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Lipase Producing Potential of Different Bacteria Species Isolated from Cooking Oil Contaminated Soils

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***Abstract*—** *In this study, we investigated the lipolytic activity of different bacteria strains isolated from different cooking oil contaminated soils.This was with a view to finding the isolate with the highest lipase-producing potential.A total of 70 bacteria strainswere isolated from the soil samples and the morphological examination ofeach isolate was done and characterized. 20 bacterial isolates were screened for lipase activity using chromogenic composed medium and the lipase producing microorganisms produced clear zones on this media. The isolate showing maximum activity was identified as Pseudomonas sp using Berger's manual. The result showed that Pseudomonas sp. has the highest lipase activity with the largest diameter of 20.4 mm, 20.4 mm and 20.9 mm at 24, 48 and 72 hours of incubation while Escherichia coli, Klebsiella sp., Alcaligens sp., Citrobacter sp., Streptococcus sp., and Serratia sp. showed no lipase activity with 0.00 mm within the hours of incubation.The prevalence of lipase activity of each suspected bacterial isolate was investigated. Pseudomonas sp. has the highest prevalence of lipase activity with a value of 32.3% and the bacterial isolates with the lowest prevalence rate include Klebsiellasp, Escherichia coli, Serratia sp., Citrobacter sp., Streptococcus sp., and Alcaligens sp. with 4.2% each.. Staphylococcus sp. has a prevalence rate of 21.2%, Bacillus sp. with 6.1% prevalence rate while Bifidobacterium sp. and Corynbacterium sp. has 7.6% each.*

Keywords*— *Lipase, Isolate, Pseudomonas, cooking oil, contaminated soil.

I INTRODUCTION

Lipases are the enzymes that play a principal role in the hydrolysis and synthesis of esters formed from long-chain fatty acids and glycerol (Sharma *et al*., 2001; Svendsen*et al*., 2000). Several studies have reported the ability of some microorganisms to produce specializeenzyme (Itakorode*et al*., 2019; Oyedeji*et al*., 2013). Large quantities of useful metabolites are mostly obtained from microorganisms with extracellular lipases producing potential. About 98% of the world's microorganisms have not been explored as enzyme sources. Soil, a region where biology, geology and nature meet provides a home to animal, plants and microbial life. The soil is also a region on the earth's crust that teems with both microscopic and macroscopic life. The different kinds of microorganisms present in soil depend on many environmental factors such asthe number of nutrients available, the degree of aeration, pH, temperature and available moisture (Akpor*et al*., 2006). Soil bacteria and fungi play prominent roles in various biochemical cycles most especially in recycling of organic compounds. It also influencesecosystems by contributing to plant nutrition, plant health, soil structure and soil fertility (O'Donnell *et al*., 2001). Lipase (EC 3.1.1.3), an ester hydrolase catalyzes the hydrolysis of its substrate (triacylglycerol) to glycerol and fatty acids (Sharma *et al*., 2001). Lipases are ubiquitous and are produced by many microorganisms and higher eukaryotes (Kamimura*et al*., 2001; Elibol and Ozer, 2000). Several attempts have been made to isolate an organism with lipase producing potential since this enzyme is becoming popular in numerous biotechnological processes most especially wastes management industries(Sharma *et al*., 2001). The use of microorganism for lipase production have varieties of advantages over higher eukaryotes that are lipase producers. Microorganisms have avariety of enzymatic activities, high production within a short period of time and the ease with which they can be genetically manipulated. Microorganisms have been found to produce high yields of lipases compare to the animal and plants. This high yield might be because of their ease of production and genetic manipulation (Akoh*et al*., 2007). The oily environment (oil mill effluent) may provide a good

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environment for the isolation of lipase producing microorganisms. Bacterial lipases are mostly extracellular and are greatly influenced by nutritional and physicochemical factors, such as temperature, pH, nitrogen and carbon sources, inorganic salts, agitation and dissolved oxygen concentration (Gupta *et al*., 2004).This study, therefore, aims at isolating lipase producing bacteria from differentsoil samples that can be of use in the treatment of oil-contaminated soil.

