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Biodegradation of synthetic pyrethroid pesticides under saline conditions by a novel halotolerant *Enterobacter ludwigii*

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ABSTRACT

Being reliable and cost-effective, bioremediation of wastewater from the pyrethroid pesticide industry requires a halotolerant strain for better degradation under hypersaline conditions. A halotolerant bacterial strain isolated from pesticide industry wastewater was characterized biochemically, molecular biologically and phylogenetically as *Enterobacter ludwigii*. Biodegradation efficiency of synthetic wastewater prepared with five different pyrethroid pesticides was investigated at varying saline (1%–10%) and pesticide concentration (50–600 mg/L). Better growth rate (first-order kinetics) with effective degradation (>90%) of pyrethroid pesticide, in mixture and individual, was achieved by *E. ludwigii* strain at an optimized condition of 3% NaCl and 100 ppm substrate concentration within 8 d of treatment. Gas chromatography-mass spectrometry and enzyme studies revealed the complete degradation of pyrethroids without toxic by-products via co-metabolism strategy. Nuclear magnetic resonance and high-performance liquid chromatography analysis exposed their adaptation hypersaline condition by an accumulation of various osmolytes. Hence, the study demonstrates the novel *E. ludwigii* strain as a promising candidate for degrading pyrethroids at the saline conditions.

Keywords: Halotolerant; Pyrethroid; Pesticide; Biodegradation; Osmolyte

1. Introduction

Synthetic pyrethroid (SPs), a potent alternative to toxic organochlorine and organophosphate pesticides, is effectively employed in agriculture and household sector for more than 30 years in insect pest management and accounts for approximately one-fourth of the global insecticide market [1]. Pyrethroid pesticide alters the activity of the sodium channels, responsible for the signal transmissions of nerve impulses, which disrupt the functioning of the nerve cells thus leading to paralysis and the eventual death of insects [2]. The inappropriate discharge of these pesticides into the environment imparts toxicity on a wide range of non-targeted organisms including humans and their long-term exposure impaired multisystem such as reproductive, respiratory, neuronal, immune and endocrine [3–5]. Moreover, SPs were reported as possible human carcinogens that cause an allergic dermatological reaction, several chronic diseases, and damages lymph nodes and spleen [6]. The high salinity and extreme toxicity of wastewater discharged from pesticide manufacturing industries is a serious environmental challenge [7]. Such concerns have heightened the critical need for an efficient method to eliminate these contaminants from the environment.

Physiochemical pretreatment of wastewater was cumbersome, less effective, more expensive, sludge generation and accumulates toxic by-products [8]. Microbial bioremediation has attracted increasing attention as a safe, eco-friendly, effective, high removal efficiency, and cheap biotechnological approach to degrade and eliminate toxic pesticide residues [2]. Many studies have proven that pyrethroid can be successfully eliminated by diverse microorganisms that belong to different taxonomic groups [2]. Up to the present, several bacterial strains including *Clostridium* sp., *Micrococcus* sp., *Ochrobactrum* sp., *Pseudomonas aeruginosa*, *Sphingobium* sp., *Serratia* sp.,

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Streptomyces sp., and *Stenotrophomonas* sp. have been reported to degrade SPs [9–13]. Most pyrethroids could be converted to a toxic metabolite which is refractory to microbial degradation [14,15]. There are quite a few studies reported the biodegradation of pesticides, either as individual strain or as a consortium, which degrades a variety of toxic polycyclic aromatic hydrocarbons [16], endosulfan [17] and dichlorvos [18]. However, it is mandatory to explore a potent microbial strain which not only breaks the whole pesticide but also utilizes the intermediate metabolites completely through co-metabolic strategy, and reduces the accumulation of toxic final product.

Furthermore, the hypersaline nature of wastewater from the pesticide industry renders its persistent nature and resistant for biodegradation by a mesophilic microorganism. The high salinity of contaminated wastewater causes disintegration of the microbial cell due to the osmotic pressure gradient between the cell and the surrounding which consequently affects microbial activity by cell dehydration [19,20]. Whereas, halotolerant bacteria was proven to be excellent and considered to be a viable option for the biodegradation of organic pesticides in hypersaline conditions, as it adapts to the salt environment using osmoregulation mechanism [21]. In this context, search for a novel halotolerant bacterial strain which degrades the pyrethroid pesticides by utilizing it as a sole carbon source, without generating toxic metabolic wastes, is need of the hour. Nevertheless, for the effective use of microorganisms in the remediation of pyrethroid-, it is extremely important to determine their potential under optimal conditions [2].

Hence in this current study pesticide degrading halotolerant organism was identified and characterized from the pesticide industry wastewater. The optimum condition of salinity and substrate concentration for a better growth rate was scrutinized. Percent removal of five pyrethroid pesticide mixture and degradation kinetics of individual pesticide based on the first-order model at optimized condition was investigated. Furthermore, the production of various enzymes and osmolytes enhancing complete degradation of intermediate pyrethroid metabolites at increased saline conditions was examined. The adaptability of the identified strain to the hypersaline environment through compatible solute synthesizing strategy and activity of enzymes showed its excellent stability in degrading mixture of pyrethroid pesticide, which is evidenced for the first time.

