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ORIGINAL ARTICLE

High fluoride resistance and virulence profile of environmental *Pseudomonas* isolated from water sources

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Abstract

In our previous study, all *Pseudomonas* strains THP6, THP41, and OHP5 were identified as fluoride-resistant bacteria isolated from Dindigul district, Tamilnadu, India. The selected strains exhibiting a high level of fluoride resistance was determined in Luria broth (LB) medium and LB agar plates. In a further effort, fluoride-resistant organisms were tested for hemolytic activity and showed β-hemolysis on blood agar plates. The virulence factors such as *gyrB*, *toxA*, *algD* and *lasB*, *plcH*, *rhlC* and biofilm response genes (*pslA*, *pelA*, *ppyR*) were detected by PCR analysis. The putative genus-specific and species-specific PCR also confirmed that the selected fluoride-resistant strains were belonging to *Pseudomonas aeruginosa* species. Fluoride-resistance gene *crcB* was amplified by gene-specific primers. The *crcB* gene was cloned in TA vector and transformed into *E. coli* DH5α. Comparative and blast analysis of THP6, THP41, and OHP5 strains *crcB* gene sequences were high homology with *P. aeruginosa* fluoride efflux transporter *crcB* and *P. aeruginosa* putative fluoride ion transporter *crcB*. The recombinants were efficiently growing in the NaF containing LB agar plates. The fluoride tolerance of these strains was also associated with resistance to multiple antibiotics. These results can lead to the use of the fluoride resistance gene of *P. aeruginosa* for the development of a biosensor for fluoride detection.

Introduction

Fluoride (F^-) is one of the most prevalent groundwater contaminants. F^- is an anion and behaves as a protoplasmic poison in nature, and even at a small concentration of F^- can cause several biochemical alterations (Eren et al. 2005). In general, bacteria have diverse types of mechanisms to deal with toxic components. In the case of F^-, bacteria possess a special type of channel protein named putative F^- ion transporter *crcB* that help them to reduce the toxic effect of F^- on the bacterial cell (Baker et al. 2012). Two of the most common genes associated with F^- riboswitches are *crcB* and $eriC^F$. The F^- resistance gene *crcB* of *E. coli* and the $eriC^F$ gene of *Pseudomonas syringae* function as F^- transporters (Baker et al. 2012). Further studies have shown that CLC^F has a function as an F^- channel, exporting F^- to protect *E. coli* against its toxicity (Stockbridge et al. 2012).

Pseudomonas aeruginosa is a gram-negative, rod-shaped bacterium belonging to the bacterial family *Pseudomonadaceae*, a member of γ-proteobacteria (Wunderink and Mendoza 2007; Todar 2008). *P. aeruginosa* is an opportunistic pathogen capable of causing several diseases which leads to infections (Lambert 2002; Laverty et al. 2014; Skariyachan et al. 2018). The pathogenicity is caused by major virulence factors such as lipopolysaccharide, flagellum, type III and IV secretion systems, type IV Pili, exotoxin A, proteases, alginate, quorum sensing, biofilm formation, and oxidant generation (Rocha et al. 2019). *Pseudomonas* has a remarkable ability to adapt and survive in a wide range of environments, especially in the water of all origins (Silby et al. 2011; Hashish et al. 2017). Furthermore, *P. aeruginosa* is well characterized as the most important heavy metal resistant and indicator organism commonly used for the bioremediation process (Edward Raja et al. 2006; Hassan et al. 2008; Ma et al. 2016; Edward Raja 2018).

The present study aimed to characterize the F^- resistance gene and to investigate the resistance profile of various antibiotics and to explore the pathogenic potential of *P. aeruginosa* isolates identified from water sources.

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Materials and methods

Fluoride-resistant *Pseudomonas* and growth conditions

In our previous study, three different *Pseudomonas* sp. THP6, *P. aeruginosa* OHP5, and *Pseudomonas* sp. THP41 strains were identified as F^--resistant bacteria isolated from groundwater samples. They displayed high F^- resistance was determined in LB medium and LB agar plates (Sree et al. 2018). These strains were selected and used for this study.

Hemolysis

β-Hemolytic activity of F^--resistant isolates was determined on Columbia agar media containing 5% sheep blood (HiMedia, Mumbai, India). Each isolate was streaked on blood agar plates and kept at 37 °C for 24 h. After incubation, the presence of a clear colorless zone surrounding the growing colonies stated β-hemolytic activity (Smibert and Krieg 1981).

Casein and starch hydrolysis

Casein hydrolysis was determined in skim milk (SM) agar (HiMedia, Mumbai, India). The F^--resistant isolates were streaked on SM agar plates and incubated at 37 °C for 24 h. The presence of a clear zone surrounding the culture indicated the proteolysis of casein. For proteolysis, *P. aeruginosa* MTCC 2453 was used as a positive control. Starch hydrolysis was carried out in LB agar plates containing 1% starch and incubated for 24 h at 37 °C. Grams iodine crystals were used for confirming the starch hydrolysis test.

