were subculture every 15 days (Sirisha et al., 2010).

C. *Screening of the Isolates for Lipase Activity*

Lipolytic organisms were screened by qualitative plate assay. Isolates were grown on Tributyrin agar base plates and incubated at 37^0C for 2 days. Zone of clearance was observed due to hydrolysis of tributyrin (Sirisha et al., 2010).

Lipolytic Potential = hydrolytic zone diameter/colony Diameter (Pallavi et al., 2015)

D. Morphological and biochemical Characterization of the Isolates

The total isolate showed maximum zone of clearance hereby referred as L2 is selected for further analysis. Morphological and Biochemical characterization of the isolate have been studied for the identification of the isolates (Sirisha et al.,2010). Morphological characterization was done by microscopic study after staining the cultures by simple and differential staining methods.

III. RESULTS AND DISCUSSIONS

Lipase producing microorganisms have been found in diverse habitats such as industrial wastes, vegetable oil processing factories, dairies, oil contaminated soils, oil seed cakes, decaying food, compost heaps, coal tips and hot springs.

In the present investigations, the distribution of lipase producers in different oil spilled soils of Bilaspur district was determined and the results are précised in table (1and 2).

TABLE 1. Isolation of lipase producing microorganisms and morphological characterization from each sample:-

Sampling site	Lipolytic bacterial isolates	Morphological characteristics	Lipolytic Potential
Sample (1) Bilaspur (Near Gandhi chawk) Confectionary shop	S1C4(a)	Coccus , G positive	2.16
	S1C4(b)	Rod Shape,G Positive	2.6
	S1C4(c)	Rod shape , Gpositive	2
	S1C3(a)	Rod shape, Gpositive	2
	S1C3(b)	Rod shape, Gpositive	2
	S1C6(a)	Rod shape, Gpositive	1.3
	S1C8(a)	Rod shape, Gpositive	2
	S1C10(a)	Rod shape, Gpositive	2
	S1C10(b)	Rod shape, Gpositive	1.42
	S1C9(a)	Rod shape, Gpositive	1.2
	S1C1(a)	Rod shape, Gpositive	2
	S1C12 (a)	Coccus , Gnegative	1.4
Sample (2) Bilaspur (Near Gandhi chawk) garage	S2C1(a)	Rod shape , Gpositive	1.4285
	S2C15(a)	Rod shape , Gpositive	2
	S2C15(b)	Rod shape , Gpositive	1.8
	S2C10 (a)	Rod shape ,G positive	1.7
	S2C14(a)	Rod shape , Gpositive	1.8
	S2C2(a)	Rod shape , Gpositive	1.25
	S2C9(a)	Rod shape , Gpositive	1.18
	S2C8(a)	Rod shape , Gpositive	1.4
	S2C5(a)	Rod shape , Gpositive	2
	S2C7(a)	Rod shape , Gpositive	2
	S2C3(a)	Rod shape , Gpositive	1.8
	S2C11(a)	Rod shape , Gpositive	1.4
	S2C3(b)	Rod shape , Gpositive	1.2
	S2C2(b)	Rod shape , Gpositive	1.4
	S2C12(a)	Rod shape , positive	1.2
	S2C4(a)	Rod shape , positive	1.8
Sample (3) Bilaspur (near Sanichari market) Confectionary shop	S3C2(a)	Rod shape , positive	2.1
	S3C2(b)	Rod shape , positive	2.3
	S3C2(c)	Rod shape , positive	2.1

	S3C4(a)	Rod shape , positive	1.875
	S3C3(a)	Rod shape , positive	2.8
Sample (4)(near sanichari market) confectionary shop	S4C1(a)	Rod shape , positive	1.888
	S4C1(b)	Rod shape , positive	2.2857
	S4C1(c)	Rod shape , positive	1.8
	S4C1(d)	Rod shape , positive	1.538
	S4C2(a)	Rod shape , positive	1.125
	S4C2(b)	Coccus , positive	1.42
	S4C2(c)	Coccus , positive	1.75
	S4C2(4)	Rod shape , positive	1.162
Sample (5) Rajendra nagar chawk (conf. shop)	S5C1(a)	Rod shape , positive	2.142
	S5C1(b)	Rod shape , positive	1.57
	S5C1(c)	Rod shape , positive	2
	S5C2(a)	Rod shape , positive	2.33
	S5 C2(b)	Rod shape , positive	2.5
Sample (6) Oil mill, Marwahi	S6C1 (a)	Rod shape , positive	2.06
	S6C1(b)	Rod shape , positive	1.25
	S6C2(a)	Rod shape , positive	3
	S6C2(b)	Rod shape , positive	2.35
	S6C3(a)	Rod shape , positive	1.75
	S6C4(a)	Rod shape , negative	1.75

A. Isolation and Screening of Lipolytic Bacteria

A critical perusal of table 1 reveals that lipase producing bacteria are distributed in all the samples tested. 06 different soil samples collected from different oil spilled soils of Bilaspur district showed high bacterial count. A total of 52 lipolytic bacterial isolates were identified from 06 different oil spilled soils of Bilaspur district. The lipolytic microbes were further screened and characterized by their features and biochemical reactions and then all were identified as Gram positive except isolates S6C4(a) which was Gram negative and all were rod shaped except isolates S4C2(a,b) ,which were cocci in shaped.