2. Methodology

2.1. Reagents and chemicals

Chemicals and solvents used in this study were purchased from Merck, Mumbai, India. Technical grade pesticides such as deltamethrin, Cyfluthrin, cypermethrin, permethrin, and lambda-cyhalothrin were obtained from AccuStandard, USA. The mixed solution of pesticides was dissolved in acetone at a stock concentration of 10,000 ppm, filtered sterilized through a 0.45 μm syringe filter.

2.2. Enrichment and isolation of pyrethroid pesticide-degrading halotolerant bacteria

Wastewater was collected from the pesticide manufacturing industry located at Tamil Nadu, India. Typically, 5 mL of the wastewater was inoculated to 50 mL of saline mineral salt medium (MSM) as per Veenagayathri and Vasudevan [16], supplemented with 50 mg/L of pyrethroid pesticide mixture at a pH of 7.2 ± 0.2 and incubated in a rotary shaker at 120 rpm for 15 d. An aliquot of the culture suspension (5 mL) was subcultured with an increased concentration of pesticide (500 mg/L, i.e. 500 ppm) in another flask. The culture was serially diluted and spread over the MSM agar plates containing 500 mg/L of pyrethroid pesticide mixture. Discrete colonies capable of degrading a higher concentration of pesticide, obtained after 3–4 d of incubation, were selected for the current study.

2.3. Identification of pyrethroid pesticide utilizing halotolerant bacteria

Pyrethroid pesticide-degrading bacterial strain was purified and characterized morphologically scanning electron microscopy (SEM, Hitachi H-7650, Japan), physio-biochemically and molecular biologically (16S ribosomal deoxyribonucleic acid (rDNA)). Genomic DNA was extracted and the fragment of the 16S rDNA gene was amplified using 27F and 1492R primers. A single discrete polymerase chain reaction (PCR) amplicon band of 1,500 bp was resolved on 1.0% agarose gel and purified to remove contaminants. DNA sequence reaction (forward and reverse) for the PCR amplicon was carried out using the BDT v3.1 cycle sequencing kit on ABI 3730xl genetic analyzer. The consensus sequence of the 16S rDNA gene was generated using aligner software, sequences were matched using the BLAST program in National Center for Biotechnology Information (NCBI) Gen bank, and multiple sequence alignment using Clustal W. Distance matrix was generated and the phylogenetic tree was constructed using MEGA 7 software.

2.4. Preparation of inoculum for biodegradation study

The halotolerant bacterial strain was cultured in MSM medium containing 3% of NaCl supplemented with 100 ppm of SPs mixture as a sole carbon source for 3 d. The bacterial pellet was harvested by centrifugation at 4,000 rpm for 5 min, washed twice with phosphate buffer and resuspend with same. Inoculum of 2×10^5 CFU/mL was used for all experiments unless otherwise stated.

2.5. Optimization of various parameters influencing pyrethroid pesticides biodegradation

Growth experiment was performed with varying concentration of NaCl (1%, 3%, 5%, 7%, and 10%), varying concentration of pyrethroid pesticide mixture (50, 100, 200, 400, and 600 ppm) for 10 d with constant shaking as mentioned in section 2.2. Sample aliquots were withdrawn periodically (2 d once) for determining growth rate (pour plate) and residual pesticide concentration (gas chromatography (GC) analysis). All experiments were performed in triplicates and the non-inoculated medium was kept as control.

2.6. Biodegradation of individual pyrethroid pesticides at optimum conditions

The growth experiment was performed with an optimized concentration of NaCl and pyrethroid pesticide mixture for 10 d with constant shaking as mentioned in section 2.2. Samples were withdrawn periodically (2 d once) for

determining growth rate (pour plate) and residual pesticide concentration (GC analysis). All experiments were performed in triplicates and the non-inoculated medium was kept as control.

2.7. Extraction of pyrethroid pesticides from liquid medium

At every periodical sampling, 50 mL of MSM broth was collected and centrifuged at 10,000 rpm for 10 min to separate the bacterial cell mass. The supernatant was mixed with twice the volume of *n*-hexane (high-performance liquid chromatography (HPLC) grade) in a separating funnel, mixed briefly for 15–20 min, and allowed to separate the top organic layer. After 3 consecutive extractions with fresh *n*-hexane, the pooled organic phase was filtered through a column packed with glass wool, anhydrous sodium sulfate and silica gel, and condensed in a rotary evaporator. The dried residue was re-dissolved in 1 mL of *n*-hexane and filtered through a 0.45 μm millipore membrane before GC analysis.

