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ISOLATION AND IDENTIFICATION OF POTENTIAL LIPASE PRODUCING *STAPHYLOCOCCUS WARNERI* MG1 BACTERIUM FROM KAHN RIVER (SEWAGE WATER)

Science

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ABSTRACT

The bacterial culture S2 screened for lipase production was identified on the basis of morphological, cultural, physiological and biochemical characteristics. It was further confirmed and identified up to species level by Bergey's manual volume 2 & Biomerieux Vitek 2 system and designated as *Staphylococcus warneri* strain MG1 (S2). This lipase producer was isolated from Kahn river of Indore region, Madhya Pradesh. Maximum lipase activity (3.2) was observed on tributyrin medium. Cells were gram positive cocci. Colonies were small, round, translucent with golden yellow pigment and was found to be sensitive for various antibiotics. The selected bacterium MG1 was negative towards citrate utilization, indole test, methyl red and voges- proskauer tests, H_2S production, urea hydrolysis, oxidase and hemolytic activity test. The strain was catalase positive and coagulase negative. The isolate exhibited a greater zone of clearance at pH 9 on tributyrin agar medium plate which shows its alkali tolerant nature.

CONCLUSION The potential lipase producing bacterium *Staphylococcus warneri* strain MG1 was isolated and identified which can be used in detergent formulations due to its alkali tolerant nature.

KEYWORDS

Staphylococcus warneri strain MG1, screening, lipase, identification.

INTRODUCTION

Bacterial lipases are of great demand and have many applications in various industries. Lipase enzymes catalyze the hydrolysis of triacylglycerol's to glycerol and free-fatty acids. Several strains of Staphylococci have been reported earlier which produce extracellular lipases like *Staphylococcus chromogenes* (Hajek *et al.,* 1986), *S. epidermidis* (Simons *et al.,* 1998; Jaeger *et al.,* 1999; Joseph *et al.,* 2006), *S. hyicus* (Van Kampen *et al.,* 1998; Jaeger *et al.,*1999), *S. warneri* (Pandey *et al.,*1999; Van Kampen *et al.,* 2001; Walavalkar *et al*., 2002; Yokoi *et al.,*2008; Rech *et al*., 2011), *S. xylosus* (Pandey *et al.,*1999; Van Kampen *et al.,* 2001; Mosbah *et al.,* 2005; Khoramnia *et al.,* 2010; Bouaziz *et al.,* 2011), *Staphylococcus argenteus* MG2 (Golani *et al.,* 2019). The present study focuses on phenotypic and biochemical analysis of potential lipase producing *Staphylococcus warneri* strain MG1 (S2) bacterium.

MATERIALS AND METHODS

Screening of lipase producing bacteria

Lipase producing bacteria were screened by enrichment culture technique from various samples like oil spilled soil of vegetable oil packing industries and auto garage, Kahn river (domestic waste water/sewage), spoiled milk and milk cream, spoiled coconut water etc. of Indore city of Madhya Pradesh, India. These samples were enriched by inoculating in Tributyrin broth medium flask (50 ml) containing 0.5% (w/v) peptone, 0.3% (w/v) yeast extract, 1% (v/v) Tributyrin pH 7.0 and 9.0 and incubated at 37ºC for 48 h. Each enriched culture sample was isolated on Tributyrin agar medium plates (Lawrence *et al.,* 1967) (pH - 7 and 9) by sector plate method and incubated at 37ºC for 48 h. Individual bacterial colonies obtained on Tributyrin agar plates were evaluated by spot inoculation method for their lipolytic ability by measuring the zone of clearance due to hydrolysis of tributyrin by lipase. The alkali tolerant nature of isolate was checked by growing each isolate of pH- 7 Tributyrin agar medium plate to pH- 9 plate and vice versa. The bacterial colonies showing maximum zone of lipid hydrolysis were selected for further study.

Characterization of selected isolate

The bacterial isolate, MG1 (pH-7) isolated from Kahn river of Indore region of Madhya Pradesh, found to produce maximum zone of hydrolysis around the colony on Tributyrin agar medium plate was selected for further study. It was studied for its morphological, colonial, cultural and biochemical characteristics.

Identification of selected isolate

The taxonomic status of the selected bacterium MG1 was identified following the criteria laid down by Bergey's Manual of Determinative Bacteriology (Holt *et al.,* 1994). The biochemical tests (Mac Faddin 1980) such as indole production from tryptophan, methyl-red and voges–proskauer tests, simmons' citrate utilization test, urea hydrolysis, production of H_2S from cysteine, various sugar fermentation tests, oxidase activity, catalase test, hemolytic activity test, starch hydrolysis test, casein hydrolysis test were examined. The isolate was further identified up to species level and confirmed on the basis of Biomerieux Vitek 2 system. It is an automated microbiology system which is used in the identification of microorganisms. It consists of colorimetric reagent cards (64 wells) that are incubated and interpreted automatically.