Isolation of genomic DNA

Fluoride-resistant bacteria were inoculated in 5 mL Luria broth and incubated at 37 °C for overnight. Genomic DNA was isolated using the nucleospin microbial DNA kit (Macherey-Nagel GmbH and Co. KG, Germany) according to the manufacturer's instructions.

Molecular identification of *Pseudomonas* strains

Specific identification of *P. aeruginosa* was performed by PCR using two different primer sets. A first primer pair used for identification of the genus *Pseudomonas*, followed by a second primer set specific for *P. aeruginosa* (Spilker et al. 2004). Used primer details are given in Table 1. PCR amplification was carried out in Veriti 96 well thermal cycler. The following conditions 95 °C for 5 min, 35 cycles of denaturation at 94 °C for 1 min, annealing at 54 °C for 1 min, extension at 72 °C for 1 min, and a final extension at 72 °C for 5 min for the amplification of general *Pseudomonas* and 95 °C for 5 min, 35 cycles of denaturation at 94 °C for 1 min, annealing at 58 °C for 1 min, extension at 72 °C for 1 min and a final extension at 72 °C for 5 min used for precise detection of *P. aeruginosa*, respectively.

Determination of the antibiotic resistance profile

Antibiotic sensitivity of the F^--resistant isolates was determined by the disc diffusion method. Antibiotic-impregnated discs (HiMedia, Mumbai, India) were placed on Mueller–Hinton agar (MHA) plates swabbed with individual isolates and incubated at 37 °C for 24 h. The diameter of the inhibition zones around the discs was measured by the EUCAST standard. Twenty-two antibiotics were selected for the antibiotic susceptibility test. The tested antibiotics are mentioned in Table 2.

Biofilm production and biofilm-forming genes

Production of biofilm was analyzed by a micro-titer plate test. It is a quantitative and reliable gold standard method for biofilm detection (Hassan et al. 2011; Najafi et al. 2015). A sterile 96-well micro-titer plate filled with 1% inoculum of each bacterial culture was incubated for 24 h at 37 °C. Biofilm fixing, drying, and staining process was carried out by the method of Steponovic et al. (2000) with slight modifications. Besides, biofilm formation was categorized in four ways such as (0) non-adherent, (+) weak, (++) moderate, and (+++) strong production. All tests were conducted in triplicate. All *Pseudomonas* isolates were screened for the presence of genes associated with biofilm formation. Specifically, *pslA*, *pelA*, and *ppyR* genes were used in this study (Pournajaf et al. 2018). PCR condition was used as follows: initial denaturation at 95 °C for 5 min followed by 33 cycles of denaturation at 95 °C for 30 s, annealing at 65°C for 1 min, extension at 72 °C for 1 min, and final extension was done at 72 °C 5 min. The oligonucleotide primer sequences are mentioned in Table 1. The samples were amplified in a Veriti 96-well thermal cycler, and their PCR products were separated on 2% agarose gel electrophoresis. *P. aeruginosa* MTCC 2453 was used as a positive control.

Molecular identification of virulence genes

In this study, six virulence genes *gyrB*, *toxA*, *algD*, *lasB*, *plcH*, and *rhlC* were examined by PCR assays. *P. aeruginosa* MTCC 2453 was used as a reference strain for PCR. The recommended primer details are mentioned in Table 1.

Table 1 Nucleotide sequences of the primers used in this study

S.no.	Primer	Nucleotide sequence (5′–3′)	Target and size (bp)	References
1	PA-GS-F	GACGGGTGAGTAATGCCTA	*Pseudomonas species (618)*	Spilker et al. 2004
2	PA-GS-R	CACTGGTGTTCCTTCCTATA		Spilker et al. 2004
3	PA-SS-F	GGGGGATCTTCGGACCTCA	*P. aeruginosa (956)*	Spilker et al. 2004
4	PA-SS-R	TCCTTAGAGTGCCCACCCG		Spilker et al. 2004
		Virulence genes		
5	GyrPA-398	CCTGACCATCCGTCGCCACAAC	*gyrB (222)*	Lanotte et al. 2004
6	GyrPA-620	CGCAGCAGGATGCCGACGCC		Lanotte et al. 2004
7	ETA1	GACAACGCCCTCAGCATCACCAGC	*toxA (397)*	Khan & Cerniglia 1994
8	ETA2	CGCTGGCCCATTCGCTCCAGCGCT		
9	algDF	ATGCGAATCAGCATCTTTGGT	*algD (1311)*	Lanotte et al. 2004
10	algDR	CTACCAGCAGATGCCCTCGGC		Lanotte et al. 2004
11	lasBF	GGAATGAACGAAGCGTTCTC	*lasB (300)*	Lanotte et al. 2004
12	lasBR	GGTCCAGTAGTAGCGGTTGG		Lanotte et al. 2004
13	plcHF	GAAGCCATGGGCTACTTCAA	*plcH (307)*	Lanotte et al. 2004
14	plcHR	AGAGTGACGAGGAGCGGTAG		Lanotte et al. 2004
15	rhlCF	ACCGGATAGACATGGGCGT	*rhlC (570)*	Rahim et al. 2001
16	rhlCR	GCAGGCTGTATTCGGTGTC		Rahim et al. 2001
		Biofilm		
17	pslA F	TCCCTACCTCAGCAGCAAGC	*pslA (756)*	Pournajaf et al. 2018
18	pslA R	TGTTGTAGCCGTAGCGTTTCTG		Pournajaf et al. 2018
19	pelA F	CATACCTTCAGCCATCCGTTCTTC	*pelA (658)*	Pournajaf et al. 2018
20	pelA R	CGCATTCGCCGCACTCAG		Pournajaf et al. 2018
21	ppyR F	CGTGATCGCCGCCTATTTCC	*ppyR (160)*	Pournajaf et al. 2018
22	ppyR R	ACAGCAGACCTCCCAACCG		Pournajaf et al. 2018
		Fluoride-resistant gene		
23	crcBF	GCGGATCCATGTGGAAATCCATTCTCG	*crcB (384)*	Present study
24	crcBR	GGCCATGGTTTGCCCAGCATCCA		Present study