TABLE 2. Lipolytic potential of Ten best lipase producers

S.No.	lipase producers	Colony diameter in TBA	Zone of hydrolysis around lipolytic bacteria	Lipolytic potential
1.	S6 C2(a)	1.0	3.0	3cm.
2.	S3C3 (a)	0.5	1.4	2.8 cm.
3.	S1 C4 (a)	0.6	1.3	2.6 cm.
4.	S5C2(c)	0.6	1.5	2.5cm.
5.	S6C2(b)	1.7	4.0	2.35 cm.
6.	S5C2(a)	0.6	1.4	2.33cm.
7.	S3C2(b)	0.6	1.4	2.33cm.
8.	S4C1 (b)	0.7	1.6	2.28cm.
9.	S1C4(b)	0.5	1.3	2.16 cm.
10.	S5C1(a)	0.7	1.5	2.142 cm.

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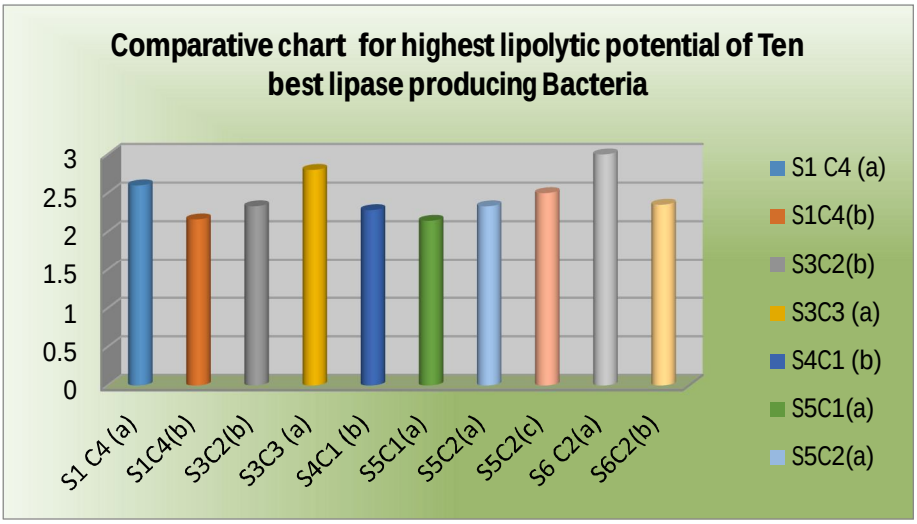


Figure 1:Lipolytic potential of Ten best lipase producers

The colony labeled as S6C2(a) showed maximum zone of clearance when plated on tributyrin agar base. Morphological and biochemical studies were done on S6C2(a) isolate. The maximum hydrolysis zone diameter at 24 hours of incubation was observed by S6C2(a) (3cm) at 24 hours incubation and least was exhibited by S2C9(a)(8mm) at 24 hours. The most widely employed primary screening method for the detection of lipolytic activity is the zone of hydrolysis in tributyrin agar medium (Limpon et al.,2006). The distribution of lipase producers in different soils of Warangal district was determined by Pallavi et al.,2015. Their investigations revealed that different soils under investigation supported lipase producers ranging from 5-48 x 10^6 CFU g^{-1} of soil. A total of 13 bacterial cultures were isolated by primary screening on tributyrin agar medium and selection of efficient strains for extracellular lipolytic activity was based on the lipolytic potential (R/r) of the isolates. They also reported that the highest hydrolytic zone was observed by LP8 (25mm) at 48 hrs. Screening of 969 microbial strains isolated from soil sample for lipolytic activity (Cardenass et al.,2001). Isolation of lipolytic microorganisms from palm oil mill wastes, garbage disposal sites and from normal soil samples (Razak et al.,1997). Results of Yan et al., (2013) revealed that 10 bacterial strains designated as X1, X2, X3, X4, X5, X6, X7, H, Y, Z. were indole-positive, whereas only strains Z and X1 were oxidase-positive and showed positive capsule staining, except for strain H. Zheng in 2018 isolated 55 samples from different regions and screened by Rhodamine B flat transparent circle method to observe lipase producing effect, among which, LHY-1, identified as *Serratia* sp. has the characteristics of fast growth, high enzyme production and stable ability. The colony of this strain is white, the edge is smooth and tidy, the surface is moist, the cell is straight, rod-shaped, gram negative, 0.1-0.2 μm in diameter and, length 0.3-0.5 μm in length. All these findings supported of our work.

