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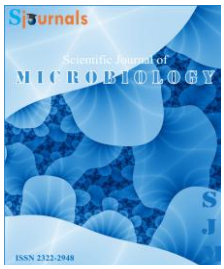

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Original article

Microbial diversity of oil spills and tar resistant bacteria isolated from beaches of Goa (India)

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ABSTRACT

Oil contaminated samples collected from seven beaches of Goa (India) were analyzed for their microbial components. The alkaline pH, the presence of oil spill and tarball residue and high salt concentration were features common of oil contaminated Goan beaches. The optimum temperature for isolating oil resistant bacteria was 23 oC. Laboratory investigation revealed the presence of diverse microbial population. Oil contaminated soil samples were found to be dominated by motile, spore-forming halophilic bacteria. Total 158 bacteria were isolated in the presence and absence of 1% phenanthrene. Of 158 isolates, 18 bacteria showing diverse characteristics were selected for further investigation. Out of 18 isolates, 16 were gram positive rods. All isolated species revealed oil resistance and salt tolerance properties.

1. Introduction

Microorganisms are the ubiquitous and important component of all ecosystems. They have inherent ability to grow and thrive under multi-stress environment, microscopic size, easy dissemination, and multiply under toxic, oxic or anoxic conditions. Diverse microorganism exhibits these characteristics because of their versatile metabolic pathways assist in using a broad range of nutrients, ability to tolerate, grow and colonize under unfavorable

conditions i.e. high or low temperature and pH, fluctuating redox potentials, high salinity, low humidity, high ion concentration and radiation etc. These extreme conditions exist on Mother Earth and manmade activities were created it. These includes hot spring, compost piles, oil spills and tar pollution, hydrocarbon pollution etc. Microorganisms thriving under these conditions called as extremophiles. Among these, oil spill incidences and tar pollution occurred mainly due to manmade activities. Clean beaches and coastal line represent the health of the surrounding area and ecosystem (Margesin and Schinner, 2001a; 2001b; Sukhdhane et al., 2013). In present research soil samples, from seven Goan beaches invaded by artificial oil spill incidence, were collected and analyzed for their oil resistance microbial components.

2. Materials and methods

2.1. Sampling site

Oil contaminated coastline of Goa was selected. Soil samples from seven Goan beaches such as Arambol, Candolim, Calangute, Colva, Dona Paula, Miramar, and Singuerim have collected studies on the presence of oil resistant microbial components. The composite sampling method was adopted from the collection of soil samples (Fig. 1-2).