II MATERIAL AND METHOD

Study Area

The study was carried out on different oil contaminated soil samples collected from different locations in Ile-Ife, Ipetumodu, Moro, and Asipa towns, Osun state.Ile-Ife is an ancient Yoruba city in south-western Nigeria. Ile-Ife city lies on the geographical coordinates of Latitude $7^0$28ˈN and Longitude $4^0$34ˈE.Moro, Ipetumodu and Asipa are towns located in Osun state, in the southwestern part of Nigeria with geographical coordinates of latitude $7^0$22'N and longitudes $4^0$30'E. Ipetumodu is also a university town of Oduduwa University. It shares a common boundary with Ile-Ife, the spiritual headquarters of the Yoruba Kingdom.

Sample Collection

Oil spilled soil samples were collected from different sites located at Ile-Ife, Ipetumodu, Moro and Asipa by adapting the aseptic technique from a depth of 5-10 cm. The samples were collected aseptically in sterilized polyethylene bags, transferred to the laboratory immediately and processed for microbiological analysis within one hour of collection.

Media

The media used included: Nutrient Agar (NA), Buffered Peptone water, Simmons Citrate Agar (SIM), Agar, Methyl-red-VogesProskuer (MR/VP) broth

Preparation of Culture Media

The medium used was prepared according to the manufacturer's specification (28g of Nutrient agar was measured into sterile conical flasks; 1000 ml of distilled water was dispensed into the conical flasks). The conical flasks were shaken very well to dissolve the medium, and then boiled on the hot-plate to homogenize. 9 ml of distilled water was dispensed into different sterile test tubes using a syringe, the test tubes were covered with cotton wool, aluminum foil and paper tape. The medium and the test tubes were sterilized in the autoclave at 121^0C for 15minutes.

Isolation of Bacteria

1g of each sample was dispensed into their respective test tubes containing 9 ml of the sterile water each, the test tubes were shaken very well to dissolve so as to prevent tiny particles from settling at the bottom. The samples were then serially diluted up to 10^{-3} (Olutiola*et al*., 2000). A volume of 1 ml of each diluent was dispensed into labelled Petri dishes and the sterilized medium was dispensed into the Petri dishes and left to solidify. The Nutrient agar plates were incubated together with one sterile Nutrient agar plate to serve as control at 37^0C for 18-24 hours to check for growth of bacteria. After 24hrs, the growth of bacteria appeared in the form of colonies and the numbers of colonies was counted using the colony counter.4 distinct colonies from each growth plate were sub-cultured on another sterile Nutrient agar plates each and incubated for 24 hours at 37^0C.

Characterization of Bacterial Isolates

The pure bacterial isolates were further identified by microscopic and several biochemical examinations. Bergey's Manual of Determinative Bacteriological (Holt *et al*., 1994) was used as a reference for the identification based on the result of various biochemical tests.

Detection of lipase activity

A chromogenic substrate medium composed of0.5 g Congo red, 10 g Peptone, 5 g Sodium chloride (NaCl), 0.1g Calcium Chloride ($CaCl_2$), 6.36 g Agar and 1 ml immersion oil in 1 litre of distilled water. The composition was dispensed into a beaker, boiled on hot-plate to homogenized and dispensed into two sterile conical flasks and autoclaved at 121^0C for 15 minutes. The sterile medium was dispensed into Petri dishes and left to solidify. The organisms were inoculated on the sterile medium plate and incubated at 37^0C for 24, 48 and 72hours. This was carried out using the modified method of Gupta *et al*. (2004). The clearance zones of inhibition and diameters of colonies were measured after 24, 48 and 72hours of incubation.

III RESULTS

A total of 11 soil samples contaminated with cooking oil were collected from different locations in Ile-Ife, Moro, Ipetumodu and Asipa. The soil samples were processed and serially diluted and pour plated using Nutrient agar medium, the growth colonies on each plate were counted and recorded as shown in Table 1. The result shows that sample A has the lowest bacterial count with 4.80×10^2 CFU/g while sample H has the highest bacterial count with 6.40×10^4 CFU/g. The bacteria strains isolated are presented in Table 2.The frequency distribution of the suspected bacteria strains as shown in Table 3 shows that *Staphylococcus sp*. has the highest occurrence in the soil sample collected while *Escherichia coli*, *Alcaligens sp*., *Citrobacter sp*., and *Serratia sp*. has the lowest occurrence. Out of the 70

