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CHARACTERIZATION OF ALKALI-TOLERANT BACTERIA FOR ALKALINE ENZYME PRODUCTION FROM INDUSTRIAL EFFLUENTS

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Abstract: Effluent samples from detergent industries are known sources of facultative alkaliphilic bacteria, which have immense potential in producing alkaline enzymes. In this study, we aimed to isolate and characterize alkali-tolerant bacteria from the effluent of Oriclean Private Limited, Cuttack, Odisha, and screen for potent enzymes. A total of twelve alkalitolerant bacteria were isolated, all of which were Gram-positive, belonging to the genera Bacillus and Paenibacillus. These bacteria not only thrived in alkaline pH but also tolerated 5% NaCl, making them halo-alkali tolerant. The alkaline enzymes produced by these isolates, such as amylase and protease, have significant applications in various industries. Further research is necessary to optimize and characterize these alkaline enzymes under standard laboratory conditions.

Keywords: alkaline enzymes, alkalitolerant, detergent industry, effluent water, Bacillus spp.

Introduction

Alkaline enzymes like amylases and proteases have extensive applications in various industries, including pharmaceutical, food, detergent, leather, textile, paper, alcohol, and brewing [1]. These enzymes can be obtained from the genus Bacillus, which are either alkaliphilic or alkali-tolerant [2]. Detergent industries are known sources of facultative alkaliphilic bacteria that have potential in producing alkaline enzymes. Therefore, we aimed to isolate and characterize alkali-tolerant bacteria from the effluent of Oriclean Private Limited, Cuttack, Odisha, and screen for potent enzymes.

Materials and Methods: Effluent samples were collected from Oriclean Private Limited, Cuttack, and subjected to alkalitolerant bacteria isolation, morphological, biochemical, and physiological characterization, and extracellular alkaline enzyme screening following established protocols [3-6].

Results and Discussion: A total of twelve alkalitolerant bacteria were isolated and found to thrive in both alkaline pH and NaCl concentrations. All isolates were Gram-positive, belonging to the genera Bacillus and Paenibacillus. These isolates have potential in producing alkaline enzymes, which have immense applications in various industries.

Conclusion: Effluent samples from detergent industries are potential sources of facultative alkaliphilic bacteria that have potential in producing alkaline enzymes. Our findings provide insight into the isolation and characterization of alkali-tolerant bacteria, which have the potential to produce valuable enzymes. Further research is necessary to optimize and characterize these enzymes under standard laboratory conditions.

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) $\times 10^3$
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.

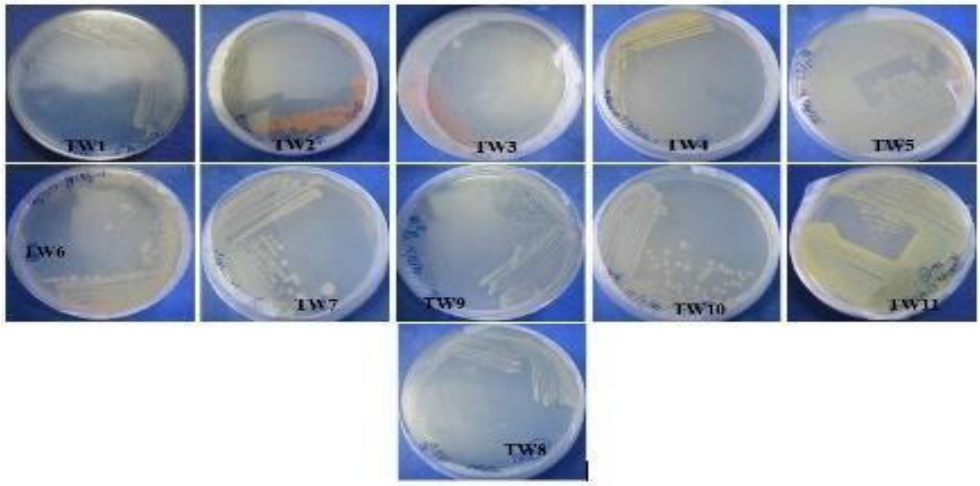

Fig.2 Isolates from effluent samples of Oriclean detergent industry

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.

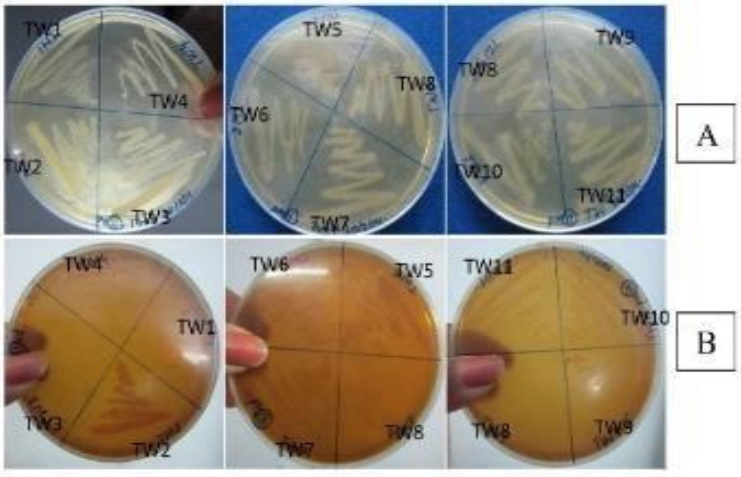

Fig. 3 Growth of the isolates on nutrient agar. A-pH 6.0, B-pH 10.0

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.

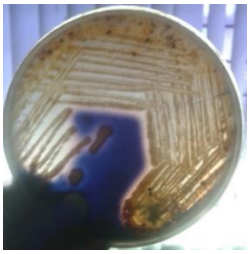

Fig. 4 Zone of clearance seen in case of TW1 isolate for alkaline amylase at pH 10.0 Table 5: Sugar utilization tests of alkaline protease producing isolates

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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