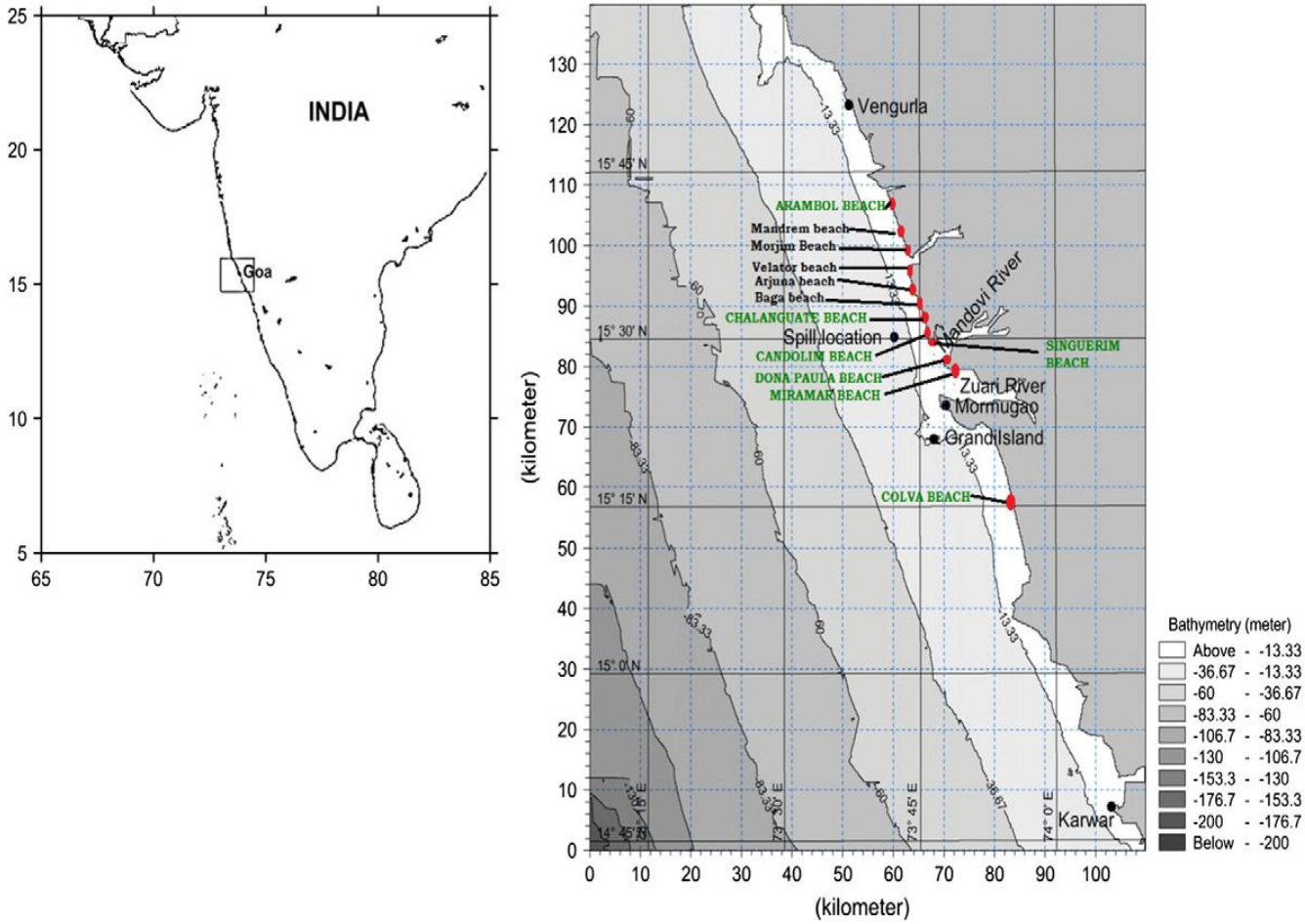

Fig. 1. Sampling site: Goa Coastline (Rekadwad and Khobragade, 2015).

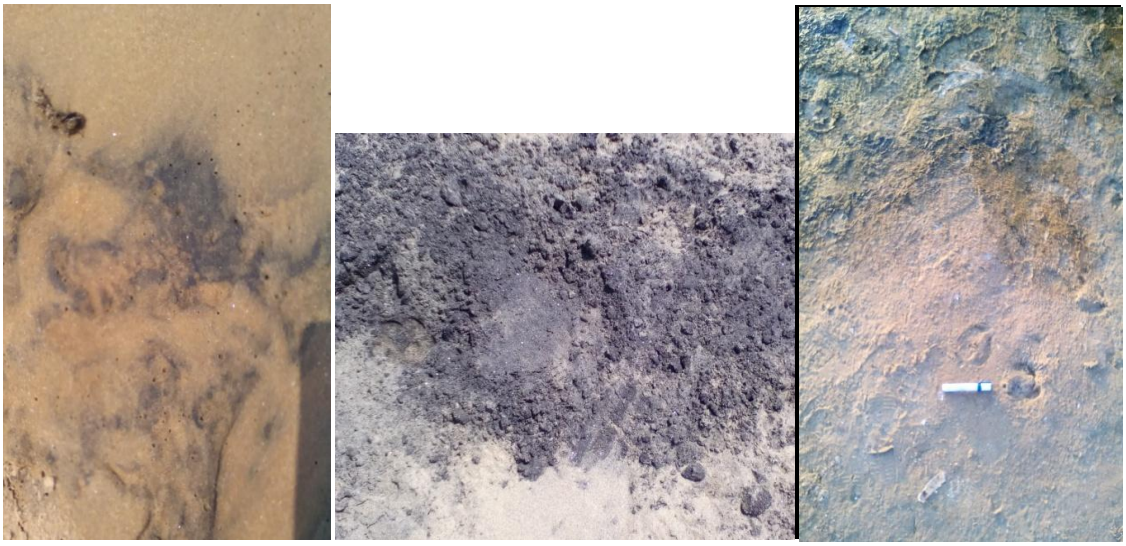

Fig. 2. Oil pollution on Arambol beach, Goa.

2.2. Collection of samples and microbial analysis

Soil/sand samples were collected in radiation sterilized polypropylene bags. All samples were analyzed within 18 hours for microbial components using standard methods.

2.3. Enumeration of microorganisms

Enumeration of viable soil/sand microbial component i.e. isolation were carried out on selective media such as R2A medium, Mannitol salt agar, and Blood agar plates spread with and without 1% phenanthrene. 1g soil/sand sample was suspended in 50 mL of sterile distilled water and make up to 100 mL. This soil suspended water was kept on shaking incubator for 1 hour and filtered. Aseptically 0.1 mL of soil suspension was spread on phenanthrene pretreated agar and kept for 48 24 h incubation at 23 ^{o}C. After incubation isolated colonies showing based on feature were selected for further study and obtained in pure form. Selected strains were biochemically characterized for the presence of desired characteristics such as salt tolerance, carbohydrate utilization and antibiotic resistance (Meyer et al., 1999). Microorganisms showing diverse morphological and biochemical characteristics were selected and stored under low temperature in the refrigerator for further investigation.

