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Isolation and Characterization of Lipase Producing Bacteria from Windrow Compost

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A B S T R A C T

Lipolytic enzymes are currently attracting significant attention because of their biotechnological potential. Most of the lipases used in industry are microbial enzymes, of both fungal and bacterial origin. Windrow composting is a controlled, self-heating, aerobic degradation of organic materials by mesophilic and thermophilic microorganisms. The present study deals with isolation of lipase producing bacteria from windrow compost. The samples were collected from compost heap at an interval of 10 days and screened for lipase producing bacterial fauna in Tributyrin agar medium after serial dilution. A clear zone of hydrolysis indicated enzyme production. Out of 73 bacterial colonies screened, 24 lipase producing bacterial strains were isolated by checking the production of zone clearance in the tributyrin agar medium. Based on the morphological and biochemical characteristics 24 bacterial strains were identified up to species levels with the help of ABIS online analysis tool. The best lipase producers Staphylococcus (WCS_1C_2) and Pseudomonas (WCS_3C_2) genus were further characterized by 16s rDNA sequencing and identified as *Staphylococcus saprophytics and Pseudomonas otidis.* These bacterial strains can be used for the production of lipase enzyme for industrial applications.

Introduction

Lipases (EC3.1.3), known as triacylglycerol acyhydrolases, are capable of catalyzing hydrolysis of long chain triacylglycerides into free fatty acids and glycerols in aqueous solutions and conducting synthetic reaction in organic media(Masomian *et. al*., 2013). Most commercially useful lipases are of microbial origin. Due to commercial importance of extracellular lipases, many microorganisms have been studied for their lipase production ability (Maia *et. al*., 2001).

Lipase producing microorganisms have been found in diverse habitats such as industrial wastes, vegetable oil processing factories, soil contaminated with oil, oil seeds and decaying food, compost heaps ,coal tips and hot springs(Wang *et al.,*1995)

The major share of industrial enzyme market is occupied by hydrolytic enzymes, such as protease, amylase, esterase, and lipase. Among these hydrolytic enzymes like

lipases furnish the greatest share in the industrial enzyme market. In wake of recent advancements in microbiology and biotechnology, lipases have emerged as key enzymes owing to their multifaceted properties which find use in a wide array of industrial applications (Benjamin and Pandey, 1996; Jaeger *et.al*., 1994).

Lipase have been isolated and purified from fungi, yeast, bacteria, plant and animal sources but bacterial lipases are more economical and stable (Snellmanet *et. al*., 2002). Bacterial lipases are extensively used in food and dairy industry, cheese ripening, flavour enhancement (Falch, 1991), detergent industry (Fujii *et. al.,* 1986), textile industry (Sharma *et. al* 2001) , for synthesis of biodegradable polymers or compounds

(Linko *et. al.,* 1998),different transesterification reactions (Hasan, *et al.,* 2006), cosmetic industry (Seitz,1974) , in pulp and paper industry (Bajpai, 1999) , in synthesis of biodiesel (Noureddini *et. al.,* 2005), and in pharmaceutical industries (Higaki, and Morohashi 2003). Currently bacterial lipases are of great demand because of potential industrial applications (Sirisha *et al.,* 2010).

Considering the ever increasing demand for the better lipases in the industry for the search for ecofriendly and economical sources of lipase producing bacteria the present study has been carried out to isolate and characterize the novel lipase producing bacteria from windrow compost.

Materials and Methods

Preparation of Compost Bed

Windrow compost was done in solid waste management unit of Mercy college campus. The compost bed was prepared by using garden wastes and kitchen waste generated at Mercy college campus. Solid waste and cow dung inoculums in the ratio 10: 1is spread in alternative layers, forming a windrow. The height of the heap is kept up to 1.5 and the moisture content is maintained at 50-60% during the composting. The waste was turned in the different sections of the windrow unit at 5- day intervals for 60 days. The windrow was covered with High Density poly Ethylene sheet silpaulin to protect from scavengers and to maintain required temperature of 60-70˚c avoiding heat dissipation. The bed is regularly monitored and proper aeration and agitation is provided. (Jayasree and Ranjini, 2012)

Collection of the Compost Sample

Compost samples were collected at an interval of 10 days for about six turns. About 10g of soil samples were collected using a sterile spatula in a sterile beaker. Temperature and pH of the sample were recorded.