Characterization of fluoride-resistant gene (*crcB*)

The genomic DNA of THP6, THP41, and OHP5 was extracted by the nucleospin microbial DNA kit (Macherey-Nagel GmbH and Co. KG, Germany). The *crcB* gene was amplified by using specific primers designed with *Bam*HI and *Nco*I restriction enzyme site. The primer details are mentioned in Table 1. PCR amplification was carried out under the following condition: initial denaturation 94 °C for 5 min, 35 cycles of denaturation 94 °C for 45 s, annealing 59 °C for 45 s, extension 72 °C for 45 s, final extension 72 °C for 5 min. Amplification was observed with 1.5% agarose gel electrophoresis. Amplified PCR fragment was gel extracted and eluted by using GeneJET gel extraction kit (Thermo Scientific, USA). Ligation and transformation were performed by using a rapid DNA ligation kit, TA cloning vector system, and transform aid bacterial transformation kit (Thermo Fisher Scientific, USA) according to the manufacturer's instructions. *E. coli* DH5α was used as a host for transformation.

Gene *crcB* was ligated with pTZ57R/T (TA cloning vector available with the kit) by rapid DNA ligation kit (Thermo Fisher Scientific, USA), according to the manufacturer's protocol. After ligation, the reaction mixture was transformed to *E. coli* DH5α. Transformation of recombinant *crcB*-pTZ57R/T was facilitated with transform aid bacterial transformation kit (Thermo Fisher Scientific, USA). Transformed cells were spread on an LB agar plate containing 0.1 mmol/L IPTG, 20 mg/mL X-Gal and 100 µg/mL ampicillin. Plates were incubated at 37 °C overnight. White colonies of pTHP6, pTHP41, pOHP5 were selected on LB agar plates containing ampicillin (100 µg/mL).

The recombinants were confirmed by PCR using M13 forward (5′GTAAAACGACGGCCA3′) and M13 reverse primer (5′CAGGAAACAGCTATGAC3′) under the cyclic condition: initial denaturation 94 °C for 5

Table 2 Antibiotic susceptibility profile of fluoride-resistant *P. aeruginosa* isolates

S.no.	Antibiotics	µg	THP6	THP41	OHP5
1	Cefixime (CFM)	5	R	R	R
2	Cefotaxime (CTX)	30	S	I	I
3	Ceftizoxime (CZX)	30	R	R	R
4	Cephalothin (CEP)	30	R	R	R
5	Co-trimoxazole (COT)	25	I	I	R
6	Doxycycline hydrochloride (DO)	30	I	I	S
7	Fosfomycin (FO)	200	R	R	R
8	Gatifloxain (GAT)	5	S	S	S
9	Imipenem (I)	10	S	S	S
10	Minocycline (MI)	30	I	I	R
11	Meropenem (MRP)	10	S	S	S
12	Methicillin (MET)	5	R	R	R
13	Nalidixic acid (NA)	30	R	R	R
14	Netillin (NET)	30	S	S	S
15	Nitrofurantoin (NIT)	300	R	R	R
16	Norfloxacin (NX)	10	S	S	S
17	Ofloxacin (OF)	5	S	S	S
18	Penicillin G (P)	2 U	R	R	R
19	Piperacillin (PI)	100	S	S	S
20	Rifampicin (RIF)	2	R	R	R
21	Trimethoprim (TR)	10	R	R	R
22	Vancomycin (VA)	10	R	R	R

R resistance, *S* sensitive, *I* intermediate, *U* unit

min, followed by 35 cycles denaturation 94 °C for 45 s, annealing 55 °C for 45 s, extension 72 °C for 45 s, final extension 72 °C for 5 min. Amplification was observed with 1.5% agarose gel electrophoresis. TA vector served as a negative control.