2.8. GC Analysis

The pesticide residues in culture filtrate were analyzed on Thermo GC 600 system equipped with DB-5 (J&W Agilent, India) 5% Phenyl capillary column (30 m × 0.25 mm × 0.25 μm), flame ionization detector (FID) detector, and nitrogen as the carrier gas (Thermo Fisher Scientific Private Limited, India). The GC oven temperature program was set as follow: 150°C (held for 1 min), ramped to 300°C at a rate of 7°C min^{-1}, and 305°C at a rate of 1°C min^{-1}. The injection volume was 1 μL and the detector temperature was 315°C.

Metabolites of the pyrethroid pesticides were detected using gas chromatography-mass spectrometry (GC-MS) (GC-Agilent 7890 B; MS-Agilent 7000C triple code, USA) equipped with autosampler, HP-5 MS 5% phenyl methyl siloxane columns (Column 1: 30 m × 250 μm × 0.25 μm; Column 2: 0.7 m × 150 μm × 0.33 μm), with a flow rate of 1.6 mL/min (column 1), 3.23 mL/min (column 2), and helium as carrier. The oven temperature program was set as follow: 50°C (held for 1 min), ramped to 170°C at a rate of 40°C/min^{-1} (held for 3.75 min) and to 310°C at a rate of 10°C min^{-1} (held for 3 min) with a total time of 20.75 min. The injector temperature was raised to 85°C (held for 0.09 min) and ramped to 300°C at a rate of 60°C min^{-1} (held for 5 min).

2.9. Biodegradation kinetics

Pesticide biodegradation was determined by plotting the concentration of residues against time and the maximum correlation coefficients obtained were used to determine the equations of best-fit curves. For all the samples studied, the biodegradation pattern corresponded to the first-order rate equation. Confirmation of the first order was further made graphically from the linearity of the plots of 'log C' against time. The rate equation was calculated from the first-order rate equation:

$$C_t = C_0\, e^{-kt} \tag{1}$$

where C_t represents the concentration of the pesticide residue at time *t*.

C_0 represents the initial concentration and '*k*' is the rate constant per day.

The half-life values ($t_{1/2}$) were determined from the '*k*' value of each experiment.

The data obtained from the experiments were subjected to simple linear regression analysis taking the residue levels as the dependent variable and sampling day as the independent variable. The following Eq. (2) was used for verifying the fitness of the first-order kinetics of degradation [12].

$$\log y = a + bx \tag{2}$$

where the due level in μg/ml x = interval in the day; '*a*' (intercept) and '*b*' (slope) are regression constants and it is derived from the linear regression analysis.

From Eq. (2) the rate constant (*k*) was calculated. The rate constant is used to calculate the half-life by the following equation:

$$t_{1/2} = \ln 2/k \tag{3}$$

2.10. Enzyme assay

Cells from the medium drawn periodically at every sampling interval were harvested by centrifugation at 10,000 rpm for 10 min. The cell pellet was suspended in 50 mM phosphate buffer, sonicated for 5 min (Ultrasonicator – Bandelin electronics, Berlin model UW-200) and centrifuged at 10,000 rpm for 40 min to collect the supernatant. The supernatant was assessed for crude enzymes esterase, 3-phenoxy benzaldehyde dehydrogenase and 3-phenoxy benzoate dioxygenase and phenol hydroxylase spectrophotometrically at 233, 340, 295 and 340 nm respectively [9,22]. Protein concentration was examined using the Bradford method with bovine serum albumin as a standard [23].

2.11. Osmolyte identification and quantification

Cell pellets collected by centrifugation were resuspended in 70% ethanol, vortexed briefly, sonicated for 5–10 min and centrifugation at 10,000 rpm for 10 min to collect the supernatant. The extraction was repeated several times (5 times) and ethanol was removed by evaporation. The dried film was freezing to remove the excess of ethanol. Samples were suspended in D_2O and examined by ^{13}C and ^{1}H - nuclear magnetic resonance (NMR) spectroscopy [24]. Another set of freeze-dried cells was extracted with chloroform/water (1:1) and the water-soluble fraction was desalted. The samples were analyzed in HPLC with an NH_2 column and a refractive index detector. The mobile phase was acetonitrile-methanol-water (15:2:3) with a flow rate of 1 mL/min [25].

3. Results and discussion

3.1. Isolation and identification of pyrethroid pesticide degrading bacterial isolates

Physiochemical characterization of pesticide industry wastewater showed the presence of various elements, metals, and pesticides, among which pyrethroid pesticides such as deltamethrin, beta-cyfluthrin, cypermethrin, permethrin,

and lambda-cyhalothrin were present in detectable quantity. A single bacterial strain was isolated from the pesticide industry wastewater and designated as strain VCRAM-2. Conditions for enrichment and screening of microorganisms from the environment are crucial in the selection of desired bacteria for degradation studies [8]. Therefore, in the current study, screening of pesticide degrading bacteria by enrichment (MSM supplemented with SPs) allowed us to select the potent bacterial isolate (VCRAM-2) which grew well in the presence of a mixture of five pyrethroid pesticides and showed superior degradation ability.