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isolates, 20 bacterial isolates were screened for lipase activity by culturing them on a chromogenic composed medium. Table 4 shows the zone of inhibitions for the suspected bacterial isolates in diameter after 24, 48 and 72 hours of incubation; 12 bacterial isolates showed lipase activity while 8 bacterial isolates showed no lipase activity within the hours of incubation. The result shows that *Pseudomonas sp*. has the highest lipase activity with the largest diameter of 20.4 mm, 20.4 mm and 20.9 mm at 24, 48 and 72 hours of incubation while *Escherichia coli, Klebsiella sp., Alcaligens sp., Citrobacter sp., Streptococcus sp., and Serratia sp*. showed no lipase activity with 0.00 mm within the hours of incubation. This shows that lipase-producing bacteria have been isolated from the soil samples collected.

Figure 1 shows the prevalence of lipase activity of each suspected bacterial isolate. This graph shows that *Pseudomonas sp*. has the highest prevalence of lipase activity with a value of 32.3% and the suspected bacterial isolates with the lowest prevalence rate include *Klebsiella sp., Escherichia coli, Serratia sp., Citrobacter sp., Streptococcus sp*., and *Alcaligens sp*. with 4.2% each.. *Staphylococcus sp*. has a prevalence rate of 21.2%, *Bacillus sp*. with 6.1% prevalence rate while*Bifidobacterium sp*. and *Corynbacterium sp*. has 7.6% each.

Table.1: Total Number of Heterotrophic Bacterial Count in Cooking Oil Contaminated Soil Samples

SAMPLE CODE	TOTAL HETEROTROPHIC BACTERIAL COUNT CFU/g
A	4.80×10^2
B	1.42×10^4
C	1.76×10^4
D	1.96×10^4
E	1.80×10^4
F	4.95×10^4
G	5.90×10^4
H	6.40×10^4
I	2.34×10^3
J	2.63×10^4
K	2.10×10^3

CFU/ml (Colony Forming Unit per gram)

Table.2: The biochemical characteristics and probable identification of bacteria isolated from soil samples contaminated with cooking oil

Isolate code	Cat	Coa	I	Cit	Oxi	MR	VP	Probable Identity
A01	NA	NA	_	NA	_	_	+	*Serratia sp.*
A02	+	+	NA	NA	_	NA	NA	*Staphylococcus aureus*
A06	NA	NA	+	_	_	+	_	*Escherichia coli*
A13	+	_	NA	NA	_	NA	NA	*Staphylococcus sp.*
A14	+	NA	_	+	_	_	+	*Bacillus sp.*
A15	+	NA	_	+	_	_	+	*Bacillus sp.*
B01	+	NA	_	+	_	_	+	*Bacillus sp.*
B02	+	NA	_	+	+	_	+	*Bacillus sp.*
BO3	+	NA	_	+	+	_	+	*Bacillus sp.*
B04	+	+	NA	NA	_	NA	NA	*Staphylococcus aureus*
B12	+	+	NA	NA	_	NA	NA	*Staphylococcus aureus*
B13	NA	NA	_	+	+	_	_	*Pseudomonas sp.*
B14	NA	NA	_	+	+	_	_	*Pseudomonas sp.*
C01	_	_	NA	NA	_	NA	NA	*Streptococcus sp.*
C02	_	_	NA	NA	_	NA	NA	*Streptococcus sp.*
C03	_	_	NA	NA	_	NA	NA	*Streptococcus sp.*
C04	+	_	NA	NA	_	NA	NA	*Staphylococcus sp.*
C05	NA	NA	_	+	+	_	_	*Pseudomonas sp.*
C06	+	NA	_	+	_	_	+	*Bacillus sp.*
C07	+	_	NA	NA	_	NA	NA	*Staphylococcus sp.*
C08	NA	NA	_	+	_	_	+	*Klebsiella sp.*
C12	+	_	NA	NA	_	NA	NA	*Staphylococcus sp.*
D06	+	NA	_	+	_	_	+	*Bacillus sp.*
E01	_	NA	NA	+	NA	NA	NA	*Bifidobacterium sp.*