3. Results and discussion

Of seven oil spill and tar contaminated samples, five samples showing the possibility of the oil resistant bacteria were selected for further investigation i.e. Arambol beach - onshore and offshore samples, Calangute salt marsh, Calangute beach and Candolim mangrove soil samples.

All the samples were analyzed for the presence of colony forming units per 100g of the sample in presence and absence of 1% standard hydrocarbon phenanthrene. It was observed that 1% phenanthrene containing R2A and Blood agar medium supported the diversity of bacteria (Table 1). Similarly, in the absence of 1% phenanthrene, CFU was lowered. These results confirm the oil spills and tar pollution incidence occurred on Goan beaches.

Total 158 bacteria were isolated in presence and absence phenanthrene. Of 158 isolates, 18 bacteria showing diverse morphological and biochemical characteristics were selected for further investigation. Out of 18 isolates, 16 were Gram-positive and two were Gram negative motile rods. All species showed luxuriant growth at optimum 22 ^{o}C temperature, optimum pH 7.8 and at 20% salt concentration. All isolates utilized dextrose, fructose, and sucrose as a carbon source and produced acid in the medium. Of 18 isolates, six isolates utilized rhamnose, two utilized inositol, and inulin and produced acid in the medium. Of 18 strains, 16 strains showed resistance towards Ceftazidine 30µg/disc) and seven strains showed resistance against Piperallicin 100µg/disc (Fig. 3-4). All strain

isolated showed growth in the presence of 1% phenanthrene and showed positive catalase test. These obtained results revealed oil resistance and salt tolerance properties isolated species (Table 3).

Fig. 3. Dextrose utilization test.

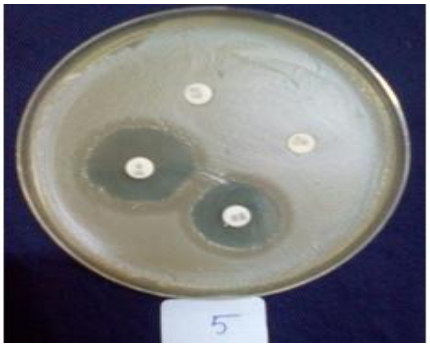

Fig. 4. Antibiotic resistant test- strain 5.

Table 1
Microbial populations in soil/sand samples from oil and tar polluted Goa coastline- Microbial populations in the presence of 1% phenanthrene (CFU/100g).

Media	Sampling sites				
	Arambol beach - onshore	**Arambol beach - offshore**	**Calanguate salt marsh**	**Calanguate beach**	**Candolim mangroove**
R2A medium	$21x10^3$	$68x10^3$	$302x10^3$	$9x10^3$	$15x10^3$
Mannitol salt agar	0	0	0	0	0
Blood agar	$289x10^3$	$30x10^3$	$184x10^3$	$2x10^3$	$11x10^3$

Table 2
Microbial populations in soil/sand samples from oil and tar polluted Goa coastline- Microbial populations in the absence of 1% phenanthrene (CFU/100g).

Media	Sampling sites				
	Arambol beach - onshore	**Arambol beach - offshore**	**Calanguate salt marsh**	**Calanguate beach**	**Candolim mangroove**
R2A medium	$42x10^3$	$74x10^3$	$242x10^3$	0	$6x10^3$
Mannitol salt agar	$7x10^3$	$22x10^3$	$122x10^3$	0	0
Blood agar	$59x10^3$	$34x10^3$	$168x10^3$	$0x10^3$	$0x10^3$

Table 3
Morphological and biochemical feature of microbial populations isolated from oil and tar polluted Goa coastline.