The confirmed recombinants were analyzed and sequenced by Agrigenome Pvt Ltd, Cochin, Kerala. The acquired sequences were matched with previously published *crcB* sequences in the NCBI databases using ADVANCED BLAST (Altschul et al. 1997).

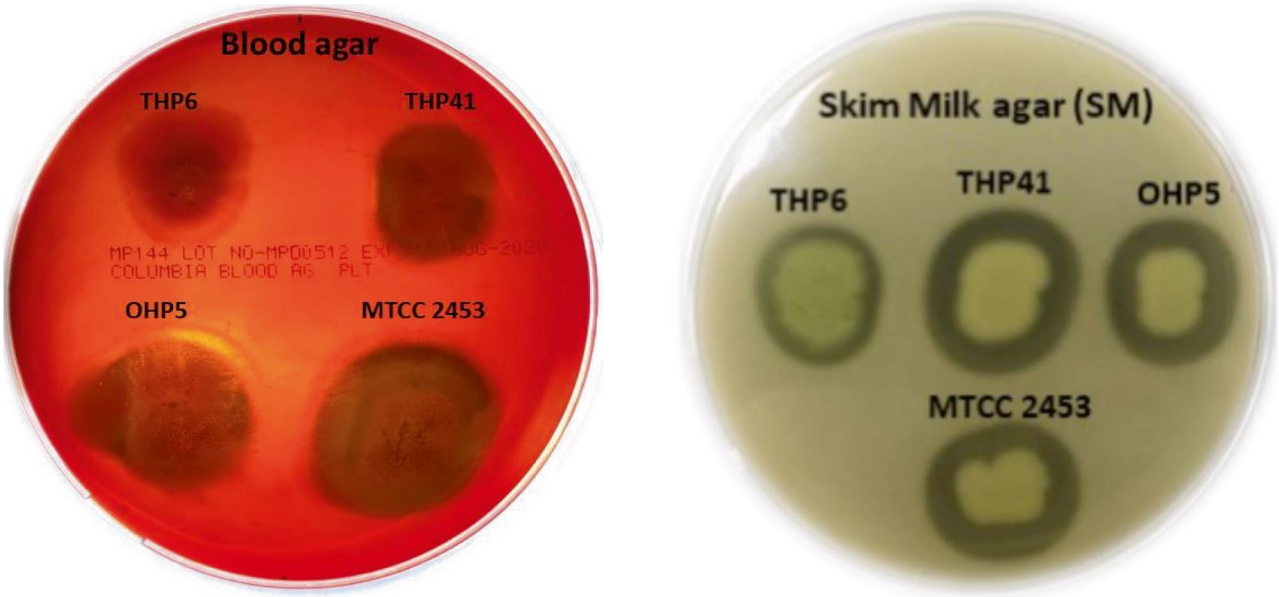
Fluoride resistance assay

The F^- resistance of recombinants was determined by the drop plate method. Minimum inhibitory concentration (MIC) was assessed until the selected recombinants were unable to grow on sodium fluoride (NaF) containing LB agar plates. Based on the evaluation, MIC was concluded at 37 °C for various time intervals. For this purpose, the TA vector is used as a negative control.

Results and discussion

Fluoride contamination in groundwater is becoming a leading and worldwide problem. In India and China, approximately 66 million and 45 million are affected by fluorosis (Adimalla and Venkatayogi 2017). Current studies proposed that 20 states in India are considered as endemic regions of fluorosis and also at risk of dental and skeletal fluorosis (Adimalla and Venkatayogi 2017). Biological methods (F^- bioremediation) are more valuable because the microbes, plants, and their products have been used to reduce and remove pollutants from the environment (Katiyar et al. 2020). In our earlier study, all strains THP6, THP41, and OHP5 were identified as F^--resistant *Pseudomonas* isolated from water samples (Sree et al. 2018). In this work, F^--resistant *Pseudomonas* isolates (THP6, THP41, and OHP5) exhibited β-hemolysis was confirmed on Columbia blood agar plates (Fig. 1a). Preceding reports evidenced that hemolysins are considered one of the significant virulence factors of *P. aeruginosa* (Majtán et al. 1991). *Pseudomonas* protease production was determined by using skim milk agar (Fig. 1b). It is also used for distinguishing *P. aeruginosa* from other *Pseudomonas* species (Wretlind et al. 1977). On the other hand, all strains showed a negative reaction to the starch hydrolysis test. Previously, all strains (THP6, THP41, and OHP5) were identified as *Pseudomonas* sp. THP6, *Pseudomonas aeruginosa* OHP5, and *Pseudomonas* sp. THP41 (Sree et al. 2018). Apart from β-hemolysis, in this study, these isolates were recognized and identifying as *P. aeruginosa* (Fig. 2a, b). It is confirmed by PCR using V2 and V8 regions of 16S rDNA-specific primers (Spilker et al. 2004). In general, PCR is a universal technique utilized for identifying microbes by amplification of gene sequences unique to a particular organism (Saiki et al. 1988). Several PCR and DNA probe methods have been developed to detect various pathogens from food, clinical samples, groundwater, and tap water, bottled water, water tanks and water purification shops (Bej et al. 1991; Fields et al. 1992; Tekpor et al. 2017; Abada et al. 2019).