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E05	+	+	NA	NA	_	NA	NA	*Staphylococcus sp.*
E07	NA	NA	_	+	+	+	_	*Citrobacter sp.*
F01	_	NA	NA	+	NA	NA	NA	*Bifidobacterium sp.*
F03	+	NA	_	+	_	_	+	*Bacillus sp.*
F04	+	NA	_	+	_	_	+	*Bacillus sp.*
F05	_	NA	NA	+	NA	NA	NA	*Bifidobacterium sp.*
F08	+	NA	_	+	_	_	+	*Bacillus sp.*
F12	+	NA	_	+	_	_	+	*Bacillus sp.*
F13	+	_	NA	NA	_	NA	NA	*Staphylococcus sp.*
F14	+	_	NA	NA	_	NA	NA	*Staphylococcus sp.*
F15	+	_	NA	NA	_	NA	NA	*Staphylococcus sp.*
G01	NA	NA	_	+	+	_	_	*Pseudomonas sp.*
G02	NA	NA	_	+	+	_	_	*Pseudomonas sp.*
G03	+	_	NA	NA	_	NA	NA	*Staphylococcus sp.*
G04	+	_	NA	NA	_	NA	NA	*Staphylococcus* sp.
G12	NA	NA	_	+	_	_	+	*Klebsiella sp.*
G13	NA	NA	_	+	_	_	_	*Pseudomonas sp.*
G14	+	_	NA	NA	_	NA	NA	*Staphylococcus sp.*
G15	+	+	NA	NA	_	NA	NA	*Staphylococcus aureus*
H02	+	+	NA	NA	_	NA	NA	*Staphylococcus aureus*
H03	+	_	NA	NA	_	NA	NA	*Staphylococcus sp.*
H04	NA		_	+	+	_	_	*Pseudomonas sp.*
H05	+	_	NA	NA	_	NA	NA	*Staphylococcus sp.*
H06	+	+	NA	NA	_	NA	NA	*Staphylococcus aureus*
H13	+	_	NA	NA	_	NA	NA	*Staphylococcus sp.*
H14	+	_	NA	NA	_	NA	NA	*Staphylococcus sp.*
H15	+	_	NA	NA	_	NA	NA	*Staphylococcus sp.*
I01	+	+	NA	NA	_	NA	NA	*Staphylococcus aureus*
I02	+	+	NA	NA	_	NA	NA	*Staphylococcus aureus*
I03	+	NA	_	+		_	+	*Bacillus sp.*
I04	+	+	NA	NA	_	NA	NA	*Staphylococcus aureus*
I05	+	NA	_	+		_	+	*Bacillus sp.*
I06	+	_	NA	NA	_	NA	NA	*Staphylococcus sp.*
I08	+	NA	NA	_	NA	NA	NA	*Corynebacterium sp.*
J01	+	+	NA	NA	_	NA	NA	*Staphylococcus aureus*
J02	+	_	NA	NA	_	NA	NA	*Staphylococcus sp.*
J05	+	_	NA	NA	_	NA	NA	*Staphylococcus sp.*
J06	+	+	NA	NA	_	NA	NA	*Staphylococcus sp.*
J07	NA	NA	_	_	+	_	_	*Alcaligens sp.*
J08	+	NA	NA	_	NA	NA	NA	*Corynebacterium sp.*
J12	+	NA	_	+	+	_	+	*Bacillus sp.*
J13	NA	NA	_	+	+	_	_	*Pseudomonas sp.*
J15	+	NA	_	+	+	_	+	*Bacillus sp.*
K01	NA	NA	_	+	+	_	_	*Pseudomonas sp.*
K02	NA	NA	_	+	+	_	_	*Pseudomonas sp.*
K13	NA	NA	_	+	+	_	_	*Pseudomonas sp.*

Keywords: + (Positive), _ (Negative), NA (Not Applicable), MR (Methy-red test), VP (VogesProskauer test), Cat (Catalase test), Coa (Coagualse test), I (Indole test), Oxi (Oxidase test), Cit (Citrate test)

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Table.3: Frequency distribution of the suspected bacteria isolates from soil samples contaminated with cooking oil