The bacterium identified as gram-negative, facultatively anaerobic, a rod-shaped bacterium with dimensions of 720 nm × 1.31 μm (based on SEM data). In nutrient agar, it produces pinpointed, a shiny white, translucent, convex and circular colony with a smooth margin. The strain VCRAM-2 exhibits positive results for biochemical parameters such as catalase, Voges-Proskauer's, esculin hydrolysis, utilization of an array of sugar such as ornithine, trehalose, glucose, cellobiose, melibiose, mannose, maltose, lactose and raffinose; and showed negative for methyl red, indole, urease, utilization of sorbitol, sucrose and lysine. The bacteria can synthesis pyrrolidonyl arylamidase and incapable of producing α-glucuronidase, β-xylosidase, O-nitrophenyl β-D-galactopyranoside (ONPG) and phenylalanine deaminase. Phylogenetic analysis through 16S rDNA sequencing and further analysis grouped the strain VCRAM-2 among *Enterobacter species* and closely clustered with *Enterobacter ludwigii* strain BPT2 (GenBank accession number MH045945) with high identity (Fig. 1).

Previous reports evidenced the presence of *E. ludwigii* strains from the soil, rumen of migratory goat and from agricultural wastewater [26]. However, from the voluminous amount of literature survey, neither the presence of *E. ludwigii* in pesticide industry wastewater nor their efficiency in degrading pyrethroid pesticides were reported so far. Earlier reports on *E. ludwigii* states their efficiency in degrading aniline, methyl parathion, chlorpyrifos and dimethoate based pesticides [26,27]. While there are hardly any reports on biodegradation of pyrethroid pesticide in industrial wastewater by this organism. This study provides the first evidence of the facultative anaerobic *E. ludwigii* in pesticide industry wastewater.

3.2. Effect of NaCl concentration on microbial growth and pyrethroid pesticide degradation

An optimal saline (NaCl) condition for growth of halotolerant *E. ludwigii* and their efficient biodegradation of the pyrethroid pesticides mixture (cypermethrin, lambda-cyhalothrin, deltamethrin, permethrin, and cyfluthrin) was obtained by observing their growth and pesticide removal competence on culture medium (MSM) containing 100 ppm of pyrethroid pesticide mixture with varying concentration of NaCl (1%–10%). The population of halotolerant bacterium after 6 d of incubation was observed to be 1×10^7 CFU/mL on 1%, 7×10^8 CFU/mL on 3% and 3×10^6 CFU/mL on 5% NaCl supplemented medium, which was declined after 10 d. No significant increase in growth of *E. ludwigii* was observed on 7% and 10% NaCl concentrations, which evidenced a

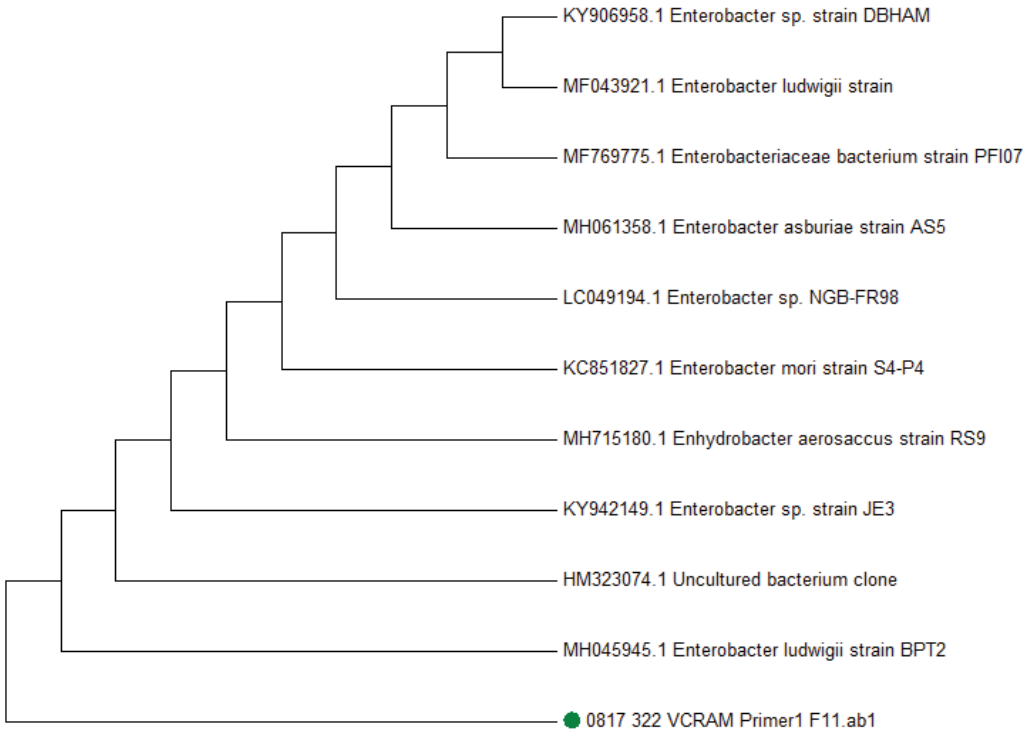


Fig. 1. Phylogenetic tree of isolated VCRAM-2 constructed using software MEGA 7. The numbers represent the sequence accession number in GenBank.

