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ISOLATION AND CHARACTERIZATION OF FACULTATIVE ALKALIPHILES FROM EFFLUENT WATER OF A DETERGENT INDUSTRY AND SCREENING FOR POTENT ENZYMES

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Abstract: Proteases are used in food, pharmaceutical, leather and detergent industry whereas α Amylases have been used in starch saccharification, detergent, textile, food, fuel alcohol, paper and brewing industries. These enzymes can be obtained from the genus *Bacillus* that are alkaliphiles or alkali-tolerant isolates. In the present study, isolation of alkali-tolerant bacteria from effluent samples of a local detergent industry, Tangi, situated at Cuttack, Odisha, having potentiality to produce alkaline enzymes was carried out.
Keywords: Alkaline enzymes, alkalitolerant, effluent water, detergent industry, Bacillus spp.

Introduction

Extremophiles are the ones found in extreme conditions which is rather impossible for normal microbes to thrive. The enzymes derived from them, also known as extremozymes have immense biotechnological applications such as bio-catalysis and biotransformation [1]. Soda soils, lakes and deserts, dilute alkaline springs, oils that contain rotting proteins or forest soil harbour Alkaliphiles- a kind of extremophile [2]. The dominant genera of bacteria producing alkaline amylase and protease is *Bacillus* due to their ability to survive at extreme alkalinity. Reports on alkaline enzyme producing bacteria isolated from the industrial effluents of Odisha are rare. The present study focuses on the isolation of facultative Alkaliphiles having the potentiality to produce alkaline enzymes. To the best of my knowledge, this is the first report on alkaline enzyme production by facultative bacterial isolates from Oriclean Private limited, at Tangi (N.H-16), District Cuttack in Odisha.

Materials and methods

Isolation of alkali-tolerant bacteria and identification

From Oriclean Private limited (Detergent Industry), Industrial effluent sediment samples were collected. The samples were placed separately in clean sterilized plastic bags and stored at 4 °C under proper laboratory conditions. Further pH, moisture content and bacterial load analysis was carried out respectively [3]. Horikoshi medium II (pH 10.3) was used to isolate

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alkalitolerant bacteria [4]. Well isolated and differentiated colonies were transferred to respective agar slants. The isolates obtained were streaked on nutrient agar plates at pH 6.0 to check the alkalitolerant nature. The isolates were then subjected to morphological, biochemical and physiological characterization. Screening for extracellular alkaline enzymes was carried out following the protocols of Priyadarshini and Ray (2015) and Priyadarshini *et al* (2020) with some modifications [5,6].

Results and discussion

Sample Characterization

Samples collection from Oriclean Private limited, Tangi, Cuttack has been shown in Fig. 1. Maximum pH, moisture content and alkali-tolerant bacterial load were recorded as 9.88, 37.4434% and 1.567×10^3 respectively (Table 1). There is a positive and significant correlation between the bacterial load with moisture content ($p<0.05$) as depicted in Table 2.

Fig. 1 Sample collection. A-ORICLEAN detergent industry, B-Sampling site

Table 1: Physico-chemical parameters and alkaliphilic load of sediment samples

Sampling Site	S. No	pH	Moisture content (%)	Bacterial load (CFU/g) $\times10^3$
Effluent sediment samples from Tangi	1	9.88	36.4910	1.133
	2	9.60	36.7341	1.533
	3	9.68	37.3301	1.543
	4	9.63	37.3321	1.455
	5	9.66	37.4434	1.567

Table 2: Pearson correlation coefficient and T-test for significant study

Parameters	Moisture content (%)	pH
pH	r=0.58162, t=2.62E-08**	
Bacterial load (CFU/g) $\times 10^3$	r=0.7337*, t=1.52E-09**	r=0.91065 t=3.39E-07**

Correlation is significant (p<0,05), **population mean is significant (two tailed)

Isolation and identification of isolates

Twelve alkalitolerant bacteria were isolated and designated as TW1-TW11 as shown in Fig. 2.