Fluoride-tolerant *Pseudomonas* strains (THP6, THP41, OHP5) showed resistance to 11 different antibiotics such as β-lactams (penicillin-G, methicillin), cephalosporins (cefixime, ceftizoxime, cephalothin), trimethoprim, rifampicin, nalidixic acid, nitrofurantoin, fosfomycin, and vancomycin. *P. aeruginosa* displays resistance to a variety of antibiotics, including β-lactams, aminoglycosides, and quinolones (Hancock and Speert 2000). *P. aeruginosa* and



(a) Blood agar **(b)** Skim Milk agar

Fig. 1 β-Hemolytic and proteolytic activity of *Pseudomonas* isolates. For blood and skim milk agar tests, *P. aeruginosa* MTCC 2453 used as a positive control

E. coli were the most commonly studied heavy metal tolerance bacteria co-occurrence of resistance to several antibiotics (Nguyen et al. 2019). Edrington et al. (2009) also reported that many environmental isolates of *P. aeruginosa* displayed resistance to ampicillin, ceftiofur, florfenicol, sulphachloropyrodazine, and sulphadimethoxine trimethoprim,

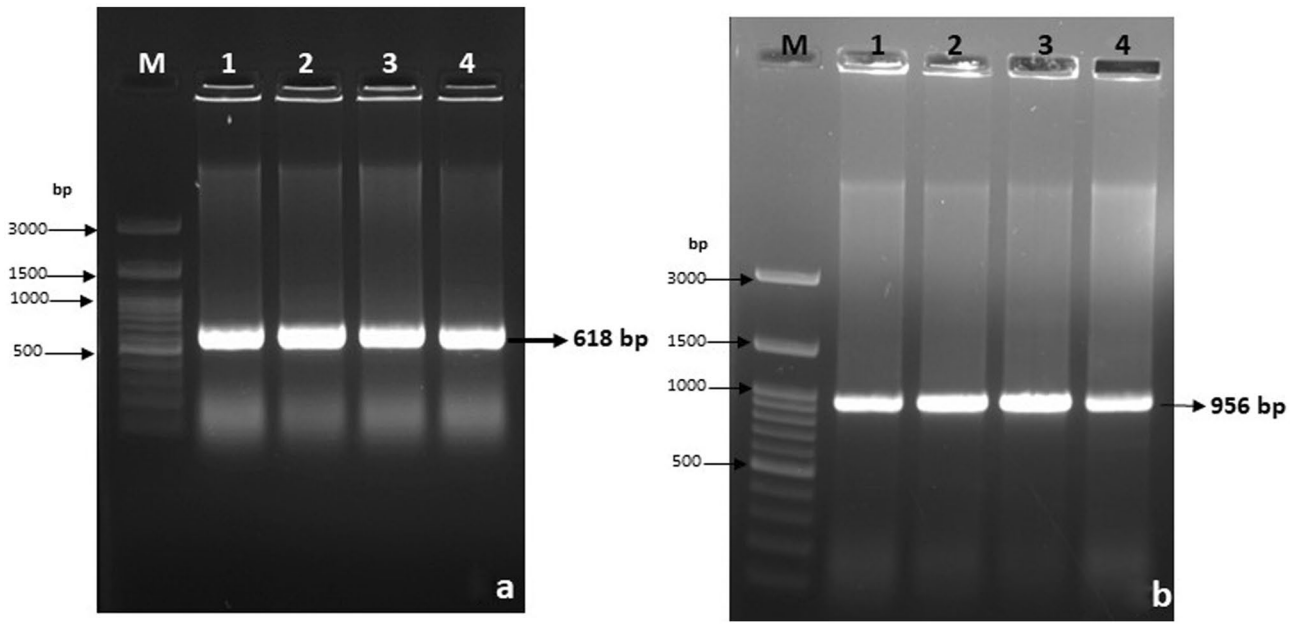


Fig. 2 PCR analysis of THP6, THP41, and OHP5 strains. **a** PCR using *Pseudomonas* genus-specific (PA GS-F; PA GS-R). **b** *P. aeruginosa*-specific primers (PA SS-F and PA SS-R). Lanes M, 100 bp ladder; lanes 1, 2, and 3, THP6, THP41, and OHP5 amplifications; lane 4, positive control (*P. aeruginosa* 2453 MTCC)