complete intolerance of the bacterium under very high saline conditions. Observation by Li et al. [28] justifies the current finding, where a decreased microbial growth was observed above 7% of NaCl. The decrease in growth might due to lysis of the microbial cell due to the osmotic stress exerted by the aqueous medium. The microbial growth at different NaCl concentrations showed that 3% NaCl concentration is suitable for the growth of *E. ludwigii* (Fig. 2a). A comparable result was reported by Roy [29] in *E. ludwigii*, isolated from the nodules of Fenugreek, which can tolerate saline up to 500 mm (2.9 %).

The residual concentration of the pesticide mixture, analyzed by GC-FID, under different saline (NaCl) conditions at varying days of incubation revealed that some of the

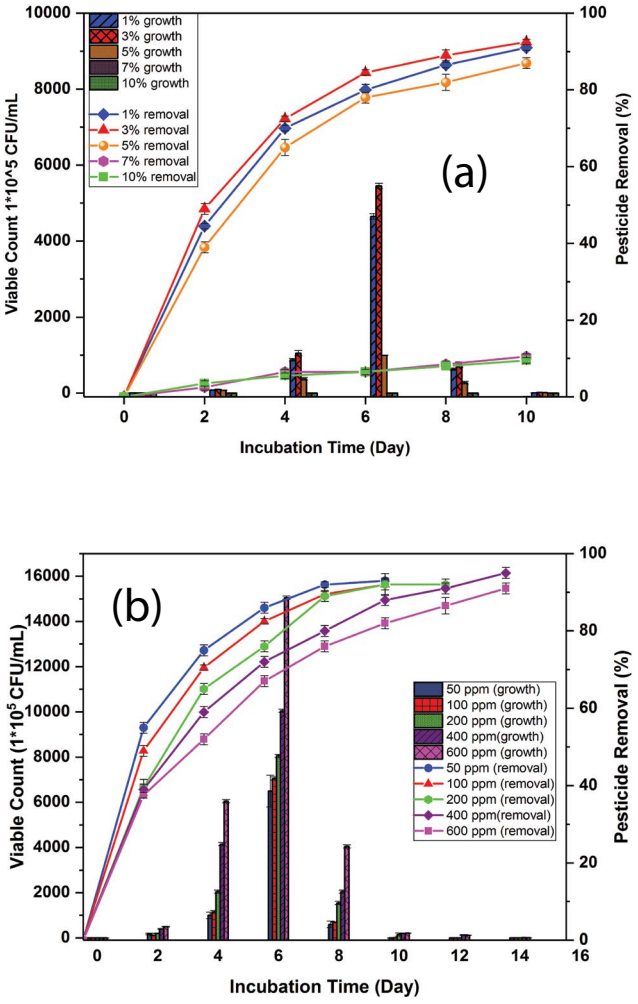


Fig. 2. Optimization of biodegradation of pyrethroid pesticides by *Enterobacter ludwigii* strain. (a) Effect of NaCl concentration. The experimental conditions were 1% to 10% NaCl at 100 ppm of pesticide concentration. (b) Effects of different concentration of pesticide. The experimental conditions were 50 to 600 ppm of pesticide concentration at 3% NaCl. Column represent the growth of bacterium (1×10^5 CFU/mL) and line indicates the pesticide removal efficiency (%). The sampling time was 2 d. Each value represents the mean of three replicates. Standard error indicates the standard deviation.

pyrethroid pesticides contain 2 or 3 isomeric forms which were combined and considered as cypermethrin and cyfluthrin. Each pesticide was identified based on its retention time, and the concentration of pesticide was calculated from the area under the peaks by comparing with standard peaks. The degradation of the pyrethroid pesticide mixture on 6th, 8th and 10th day of incubation at 1% of NaCl was 80%, 87%, and 91%; 3% of NaCl was 85%, 89%, and 93%; and 5% of NaCl was 78%, 82%, and 87%, respectively. Analysis showed that majority of the pesticides was degraded on the 8th day and the efficiency of degradation at different NaCl concentrations was in the order of 3% NaCl > 1% NaCl > 5% NaCl > 7% NaCl > 10% NaCl. The mechanism by which the *E. ludwigii* aids in the biodegradation of the pesticide compounds is that the bacterium utilizes toxic organic compound as sole carbon source, and organic compound enters into the halotolerant bacteria to equilibrate the osmotic pressure through osmosis [18].