IV. CONCLUSION

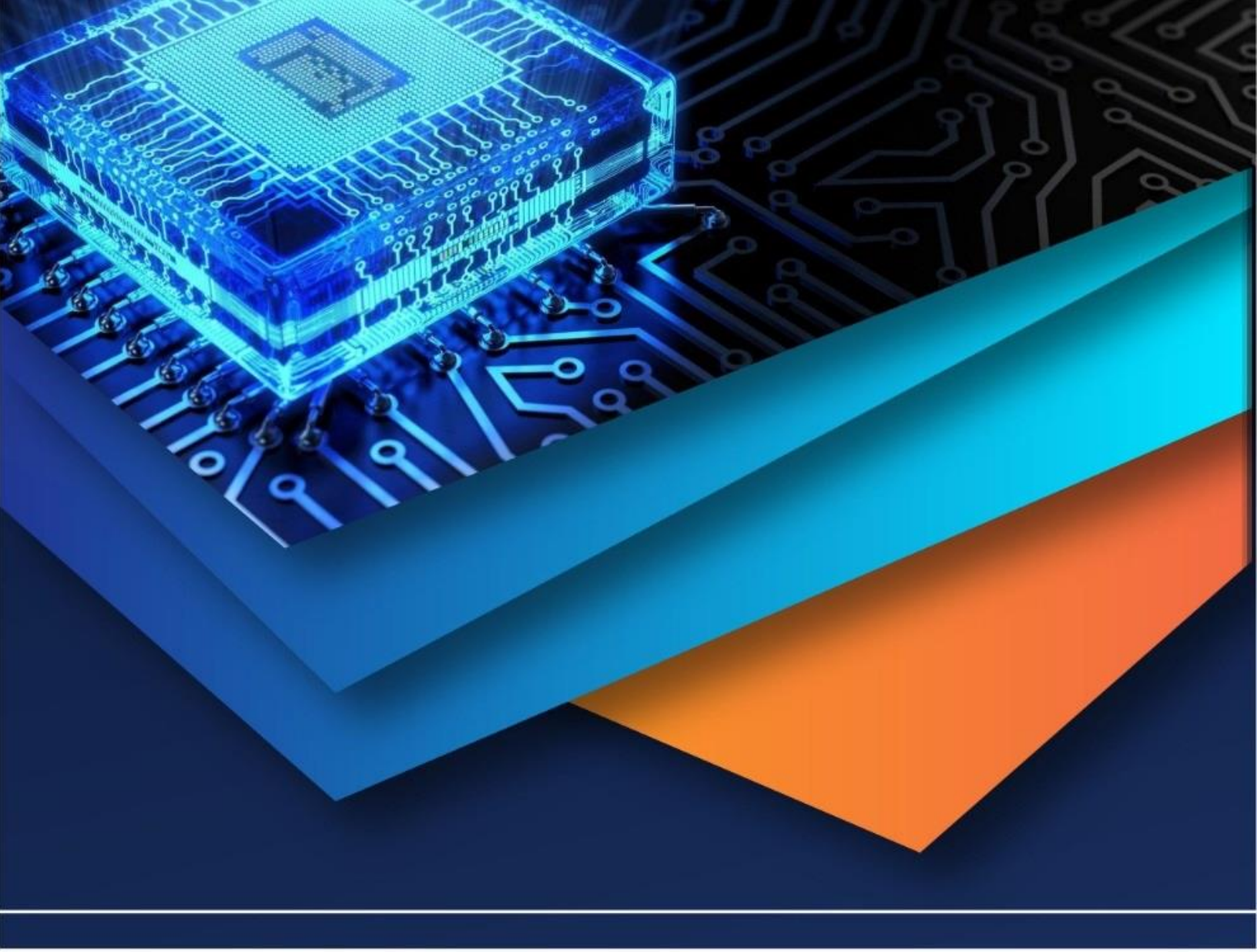
Lipases have been used for the degradation of wastewater contaminants such as olive oil from oil mills (Vitolo et al.,1998). Therefore, lipase producing isolates can be further screened for lipase production and could be employed directly as scavenger of oil contaminants in soil and waste water.

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