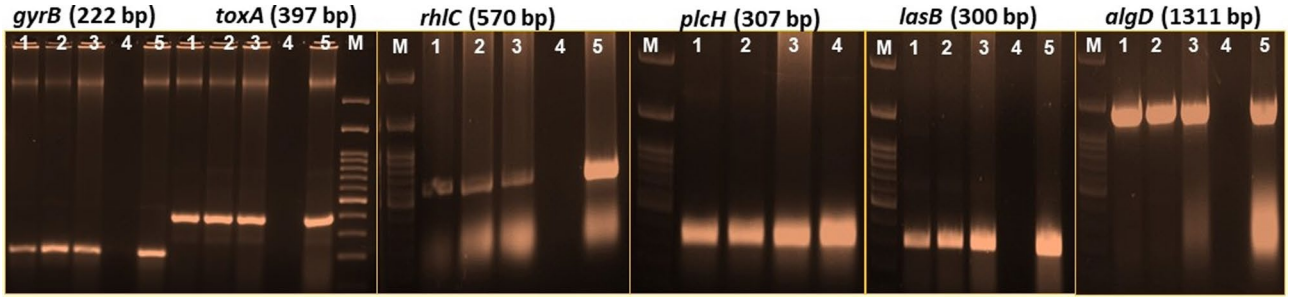


Fig. 3 Virulence profile of *Pseudomonas* strains. In all figures, lane M shows 100 bp ladder; lanes 1, 2, and 3 represent virulence genes amplification of THP6, THP41, and OHP5; lane 4, negative control (without template) and except in *plcH* gene amplification; lane 5, *P. aeruginosa* 2453 MTCC (positive control)

chlortetracycline and spectinomycin, respectively. The antimicrobial susceptibility test results are shown in Table 2.

Pseudomonas aeruginosa can secrete a variety of virulence factors including extracellular polymeric substances (EPS), lipopolysaccharides, pyocyanin, elastases, proteases, lipases, exotoxin A, exoenzyme S, rhamnolipids, and type IV pili (Wagner et al. 2007; Balasubramanian et al. 2013; Pitondo-Silva et al. 2016). In this effort, the selective virulence factors *algD* (GDP mannose 6-dehydrogenase), *gyrB* (DNA gyrase subunit B), *toxA* (exotoxin A), *lasB* (elastase), *plcH* (hemolytic phospholipase C), and *rhlC* (dirhamnosyl transferase II) were detected by PCR. *P. aeruginosa* MTCC 2453 was used as a positive control (Fig. 3). The amplification by PCR of *gyrB* gene is one of the detections and identification tools for *P. aeruginosa* strains isolated from water samples (Qin et al. 2003; Lee et al. 2011). Two research groups applied this type of screening for specific detection of *P. aeruginosa* from aquatic environments (Khan and Cerniglia 1994; Schwartz et al. 2006). Both *algD* and *lasB* virulence factors can influence pathogenesis by enhancing adhesion, colonization, and invasion of tissues causing chronic pulmonary inflammation (Lanotte et al. 2004). Likewise, the most common virulent genes (*algD*, *lasB* and *plcH*) are existing in THP6, THP41, and OHP5. Also, Martins et al. (2013) reported that these collective genes were present in *P. aeruginosa* isolated from clinical and environmental sources. Exotoxin A (*toxA*), the potential virulence factor of *P. aeruginosa*, was identified in this study. It inhibits protein biosynthesis, a necrotizing activity on tissues, and contributes to the colonization process (Michalska and Wolf 2015). Rhamnolipids are glycolipids containing L-rhamnose and β-hydroxy fatty acids moieties (Reis et al. 2011). The three key enzymes (RhlA, RhlB, and RhlC) responsible for rhamnolipid biosynthesis are found exclusively in *Pseudomonas* and *Burkholderia* species (Abdel-Mawgoud et al. 2010). Rhamnosyltransferase 2 (*rhlC*), the second glycosyltransferase involved in rhamnolipid production in *P. aeruginosa* (Rahim et al. 2001). In this work, *rhlC* gene was detected from these strains by PCR analysis.

Biofilm represents the most significant virulence factor responsible for developing structured communities of bacteria attached to a surface (Karatuna and Yagci 2010). Bacterial biofilm plays a vital role in the bioremediation of heavy metals from wastewaters (Mosharaf et al. 2018). In this work, strain THP41 is the highest biofilm producer compared to other *Pseudomonas* strains. Likewise, Hou et al. (2012) reported that not at all *P. aeruginosa* isolates were phenotypically positive for biofilm formation. More importantly, biofilm response genes *pslA*, *pelA*, *and ppyR* were present in these *Pseudomonas* isolates perceived by PCR (Fig. 4).