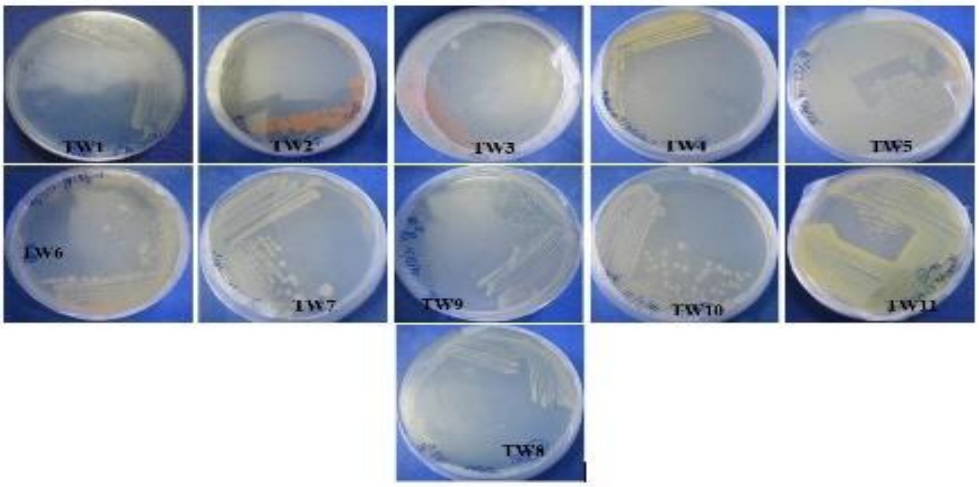

Fig.2 Isolates from effluent samples of Oriclean detergent industry

The physiological and antibiogram are depicted in Table 3. The isolates grew well at higher alkaline pH 13.0 but they also showed luxuriant growth at pH 6.0, thereby proving that they are facultative and not obligate alkaliphiles (Fig. 3). All the isolates were able to tolerate 2% NaCl. 5% NaCl also proved beneficial for the growth of the alkalitolerant bacteria except TW3 isolate. All the isolates showed positive growth at 37 °C and sensitivity to ampicillin too. The biochemical characterization has been presented in Table 4. All the isolates were negative for indole test and phosphate solubilization whereas they all showed positive results for esculin hydrolysis. All the isolates were positive for starch and maltose utilization. For the other sugars, the results were variable as shown in Table 5. Bacterial isolates were identified by cultural (colour, texture, margin, elevation, shape and size) as shown in Table 6 and morphological (Gram variability) as shown in Table 7. The results were analysed by ABIS online Software. All the isolates were found to be Gram positive rods and belonged to genus *Bacillus* and *Paenibacillus.*

Screening for extracellular alkaline enzymes

The screening for alkaline enzymes from the alkalitolerant bacteria at pH 10.0 are presented in Table 8. None of the isolates were positive for cellulase at 10 pH. All the isolates were positive for alkaline amylase (100%) whereas most of them were positive for alkaline protease (63.6363%). TW1 isolate showed >10mm diameter (+++).

Table 3 Physiological characterization, Antibiogram of the isolates

Isolates	pH			Nacl (%)				Temperature (°C)		Anaerobic growth	Antibiosis			
	6 to 11	12	13	2	5	10	15	37	55		Streptomycin	Chloramphenicol	Ampicillin	Polymyxin B
TW1	+	-	-	+	+	+	+	+	-	+	S	S	S	S
TW2	+	+	+	+	+	+	-	+	-	+	S	S	S	R
TW3	+	-	-	+	-	-	-	+	-	+	S	S	S	R
TW4	+	-	-	+	+	+	-	+	+	+	S	S	S	S
TW5	+	+	+	+	+	+	-	+	-	+	S	S	S	S
TW6	+	+	+	+	+	-	-	+	-	+	S	S	S	S
TW7	+	+	+	+	+	+	-	+	-	+	S	S	S	S
TW8	+	-	-	+	+	-	-	+	-	-	R	R	S	S
TW9	+	+	+	+	+	-	-	+	-	+	S	S	S	R
TW10	+	+	+	+	+	+	+	+	-	+	S	S	S	S
TW11	+	+	+	+	+	+	-	+	+	+	S	S	S	R

S: sensitive; R: resistant; (+): Growth Positive; (-): Growth Negative.

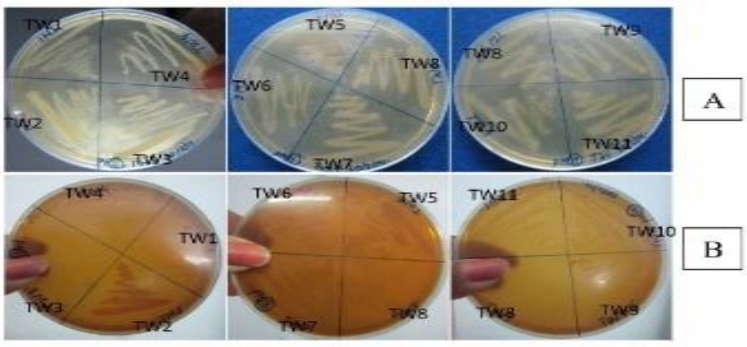

Fig. 3 Growth of the isolates on nutrient agar. A-pH 6.0, B-pH 10.0

Table 4 Biochemical characterization of alkaline amylase producing isolates

Isolates	MR	VP	In	Ci	Ca	Oxi	Es	NR	Ur	PS	Mo
TW1	+	+	-	-	+	-	+	-	-	-	+
TW2	+	-	-	-	+	-	+	+	-	-	+
TW3	+	+	-	-	+	-	+	+	-	-	+
TW4	+	-	-	-	+	-	+	-	-	-	+
TW5	-	-	-	-	+	-	+	-	-	-	-
TW6	+	+	-	+	+	-	+	+	+	-	+
TW7	+	+	-	+	+	+	+	+	+	-	+
TW8	+	-	-	+	+	+	+	+	-	-	+
TW9	+	-	-	+	+	+	+	+	-	-	+
TW10	-	+	-	+	+	-	+	+	-	-	+
TW11	+	-	-	-	+	-	+	-	-	-	+