Suspected Bacterial Isolate	Frequency	Percentages
Serratia sp.	1	1.4%
Escherichia coli	1	1.4%
Bacillus sp.	15	21.4%
Pseudomonas sp.	11	15.7%
Staphylococcus sp.	30	42.9%
Bifidobacterium sp.	3	4.3%
Klebsiella sp.	2	2.9%
Corynebacterium sp.	2	2.9%
Streptococcus sp.	3	4.3%
Citrobacter sp.	1	1.4%
Alcaligens sp.	1	1.4%
Total	**70**	**100**

Table.4: Screening of the bacterial strains for lipase production on solid agar

		Lipase activity in diameters(mm)		
		Incubation time (hours)		
Isolates code	**Probable Identity**	**24**	**48**	**72**
A01	*Serratia sp.*	0.00	0.00	0.00
A06	*Escherichia coli*	0.00	0.00	0.00
B01	*Bacillus sp.*	0.00	10.2	20.0
B13	*Pseudomonas sp.*	20.1	20.1	20.1
B14	*Pseudomonas sp.*	20.3	20.4	20.9
C05	*Pseudomonas sp.*	10.8	20.1	20.1
E01	*Bifidobacterium sp.*	0.00	0.00	0.00
F01	*Bifidobacterium sp.*	0.00	10.9	20.5
F13	*Staphylococcus sp.*	0.00	20.1	20.1
G12	*Klebsiella sp.*	0.00	0.00	0.00
H02	*Staphylococcus aureus*	0.00	0.00	0.00
H04	*Pseudomonas sp.*	10.6	10.8	20.3
I04	*Staphylococcus aureus*	0.00	10.7	20.5
I08	*Corynebacterium sp.*	0.00	10.9	20.5
J05	*Staphylococcus sp.*	10.8	10.8	20.0
J07	*Alcaligens sp.*	0.00	0.00	0.00
J13	*Pseudomonas sp.*	0.00	10.5	10.9
K01	*Pseudomonas sp.*	20.2	20.2	20.2
E07	*Citrobacter sp.*	0.00	0.00	0.00
C03	*Streptococcus sp.*	0.00	0.00	0.00

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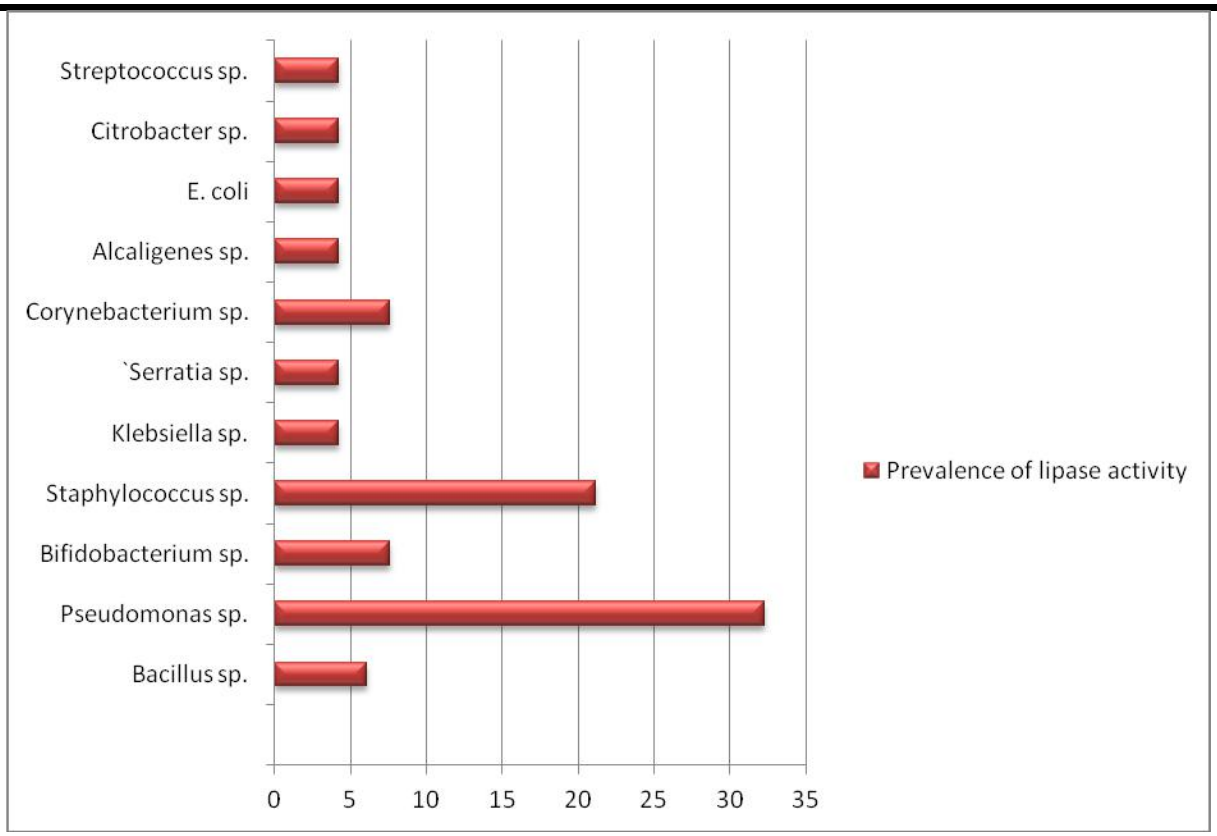