Antibiotic susceptibility test

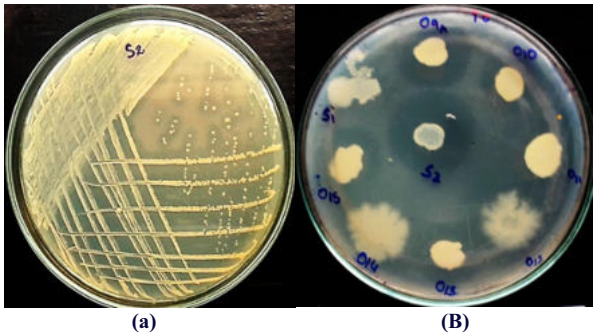
Disc diffusion assay was performed to assess the antibiotic resistance/sensitivity pattern. The susceptibility of each of the study organisms to selected antibiotics (Hi Media) namely, Ampicillin (AMP 10 mcg), Amikacin (AK 30 mcg) Gentamycin (GEN 10 mcg), Cefoperazone (CPZ 75 mcg), Bacitracin (B 10 mcg) and Clindamycin (Cd 2 mcg) was determined.

The test bacterial culture was spreaded on the Mueller Hinton agar medium using sterile swabs. The antibiotic discs were placed on the surface of the inoculated medium and plates were allowed to incubate at 37±2°C for 48 h. Clear zones of growth inhibition observed around the discs after incubation, were measured in millimeters. The bacterial isolates were recorded resistant or sensitive by measuring the growth inhibitory zone and comparing it with the standard interpretative chart (Table - 1).

RESULTS

Screening of lipase producing bacteria

Various lipolytic bacterial isolates were screened from diverse samples. Among these isolates, MG1 (pH-7) showed maximum zone of hydrolysis (3.25 mm) around colony (Fig. 1) and was also able to grow at pH-9 with maximum lipase activity which shows its alkali tolerant nature and was selected for further studies.



(a) (B)

Fig. 1 Zone of hydrolysis by lipase producing *Staphylococcus warneri* MG1 on Tributyrin agar plate (a) isolated colonies (b) patch test (in center S2)

Characterization of selected bacterial isolate

The isolate MG1 was found to be gram positive cocci. The colonial characters of isolate MG1 were small, round, even, regular, low

convex, smooth, translucent, and golden yellow pigmented.

Identification of selected isolate

The taxonomic status of the selected bacterium MG1 was identified following the criteria laid down by Bergey's Manual of Determinative Bacteriology (Holt *et al.,* 1994). The selected bacterium MG1 was negative towards citrate utilization, indole test, methyl red and voges-proskauer tests, H_2S production, urea hydrolysis, oxidase and hemolytic activity test (Fig. 2, 3). The isolate MG1 was not able to produce amylase and protease enzyme. The strain was catalase positive and coagulase negative (Fig. 2). It was able to ferment various sugars like sucrose, maltose and lactose. The isolate was further identified up to species level and confirmed on the basis of Biomerieux Vitek 2 system. This system showed 94% probability of *Staphylococcus warneri* bacterium with bio number – 010000002020231. Results are shown in Table 2.

Fig. 2 Catalase, coagulase tests & hemolytic activity (no hemolysis) of *Staphylococcus warneri* MG1

Urease VP MR Indole Citrate TSI

Fig. 3 Biochemical tests of *S. warneri* MG1

Antibiotic susceptibility test

The isolate MG1 was found to be sensitive for the entire selected antibiotics which indicates that the isolate MG1 is non-pathogenic strain (Table -1) (Figure - 4).

Table -1 Zone of inhibition (mm) of various antibiotics of isolate Mg1

S. No.	Antibiotic	Symbol	Resistant (mm) or less	Intermediate (mm)	Sensitivity (mm) or more	Zone diameter (mm)	Sensitive (S) / Resistant (R)
1.	Ampicillin	AMP	28	–	29	30	S
2.	Amikacin	AK	16	16 – 17	18	24	S
3.	Gentamycin	GEN	18	–	18	23	S
4.	Cefoperazone	CPZ	15	16 – 20	21	22	S
5.	Bacitracin	B	8	9 – 12	12	12	S
6.	Clindamycin	Cd	14	15 – 20	21	26	S