Earlier, the *crcB* and *eriC*^{F} genes were well characterized in *E. coli* and *Pseudomonas syringae*. Both act as F^- transporters (Baker et al. 2012). In this study, we identified *crcB* gene of *Pseudomonas* isolates by PCR assays (Fig. 5a). The purified PCR products were cloned in the TA vector using *E. coli* DH5α as a host. A total of 10 recombinants were selected and confirmed by PCR using plasmid DNA as a template (Fig. 5b). Also, clones were reconfirmed by sequencing using M13 forward and reverse primers. Sequence matching analysis of *crcB* was compared with previously submitted gene sequences in the NCBI database. Blast analysis is given in Table 1S. Based on results, 22 different *P. aeruginosa* strains were matched as 100% homology to F^- efflux transporter *crcB* and 8 different *P. aeruginosa* strains were corresponding as putative F^- ion transporter *crcB* (Table 2S).

The F^- resistance of recombinants was determined on LB agar plates supplemented with different concentrations of NaF. The concentration was selected starting from 50 to 300 mmol/L. At 50 mmol/L and 100 mmol/L concentration, there were no significant variations in the growth pattern of recombinants and TA vector plasmid. The result is described in Fig. 6. After that, recombinants were efficiently growing at 250 and 300 mmol/L NaF plates. On the other side, MIC of the TA vector against NaF was found at 150 mmol/L NaF concentration, respectively.

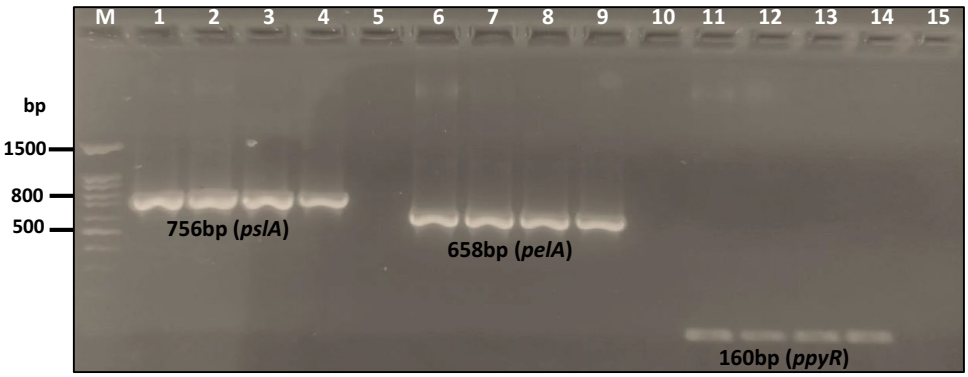


Fig. 4 Detection of biofilm-forming genes of *Pseudomonas* strains by PCR analysis. Lane M, 100 bp DNA ladder RTU (Gene DireX); lanes 1–4, *pslA* amplification; lanes 6–9, *pelA* amplification; lanes 11–14, *ppyR* amplification; lanes 4, 9, and 14, positive control (*P. aeruginosa* MTCC 2453); lanes 5, 10, and 15, negative control (without template DNA)