Screening of Lipolytic Bacterial Strains

Serial dilutions of the samples were made up to 10^{-7} and each dilution were pour plated using Tributyrin agar media and the plates were incubated at 37^0C for 24, 48 and 72 hours. The morphological characteristics of the colonies were observed and the colonies with zone of clearance were selected (Sirisha *et al.,* 2010). The diameter of the zone of clearance was noted at 24, 48, and 72 hours. The colonies with one of clearance were inoculated into nutrient broth and stored for further characterization.

Phenotypic Identification

Phenotypic identification of lipase producing bacteria was characterized via conventional morphological observation (Barrow and Feltham, 1993).

Biochemical Characterization

The isolated positive colonies were identified by biochemical analysis following the methods described in Bergey's manual of systemic bacteriology (Claus and Berkeley, 2011). Gram staining reaction, biochemical tests like catalase test, oxidase test ,indole production, methyl production test, vogues proskauer test, citrate utilization test, macconkey agar test, mannitol salt agar, motility test, H_2S Production test, urease test, potassium hydroxide test, acetate utilization test, lactose fermentation test, starch hydrolysis test, gelatin hydrolysis test and nitrate reduction tests were done as per the procedure described in Bergey's manual.

Lipolytic Activity Assay

Lypolytic activity assay was detected by screening zone of hydrolysis around colonies growing on a solid basal T BA medium (peptone 5g, beef extract 3g, tributyrin 10ml and agar 20g. The medium was prepared in distilled water and pH 7.2) All of the isolates were inoculated for lipolytic activity and were incubated at 37˚c for 24hrs. Lipolytic activity of the isolates was detected by appearance of an opaque zone around the colonies. Total halo diameter, minus the diameter of the colony was considered to be proportional to the lipolytic activity (Jaeger, 1994).

Identification of Isolated Bacterium

Lipolytic bacterial isolates were identified up to the species level by ABIS online software. The two highest lipase producers were further characterized by 16s rDNA sequencing. 16SrDNA fragment was amplified by PCR from bacterial genomic DNA using 16S rDNA universal primers: The primers used for the forward and backward reaction are :

8F: 5'-AGAGTTTGATCMTGG-3'

1492R: 5'-ACCTTGTTACGACTT-3'

PCR was carried out in a final reaction volume of 25 µl in 200 µl capacity thin wall PCR tube. PCR tubes containing the mixture were tapped gently and spin briefly at 10,000 rpm. The PCR tubes with all the components were transferred to thermal cycler. The PCR protocol designed for 30 cycles for the primers used for 16SrDNA amplification.

Results and Discussion

Out of 73 bacterial colonies screened, 24 bacterial strains were identified with the help of ABIS online software after submitting the results of various biochemical tests as lipase producers (Tembhurkar *et al.,* 2012). Several workers studied the qualitative lipolytic activity in bacterial isolates and determined it on tributyrin containing agar plates (Jaeger *et al.,* 1999 and Kim, *et al.,* 2002).

Screening of lipase producers on agar plates using tributyrin is usually favored as the substrate in plate assay technique has been reported (Brockerhoff and Jensen, 1974). In this study the isolates WCS_1C_2, WCS_3C_2, WCS_5C_1, WCS_5C_3, WCS_6C_4 and WCS_6C_3 showed significant lipolytic activity on tributyrin agar plates (Fig.1, 2,)similar studies of Patcha and Wiyada., 2013. The 24 isolates identified by morphological and biochemical characteristics were tabulated in (Table1,2)(Claus and Berkeley, 2011). *Pseudomonas* spp and *chromobacterium* spp have been well exploited for lipase production (Ghosh *et at.*, 1996). Among bacterial lipases, attention has usually been focused on particular classes of enzymes such as the lipases from the genus *Pseudomonas*, which are especially interesting for biotechnology (Gilbert, 1993).