Mo: Motility test; MR: Methyl red test; VP: Voges-Proskauer test; In: Indole production; Ci: Citrate Utilization; Ca: Catalase; Oxi: Oxidase; Es: Esculin Hydrolysis; Ur: Urease test; PS: Phosphate solubilization; NR: Nitrate reductase; (+): Positive; (-): Negative.

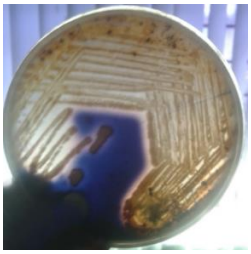

Fig. 4 Zone of clearance seen in case of TW1 isolate for alkaline amylase at pH 10.0

Table 5: Sugar utilization tests of alkaline protease producing isolates

Isolates	Glucose	Starch	Fructose	Lactose	Xylose	Maltose	Salicin	Mannose
TW1	+	+	+	-	-	+	-	-
TW2	-	+	-	+	-	+	+	-
TW3	+	+	+	+	+	+	+	+
TW4	+	+	+	+	-	+	+	+
TW5	+	+	+	-	-	+	+	+
TW6	+	+	+	+	-	+	-	+
TW7	+	+	+	+	+	+	+	+
TW8	+	+	+	+	+	+	+	-
TW9	+	+	+	+	+	+	+	+

TW10	+	+	+	-	+	+	+	-
TW11	+	+	-	+	-	+	-	+

Table 6: Micro-and macro-morphological study of the isolates

Organism	Colour	Size	Shape	Margin	Texture	Elevation
TW1	off-white	medium	round	regular	rough	elevated
TW2	Orange pigmented	medium	round	regular	smooth	Slightly elevated
TW3	Brick red pigmented	medium	round	regular	smooth	flat
TW4	Yellow pigmented	small	round	regular	Smooth, shiny	flat
TW5	creamy	medium	round	regular	smooth	flat
TW6	white	medium	oval	regular	rough	Slightly elevated
TW7	creamy	medium	oval	regular	rough	Slightly elevated
TW8	creamy	small	oval	regular	smooth	flat
TW9	creamy	small	Pinhead, tiny	regular	Smooth	flat
TW10	creamy	medium	oval	regular	smooth	flat
TW11	yellow	small	Pinhead	regular	smooth	flat

Table 7 Gram staining and identification of isolates

Isolate code	Gram staining	Identification of isolates	Similarity %
TW1	Gram Positive	*Bacillus cereus*	~90
TW2	Gram Positive	*Bacillus siamensis*	~82.5
TW3	Gram Positive	*Paenibacillus polymyxa*	~96.7
TW4	Gram Positive	*Bacillus circulans*	~93.2
TW5	Gram Positive	*Bacillus coagulans*	~85
TW6	Gram Positive	*Bacillus licheniformis*	~87.3
TW7	Gram Positive	*Bacillus licheniformis*	~98.3
TW8	Gram Positive	*Bacillus megaterium*	~98
TW9	Gram Positive	*Paenibacillus glucanolyticus*	~97.6
TW10	Gram Positive	*Bacillus thuringiensis*	~98
TW11	Gram Positive	*Bacillus circulans*	~85.3

Table 8: Enzymatic tests of isolates at pH 10.0

Isolates	TW1	TW2	TW3	TW4	TW5	TW6	TW7	TW8	TW9	TW10	TW11
Amylase	+++	+	+	+	+	+	+	+	+	++	+
Protease	-	+	+	+	-	-	++	+	+	-	+
Cellulase	-	-	-	-	-	-	-	-	-	-	-

>10mm diameter (+++); ≤ 10mm diameter (++); <5mm diameter (+); no activity (-)

Conclusion

From the above study, it was observed that industrial effluents are the source of various facultative alkaliphilic bacteria from which alkaline enzymes like amylase, protease can be obtained. All the isolates obtained were Gram Positive. The isolates were not only active in alkaline pH but also tolerated 5% NaCl, except TW3 isolate which makes them halo-alkali tolerant bacteria. The alkaline enzymes produced from the above isolates have immense applications in various fields of sciences and industries. Further research needs to be undertaken for the production, optimization and characterization of alkaline enzyme by the potent isolate under standard laboratory conditions.

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