Though biodegradation of the pesticide mixture was appreciable under all the three NaCl concentrations, degradation was more efficient under 3% NaCl concentration (Fig. 2a). The lower degradation efficiency of *E. ludwigii* under 1% NaCl concentration may be attributed to its halotolerant nature, which perhaps demands more salinity, indicates that excess salinity is required for the growth of the bacterial consortium. On the other hand, the decreased performance of the bacterium under 5% NaCl conditions might due to the onset of intolerance under higher saline conditions [30]. Shapir et al. [31] findings support this point of view, where it was proved that *Pseudomonas* sp. strain ADP was capable of efficiently degrading 25 ppm of atrazine pesticide at 3% NaCl concentration after 8 d, whereas, 5% NaCl concentration was found to inhibit the growth of *Pseudomonas* sp. ADP. High salt concentrations cause disintegration of cells because of the loss of cellular water (plasmolysis) or recession of the cytoplasm which is induced by an osmotic difference across the cell wall and cause of the outward flow of intracellular water resulting in the loss of microbial activity and cell dehydration [32]. It was concluded that 3% NaCl was the optimum concentration for the growth and effective biodegradation of the pyrethroid mixture by the identified *E. ludwigii* strain. The bacterial growth pattern in the present research was consistent with similar studies for bioremediation and treatment of organic matter [32].

3.3. Effect of substrate concentration on microbial growth and pyrethroid pesticide degradation

The growth pattern of the halotolerant bacterium *E. ludwigii* on MSM medium supplemented with different concentrations of pyrethroid pesticides (50, 100, 200, 400, and 600 ppm), at 3% NaCl revealed that *E. ludwigii* was capable of growing at all the five tested concentrations (Fig. 2b). Growth of the bacteria at 100, 200, 400 and 600 ppm of pesticide mixture increases up to 7×10^8 CFU/mL, 12×10^8 CFU/mL, 1×10^9 CFU/mL and 2×10^9 CFU/mL, respectively, after 6 d of incubation. The growth of bacterial species increases with the period of incubation, irrespective of pesticides concentration studied. It was clear from the results that the increasing concentration of pesticide does not inhibit the growth of *E. ludwigii* under saline condition, even at the highest

pesticide concentration of 600 ppm. An earlier report on *Streptomyces aureus* strain HP-S-01 justifies that the microbial biomass increases with the increasing concentration of deltamethrin [12].

The *E. ludwigii* degraded 96% of aniline (100 mg/L) in 7 d under non-saline condition [27] and degraded oxamyl (200 ppm) after 6 d of incubation [33]. The isolated strain degraded pyrethroid pesticides at a concentration of 100 ppm and 200 ppm with an efficiency of 95% and 92%, respectively, in 10 d of incubation without other carbon sources (Fig. 2b). The current result was appreciable when compared to previous findings by Gur et al. [34] on *Stenotrophomonas maltophilia* OG2, which required an additional carbon source (glucose) for achieving a degradation of 81% for pyrethroid pesticides. Effective biodegradation up to 92% was achieved on higher pesticide concentrations of 400 ppm and 600 ppm when incubated for 14 d (Fig. 2b). Findings on biodegradation of deltamethrin by *Streptomyces aureus* Strain HP-S-01 showed complete degradation of 100–300 ppm concentration within 5–7 d under non-saline condition, which required an extended period for complete degradation when concentration increases to 400–800 ppm [12]. This might due to the extended lag phase of the strain for acclimatizing to a higher concentration of pesticide, before their accelerated degradation [35]. Hu et al. [36] reported that the degradation of beta-cypermethrin by *Bacillus cereus* strain BCC01 slowed down when the growth of the strain reaches the stationary period, suggesting the pyrethroid-degrading ability of BCC01 was closely related to the tendency of bacterial proliferation. However, it is important to note that the *E. ludwigii* strain was capable of degrading almost all the substrate (pesticide) concentrations studied.

3.4. Biodegradation of individual pyrethroid pesticides under optimized condition

The biodegradation pattern of all the five pyrethroid pesticides was assessed individually under optimal NaCl (3%) and pesticide concentrations (100 ppm). The biodegradation percentage of lambda-cyhalothrin, permethrin, beta-cyfluthrin, cypermethrin, and deltamethrin was detected to be 93%, 94%, 99%, 97% and 94%, respectively, after 10 d of incubation (Fig. 3). The rate of biodegradation of pyrethroid pesticides followed a pattern where beta-cyfluthrin > cypermethrin > permethrin > lambda-cyhalothrin and >deltamethrin. This observation was in agreement with the previous findings in a non-halotolerant bacterium *Ochrobactrum lupini* DG-S-01, which effectively degrade each pyrethroid pesticide in the following manner: beta-cypermethrin > cyfluthrin and fenpropathrin > cyhalothrin and deltamethrin [11]. Whereas, *Bacillus thuringiensis* strain ZS-19 degrade the pyrethroids in a reverse pattern: cyhalothrin > fenpropathrin > deltamethrin > cyfluthrin > beta-cypermethrin > bifenthrin [37]. This prototype of degradation was reported to be based on the chemical and structural features of the pyrethroid pesticides which favor the degradation of compounds by the bacterial species. It was found that there was no substrate specificity for a wide variety of pyrethroids and the order of pyrethroids utilization might be changed depending on the ability of enzymes produced by the bacterial strain.