Table.1 Morphological Characteristics of Dominant Bacteria in the Windrow Compost

Samples	Appearance	Margin	Elevation	Colour
WCS1-C1	Round,L-Form	Smooth	Convex	Cream
WCS1-C2	L-Form	Smooth	Convex	Cream
WCS1-C3	Round	Smooth	Convex	Cream
WCS2-C1	Round	Smooth	Convex	Cream
WCS2-C2	Raised	Wavy	Wavy,Convex	Cream
WCS2-C3	Irregular Spreading	Wavy,Smooth	Convex	Cream
WCS2-C4	L-Form	Smooth	Convex	Cream
WCS2-C5	Round	Smooth,Wavy	Transluscent,Wavy	Cream
WCS3-C1	L-Form	Smooth	Convex	Cream
WCS3-C2	Round,L-Form	Smooth,Wavy	Wavy,Convex	Cream
WCS3-C3	Round,Rhizoid	Smooth,Branching	Convex,Flat	Cream
WCS3-C4	Round	Smooth	Convex	Cream
WCS4-C1	Rhizoid	Branching	Flat	Cream
WCS4-C2	Round,L-Form	Smooth	Convex	Cream
WCS4-C3	Round	Smooth	Convex	Cream
WCS4-C4	L-Form	Smooth	Convex	Cream
WCS4-C5	Round,L-Form	Smooth,Wavy	Convex	Cream
WCS5-C1	L-Form	Smooth	Convex	Yellowish
WCS5-C2	Raised	Wavy	Wavy,Convex	Cream
WCS5-C3	Round	Smooth,Wavy	Convex,Wavy	Cream
WCS6-C1	Irregular Spreading	Smooth,Wavy	Convex	Cream
WCS6-C2	Round	Smooth	Convex	Cream
WCS6-C3	Round,L-Form	Smooth	Convex	Cream
WCS6-C4	L-Form	Smooth	Convex	Cream