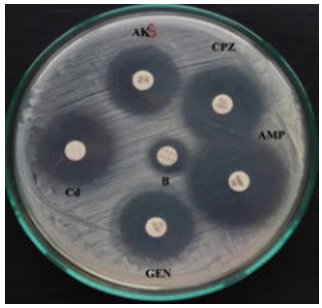


Fig. 4 Antibiotic susceptibility test of *S. warneri* MG1

Table: 2 Results of test substrate on GP card of Biomerieux Vitek 2 system

Well	Test	Mnemonic	Amount/Well	Results
2	D-AMYGDALIN	AMY	0.1875 mg	-
4	PHOSPHATIDYLINOSITOL PHOSPHOLIPASE	PIPLC	0.015 mg	-
5	D-XYLOSE	aXYL	0.3 mg	-
8	ARGININE DIHYDROLASE 1	ADH1	0.111 mg	+
9	BETA-GALACTOSIDASE	BGAL	0.036 mg	-
11	ALPHA-GLUCOSIDASE	AGLU	0.036 mg	-
13	Ala-Phe-Pro ARYLAMIDASE	APPA	0.0384 mg	-
14	CYCLODEXTRIN	CDEX	0.3 mg	-
15	L-Asparate ARYLAMIDASE	AspA	0.024 mg	-
16	BETA GALACTOPYRANOSIDASE	BGAR	0.00204 mg	-
17	ALPHA-MANNOSIDES	AMAN	0.036 mg	-
19	PHOSPHATASE	PHOS	0.0504 mg	-
20	Leucine ARYLAMIDASE	LeuA	0.0234 mg	-
23	L-Proline ARYLAMIDASE	ProA	0.0234 mg	-
24	BETA-GLUCURONIDASE	BGURr	0.0018 mg	-
25	ALPHA-GALACTOSIDASE	AGAL	0.036 mg	-
26	L-Pyrrolidonyl-ARYLAMIDASE	PyrA	0.018 mg	-
27	BETA GLUCURONIDASE	BGUR	0.0378 mg	-
28	Alanine ARYLAMIDASE	AlaA	0.0216 mg	-
29	Tyrosine ARYLAMIDASE	TyrA	0.0276 mg	-
30	D-SORBITOL	dSOR	0.1875 mg	-
31	UREASE	URE	0.15 mg	-
32	POLYMIXIN B RESISTANCE	POLYB	0.00093 mg	-
37	D-GALACTOSE	dGAL	0.3 mg	-
38	D-RIBOSE	dRIB	0.3 mg	-
39	L-LACTATE alkalinization	ILATk	0.15 mg	+
42	LACTOSE	LAC	0.96 mg	-
44	N-ACETYL-D-GLUCOSAMINE	NAC	0.3 mg	-
45	D-MALTOSE	dMAL	0.3 mg	-
46	BACITRACIN RESISTANCE	BACl	0.0006 mg	-
47	NOVOBIOCIN RESISTANCE	NOVO	0.000075 mg	-
50	GROWTH IN 6.5% Nacl	NC6.5	1.68 mg	+
52	D-MANNITOL	dMAN	0.1875 mg	-
53	D-MANNOSE	dMNE	0.3 mg	-
54	METHYL-B-D-GLUCOPYRANOSIDE	MBdC	0.3 mg	-
56	PULLULAN	PUL	0.3 mg	-
57	D-RAFFINOSE	dRAF	0.3 mg	-
58	O/129 RESISTANCE [COMP.VIBRIO]	O129R	0.0084 mg	+
59	SALICIN	SAL	0.3 mg	-
60	SACCHAROSE/SUCROSE	SAC	0.3 mg	+
62	D-TREHALOSE	dTRE	0.3 mg	-
63	ARGININE DIHYDROLASE 2	ADH2s	0.27 mg	+
64	OPTOCHIN RESISTANCE	OPTO	0.000399 mg	+

The morphological, cultural, physiological and biochemical characteristics suggested that the isolate MG1 was close to a bacterium ***Staphylococcus warneri***. Hence this strain was identified and named as ***Staphylococcus warneri* MG1 (S2).**

DISCUSSION

In present study a potential lipase producing bacterium *Staphylococcus warneri* strain MG1 was screened from Kahn river of Indore region which was confirmed and identified up to species level

by Bergey's manual volume 2 (Bergey's manual, 1974) & Biomerieux Vitek 2 system.

The strain MG1 was also studied by Walavalkar *et al*., 2002 which was isolated from Indian sweets viz. basundhi and was found to be similar in all the characteristics. Van Kampen *et al*., 2001 characterized *Staphylococcus warneri* lipase 2, similarly Talon *et al*., 1995 also characterized the same bacterium. Rech *et al*., 2011 studied *Staphylococcus warneri* EX17 and optimized lipase production in bioreactor cultures.

In our study, the isolate *Staphylococcus warneri* strain MG1showed non-hemolytic activity and was also coagulase negative. All biochemical characteristics as well as sensitivity towards antibiotics indicates that this strain MG1 is non-pathogenic and can be used for lipase production. This strain was observed as potential lipase producer.

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