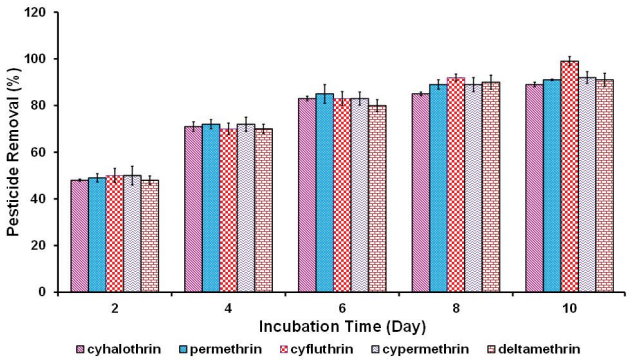


Fig. 3. Degradation of pyrethroid pesticide by *Enterobacter ludwigii* strain under optimized condition. The experimental conditions were 100 ppm of pesticide concentration, pH of 7.0 and 3% NaCl. The sampling time was 2 d. Each value represents the mean of three replicates. Standard error indicates the standard deviation.

3.5. Biodegradation kinetics

To quantify the degradation effects of a mixture of pyrethroids by *E. ludwigii* half-life ($t_{1/2}$) and degradation rate constant (k) from the first-order kinetic model was calculated. Significantly, that the biodegradation rate and the pesticide concentration were directly proportional and the reaction followed to study the first-order kinetics. From the kinetic data, it was clear that the biodegradation kinetics fitted well for the first-order rate equation with the regression coefficient in the range of 0.9667–0.9979 (Table 1). A half-life period of 26 and 28 d was observed for the biodegradation of 100 ppm of pesticide mixture at 7% NaCl and 10% NaCl, respectively (Table 1). This shows that 7% NaCl and 10% NaCl was the most intolerable saline condition for the biodegradation of the pesticides by this halotolerant *E. ludwigii* strain. Under optimum conditions, the rate constants of different pyrethroids such as cypermethrin, beta-cyfluthrin, deltamethrin, lambda-cyhalothrin, and permethrin in the pyrethroid pesticide mixture were found to be: 0.3367, 0.5054, 0.282, 0.2644, and 0.2843 d^{-1}. Similarly, the half-life of the above said pyrethroids were observed to be 2.0585, 1.3714, 2.4578, 2.6214 and 2.4379 d, respectively (Table 1). An earlier report by Chen et al., [11] in *Ochrobactrum lupini* reported a half-life of 1.9, 2.3, 2.7, 6.5 and 8.2 d interval under non-saline condition. This reveals that the halotolerant bacterium *E. ludwigii* is capable of biodegrading pyrethroid pesticides more rapidly than non-halotolerant bacterial species.

3.6. Identification of metabolites of pyrethroid pesticide on degradation

The intermediates of the pesticide biodegradation were identified by GC-MS, and the compounds tabulated in Table 2 shows metabolites of all the five SPs employed. Twenty different metabolites were observed from the analysis of which seven compounds (from compound 14–20) were observed as whole or slight modified pyrethroid pesticide, and thirteen compounds were their metabolites. Detection of 3-phenoxy benzaldehyde revealed that the composite structure of the pyrethroid pesticides included a carboxyl ester linkage, which was hydrolyzed by the microbial activity

Table 1
Kinetic parameters for degradation of pyrethroid pesticides by *Enterobacter ludwigii*

S. No	Study parameters	Kinetic model	k d^{-1}	$t_{1/2}$ (Day)	R^2 value
1	1% NaCl	$Y = 0.193X + 0.0581$	0.193	1.7953	0.9667
2	3% NaCl	$Y = 0.3027X + 0.0281$	0.3027	2.2894	0.9957
3	5% NaCl	$Y = 0.2417X + 0.0581$	0.2417	1.4336	0.9809
4	7% NaCl	$Y = 0.0133X - 0.0014$	0.0133	26.0526	0.9845
5	10% NaCl	$Y = 0.0126X + 0.0071$	0.0126	27.6096	0.9878
6	50 ppm	$Y = 0.272X + 0.1947$	0.272	2.5481	0.969
7	100 ppm	$Y = 0.3027X + 0.0281$	0.3027	2.2894	0.9957
8	200 ppm	$Y = 0.2526X + 0.0195$	0.2526	2.7435	0.9979
9	400 ppm	$Y = 0.2029X + 0.03$	0.2029	2.2894	0.9889
10	600 ppm	$Y = 0.1644X + 0.0692$	0.1644	4.2153	0.9951
11	Cypermethrin	$Y = 0.3367X + 0.0552$	0.3367	2.0585	0.9878
12	Beta-Cyfluthrin	$Y = 0.5054X + 0.3805$	0.5054	1.3714	0.8984
13	Permethrin	$Y = 0.282X + 0.0367$	0.282	2.4578	0.9937
14	Lambda-Cyhalothrin	$Y = 0.2644X + 0.0995$	0.2644	2.6214	0.9936
15	Deltamethrin	$Y = 0.2843X + 0.0886$	0.2843	2.4379	0.9933