Fig.1 TBA Plates with Highest Lipase Producing Bacterial Isolates

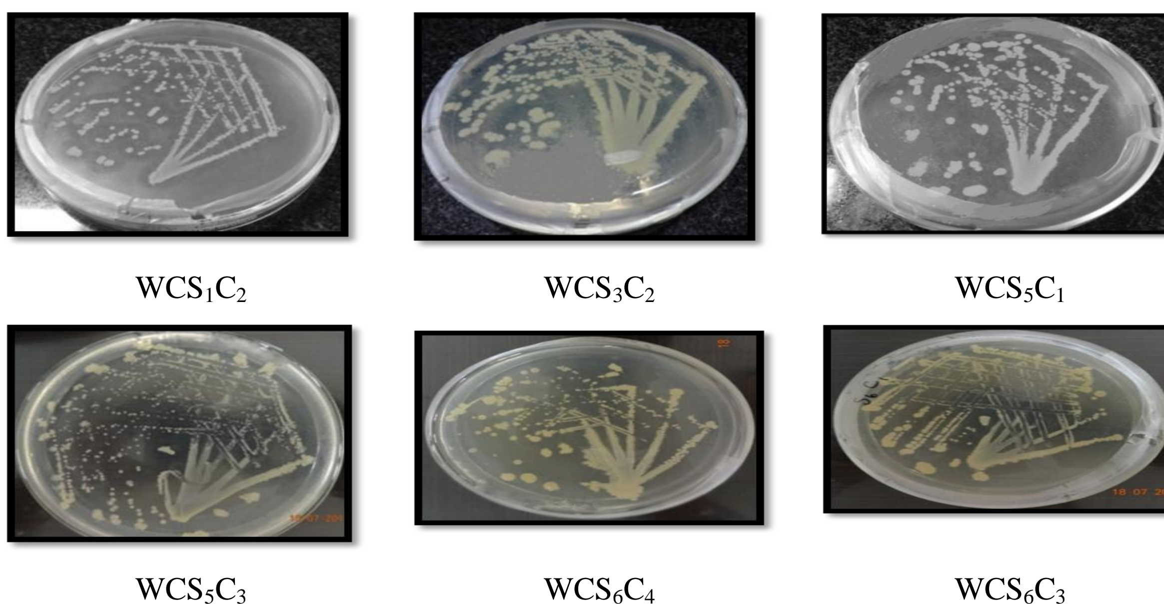
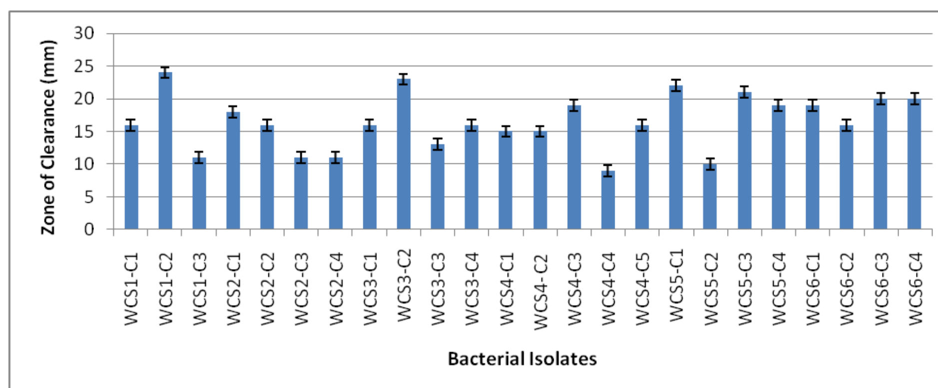


Table.2 Physiological and Biochemical Characteristics of Dominant Bacteria in the Windrow Compost

Windrow compost	GR	CT	OT	IP	MR	VP	CU	MC	ML	MT	HP	UT	K	AUT	LF	SH	GH	NRT	Probable organisms
WCS1-C1	-	+	-	-	-	-	-	-	-	-	-	+	-	-	-	-	+	+	***Pseudomonas aerogenosa***
WCS1-C2	+	+	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	+	***Staphylococcus saprophyticus***
WCS1-C3	+	+	+	-	-	+	-	-	-	+	-	+	-	-	-	-	-	+	***Streptococcus***
WCS2-C1	-	+	+	-	-	+	-	-	+	-	-	+	-	-	-	-	+	+	***Actinobacillus scotiae***
WCS2-C2	-	-	-	-	-	+	-	+	+	+	-	+	-	+	+	-	-	+	***Klebsiella pneumoniae***
WCS2-C3	-	+	-	-	-	+	-	-	-	-	-	+	-	-	-	-	+	-	***Bacillus niacini***
WCS2-C4	-	+	+	-	-	+	-	+	-	-	-	+	-	-	+	-	+	+	***Yersinia pseudotuberculosis***
WCS2-C5	+	+	-	-	-	-	-	+	-	+	-	-	-	-	+	+	+	+	***Bacillus siamensis***
WCS3-C1	+	+	-	-	+	-	-	+	+	+	+	-	-	-	+	-	+	+	***Pseudomonas otidis***
WCS3-C2	-	+	+	-	+	-	-	+	-	-	+	+	-	-	+	-	+	+	***Bacillus neizhouensis***
WCS3-C3	+	+	-	-	-	+	-	+	-	-	-	+	-	+	+	+	+	+	***Lysine bacillus massiliensis***
WCS3-C4	-	-	-	-	-	+	-	+	+	+	-	-	-	-	+	+	+	+	***Paenibacillus polymyxa***
WCS4-C1	+	-	-	-	+	+	-	+	+	-	-	-	-	-	+	-	-	+	***Buttiauxella ferragutiae***
WCS4-C2	+	-	-	-	-	+	-	+	-	-	-	-	-	-	+	-	-	+	***Obesumbasterum proteus***
WCS4-C3	+	+	-	-	-	-	-	+	-	+	-	-	-	-	+	-	+	+	***Bacillus horneckiae***
WCS4-C4	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	+	+	***Viridibacillus neidei***
WCS4-C5	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	***Bacillus acidiceler***
WCS5-C1	-	-	-	-	-	-	-	+	-	-	-	-	-	-	+	-	+	+	***Corynebacterium pseudodiphtheria***
WCS5-C2	+	+	+	-	+	-	-	-	-	-	-	-	-	-	-	-	+	+	***Corynebacterium matruchotii***
WCS5-C3	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	***Avibacterium gallinarum***
WCS6-C1	+	-	+	-	+	-	-	-	-	-	-	+	-	-	+	-	-	-	***Allivibrio fischeri***
WCS6-C2	-	-	+	-	-		-	+	+	-	-	-	-	-	-	+	+	-	***Bacillus vietnamensis***
WCS6-C3	-	+	+	-	+		-	-	-	-	-	-	-	-	-	-	+	-	***Aneurinibacillus aneurinilyticus***
WCS6-C4	-	+	+	-	+		-	-	-	-	-	+	-	-	-	-	+	+	***Lactobacillus ceti***