Fig.1: Prevalence of the lipase activity of suspected bacteria isolates in percentage

IV

DISCUSSION

Lipases are serine hydrolases that catalyze both the hydrolysis and synthesis of long-chain triacylglycerol. Extracellular lipases are produced by microorganisms, fungi and bacteria and the use of bacteria lipases in a variety of biotechnological applications is becoming popular. Pseudomonas lipases have proved to be the most important ones which have a significant potential in detergent industry and organic chemistry.Bacteria isolated from the environmental soil samples contaminated with cooking oil were screened for their lipase producing ability on solid agar. A total of 11 bacteria genus (*Pseudomonas sp., Bacillus sp., Corynebcaterium sp., Alcaligens sp., Staphylococcus sp., Streptococcus sp., Bifidobacterium sp., Klebsiella sp., Citrobacter sp., Serratia sp. and Escherichia coli*) were isolated from different soil samples contaminated with cooking oil collected from different locations in Ile-Ife, Asipa, Moro and Ipetumodu towns. The total heterotrophic bacteria count per gram of each soil sample ranged from 6.40×10^4 CFU/g of sample H and 4.80×10^2 CFU/g of sample A. Table 3 shows the percentage frequency of occurrence of the different bacteria isolated from the samples. *Staphylococcus sp.* had the highest frequency of occurrence (42.9%), followed in order by *Bacillus sp.* (21.4%), *Pseudomonas sp.* (15.7%), *Streptococcus sp.* (4.3%) and *Bifidobacterium sp.* (4.3%), *Klebsiella sp.* (2.9%) and *Corynebacterium sp.* (2.9%), *Escherichia coli, Serratia sp., Alcaligens sp., Citrobactersp* (1.4%). Similar results have been reported by Riaz*et al.* (2010) and Pandey *et al.* (1999). The screening of bacterial isolates for lipase production on solid agar is shown in Table 4. The lipolytic activity ranged within 10.6-20.3 mm, 10.2-20.4 mm, and 10.9-20.9 mm at 24, 48 and 72 hours of incubation respectively. *Pseudomonas sp.* (B14) had the highest activity at different hours of incubation followed by *Staphylococcus sp.*The rest of the isolate showed little lipolytic activity (Figure 1).Sirisha,*et al.* 2010 also reported the lipase producing potential of *Staphylococcus sp.*

V. CONCLUSION

Lipase producing microbes have been found in diverse habitats such as industrial wastes, vegetables, oil contaminated soil, oilseeds, and decaying food. Lipases are important enzymes in the chemical industry and the production of wine chemicals.In this study, lipase producing bacteria were isolated from the soil contaminated with

cooking oil and the bacteria were identified as *Pseudomonas sp.*,*Staphylococcus sp., Bacillus sp.*, *Bifidobacterium sp.*, and *Corynebacterium sp*. The high lipase producing potential of *Pseudomonas sp*. suggests that the organism might be of use in waste management processes and in chemical industries.

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