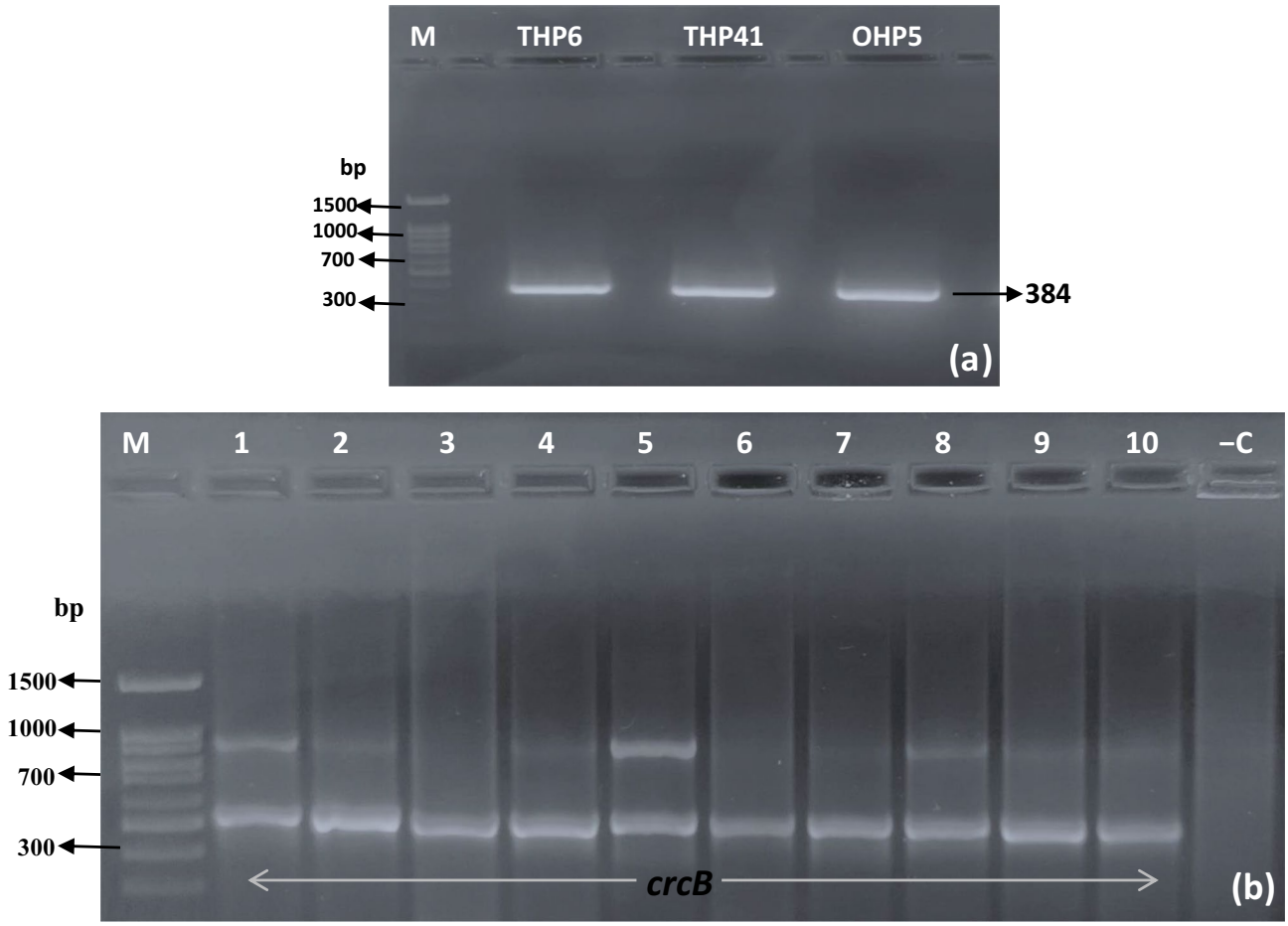


Fig. 5 **a** Amplification of *crcB* by PCR. Lane M, 100 bp DNA ladder RTU (Gene DireX); lanes THP6, THP41, and OHP5, *crcB* gene amplified products of *Pseudomonas* species. **b** The confirmation of *crcB* in recombinants by PCR. Lane M, 100 bp ladder; lanes 1 and 2, *crcB* gene amplification of pTHP6C1 and pTHPC2; lanes 3–5, gene amplifications of pTHP41C1, C2, and C3; lanes 6–10, gene amplifications of pOHP5C1, C2, C3, C4, and C5; lane -C, negative control (TA vector). The PCR product 535 bp (*crcB* gene, 384 bp; TA vector backbone, 151 bp) separated on 1.5% agarose gel electrophoresis

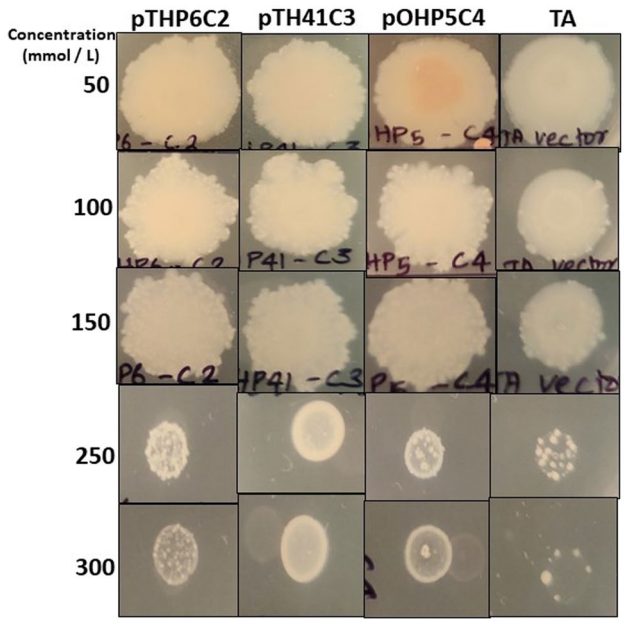


Fig. 6 MIC determination of recombinants carried out by the drop plate method. The concentration of NaF was taken from 50 to 300 mmol/L. TA vector used as a negative control

Conclusion

In this study, we identified the F^- resistance gene (*crcB*) from environmental *Pseudomonas aeruginosa* isolates. These isolates exhibited high F^- tolerance along with multiple antibiotic resistances. The pathogenic profile of *Pseudomonas* isolates was confirmed by β-hemolysis, virulence factors, and biofilm response genes. In further, this work will continue for the construction of GFP-based biosensor associated *crcB* gene in the expression host-vector system. This biosensor will be useful for the detection of fluoride in water bodies.

Supplementary information The online version contains supplementary material available at https://doi.org/10.1007/s12223-021-00867-z.

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Declarations

Ethical approval This article does not contain any studies with human participants or animals performed by any of the authors.

Conflict of interest The authors declare no competing interests.

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