Fig.2 Lipase Producing Bacterial Isolates Showing Zone of Clearance



Lipases form an important group of enzymes in biotechnology with a wide range of applications in food, dairy, detergents, textile and some other industries, which are produced by microorganisms, and especially bacteria. Bacillus, Pseudomonas, and Burkholderia are the most important lipase producing bacteria (Gupta *et al.,* 2004). However, many researches are on going to introduce new bacterial sources of lipases, and use lipases as the biocatalysts in different industries (Cristian Ruiz *et al.,* 2004). Amongst, the lipases of B. subtilis and B. megaterium are small extracellular enzymes and classified to the subfamily 1.4 of bacterial lipases, based on amino acid sequence similarity (Travers,*et al.,*1987). The subfamily 1.4 lipases are mesophilic, have pH optima in a neutral to alkaline range (pH 7–11), and preferentially hydrolyze substrates with medium or short-chain fatty acids (Travers, *et al.,*1987). Staphylococcus is the genera shown the potential of lipase production. Staphylococcal lipases are classified as true lipases (Jaeger *et al.,* 1999; Rosenstein and Gotz, 2000; Simons *et al.,* 1996). In the present study, based on the zone of clearance *Staphylococcus* (WCS_1C_2) and *Pseudomonas* (WCS_3C_2) genus were found to be the highest lipase producers. The two highest lipase producing isolates were further characterized by 16SrDNA sequencing identification they are classified as *Staphylococcus saprophytics* and *Pseudomonas otidis.*

Molecular techniques utilizing amplification of target DNA provide alternative methods for diagnosis and identification (Veerapagu *et al.,* 2014). Khataminezhad M Reza *et al* 2014 identified *Bacillus megaterium* 37-1, *Bacillus safensis* 1-1, *Bacillus pumilus* KN-Lip2, *Bacillus subtilis* KN-Lip3,and *Lysinibacillus fusiformis* KN-Lip4 using 16S rDNAs sequencing.

The isolates WCS_1C_2 (*Stapylococcus saprophyticus*), WCS_3C_2(*Pseudomonas otidis*), WCS_5C_1 (*Corynebacterium xerosis*), WCS_5C_3 (*Corynibacterium matruchotii*) and WCS_6C_4(*Lactobacillus ceti*) were the best lipase producers . The two best lipase producers were further characterized by 16s rDNA technology and identified as *Staphylococcus saprophytics and Pseudomonas otidis.* These bacterial strains can be used for the production of lipase enzyme for industrial applications.

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