Strain	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**17**	**18**
Cell shape	LR	LR	Rd	Rd	Rd	Rd	Rd	Rd	LR	LR	TSR	SR	Rd	Rd	Rd	LR	Rd	Rd
Gram staining	G+	G+	G+	G+	G+	G+	G+	G-	G+	G+	G+	G+	G+	G+	G+	G+	-	G+
Cell arrangement	C	C	C	Vlp	C	C	C	Spt	C	C	C	C	Spt	Spt	C	C	Vlc	C
Endospore	T	T	T	A	T	T	T	T	T	Ctl	T	T	T	T	Ctl	T	T	T
Motility	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M
Color of colony on BHI	W	W	W	W	FB	FB	W	W	W	Bl	Bl	W	W	T	Y	Bl	Y	W
Color of colony on ZMA	W	W	W	W	DW	DW	DW	FY	CW	CW	DW	CW	W	LO	CW	W	CW	Y
Size of colony (mm)	2	1	2	3	3	2	2	4	3	4	4	3	2	3	3	4	4	4
Shape of colony	Cr	Cr	Cr	Cr	Cr	Cr	Cr	Rnd	Rnd	Rnd	Rhi	Rnd	Ir	Cr	Rnd	Ir	Rnd	Cr
Margin of colony	Sr	E	E	Ir	Ir	E	E	Sr	Sr	Ir	Sr	Sr	Ir	E	Sr	E	Sr	E
Elevation of colony	U	El	Fl	El	El	El	El	Cx	Cx	Fl	Sle	U	Fl	El	U	Fl	U	Cv
Opacity	O	O	O	O	O	O	O	O	O	O	O	O	Tp	O	O	O	O	O
Consistency	NS	St	St	St	St	St	St	St	NS	NS	NS	St	NS	St	NS	NS	NS	NS
Hard/Smooth	Sm	Sm	Hd	Sm	Sm	Sm	Sm	Sm	Sm	Sm	Hd	Sm	Sm	Sm	Sm	Sm	Sm	Sm
Growth in Broth Medium	Uf	Uf	Sd	Sd	Uf	Uf	Uf	Sd	Sd	Uf + Sd	Sd	Sd	Sd	Sd	Sd	Uf	Sd	Sd
Tolerance of NaCl (%)	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20
Optimum temp.(^{o}C)	22	22	22	22	22	22	22	22	22	22	22	22	22	22	22	22	22	22
Optimum pH	7.8	7.8	7.8	7.8	7.8	7.8	7.8	7.8	7.8	7.8	7.8	7.8	7.8	7.8	7.8	7.8	7.8	7.8
Carbohydrate utilization after 96 h																		
Dextrose	(+)	(+)	(+)	(+)	(+)	(+)	(+)	(+)	(+)	(+)	(+)	(+)	(+)	(+)	(+)	(+)	+	(+)
Fructose	(+)	(+)	(+)	(+)	(+)	(+)	(+)	(+)	(+)	(+)	(+)	(+)	(+)	(+)	(+)	(+)	(+)	(+)
Sucrose	(+)	(+)	(+)	(+)	+	+	(+)	(+)	(+)	(+)	+	(+)	(+)	(+)	(+)	+	(+)	(+)
Inositol	-	-	-	-	-	-	-	-	-	-	-	-	+	+	-	-	-	-
Inulin	-	-	-	-	-	-	-	-	-	-	-	-	+	+	-	-	-	-
Rhamnose	-	-	-	-	+	+	+	-	-	+	+	+	-	-	-	-	-	-
Catalase	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

Antibiotic test (µg per disc)																		
Ceftazidine (30)	0.275	0.225	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R
Amikacin (30)	3.374	1.45	1.45	0.95	0.7	0.725	0.6	1.05	1.25	0.625	0.75	1.4	1.175	1.075	1.125	0.65	1.075	4
Piperallicin (100)	0.4	0.875	0.825	0.325	R	R	R	0.275	0.45	R	R	0.05	0.2	0.375	0.35	R	R	0.375
Gentamycin (120)	1.6	1.95	1.95	1.125	0.9	0.825	0.975	1.5	1.6	4	0.95	1.55	1.45	1.675	1.3	1.075	1.6	1.05

Keywords: LR = Long rod; Rd = Rod; TSR = Thick short rod; SR = Short rod; G+ = Gram positive; G- = Gram negative; C = Chain; Vlp = Very long spiral; Spt = Separate; Vlc = Very long chain; T = Terminal; A = Absent; Ctl = Central; M = Motile, NM = Nonmotile; W = White; Y = Yellowish; FB = Faint blue; Bl = Bluish; CW = Creamy white; FY= Faint yellow; DW = Dirty white; mm = Millimeter; Sr = Serrate; Cr = Circular; E = Entire; Ir = Irregular; U = Umbonate; El = Elevated; Fl = Flat; O = Opaque; Tp = Transparent; NS = Non-sticky; St = Sticky; Sm = Smooth; Hd = Hard; Uf = Uniform; Sd = Sediment; +, (+) = Positive test; - = Negative test; µg = microgram; R = Resistant; LO = Light orange yellow; BHI = Brain Heart Infusion agar; ZMA = Zobell Marine agar; Rnd = Round; Rhi = Rhizoidal; Cx = Concex; Cv = Concave; Sle = Slightly elevated.

4. Conclusion

From the results, it is concluded that oil spill and tar polluted beaches of the Goa coastline were dominated by the diversity of oil resistant and salt tolerant bacteria. These may have potential in bioremediation of tar-ball deposition on the seashore, Goan beaches, and other oil-polluted sites.

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