Table 2
Mass spectrometric detection of metabolites produced during the degradation of pyrethroid pesticides by *Enterobacter ludwigii*

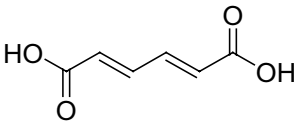
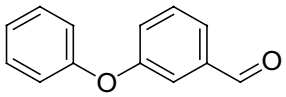
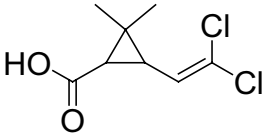
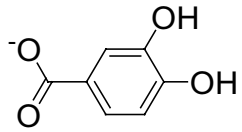
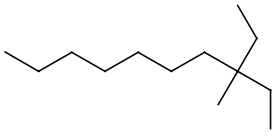
S. No	Retention time	Molecular formula	Molecular weight	Identified molecular structure and compound name
1	10.89	$C_6H_6O_4$	142	 Muconic Acid
2	12.85	$C_{13}H_{10}O_2$	198	 Benzaldehyde, 3-phenoxy-
3	15.01	$C_9H_{13}Cl_2O_2$	224	 3-2,2-Dichloro-vinyl-2,2-dimethyl cyclopropane carboxylic acid
4	17.50	$C_7H_5O_4$	153	 3,4-Dihydroxy benzoate
5	18.97	$C_{13}H_{28}$	184	 Decane, 3-ethyl-3-methyl-

Table 2 *(Continued)*

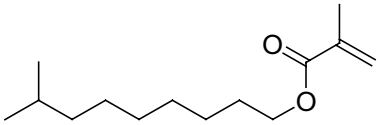
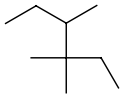
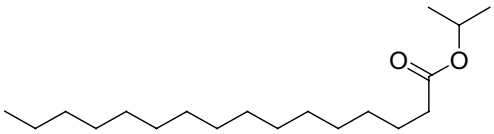
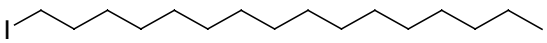
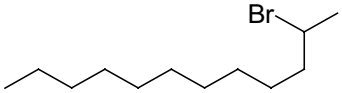
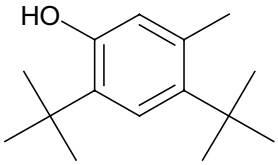
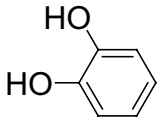
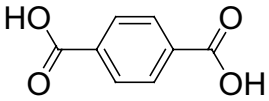
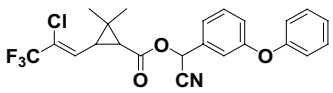
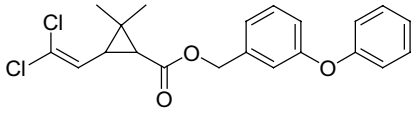
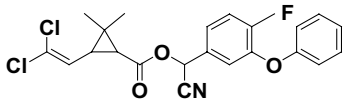
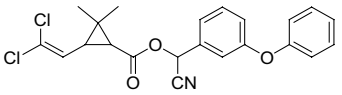
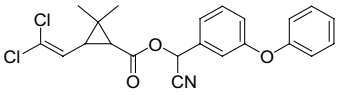
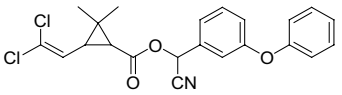
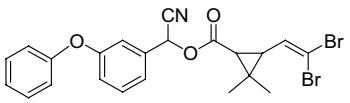
S. No	Retention time	Molecular formula	Molecular weight	Identified molecular structure and compound name
6	19.06	$C_{14}H_{26}O_2$	226	 Isodecyl methacrylate
7	19.15	C_9H_{20}	128	 Hexane, 3,3,4-trimethyl-
8	20.02	$C_{19}H_{38}O_2$	298	 Isopropyl Palmitate
9	23.00	$C_{16}H_{33}I$	352	 Hexadecane, 1-iodo-
10	25.90	$C_{12}H_{25}Br$	248	 2-Bromo dodecane
11	28.50	$C_{15}H_{24}O$	220	 4,6-di-tert-Butyl-m-cresol
12	31.20	$C_6H_6O_2$	110	 Benzene- 1,2-diol
13	36.20	$C_8H_6O_4$	166	 Terepthalic Acid
14	39.24	$C_{23}H_{19}ClF_3NO_3$	449	 3-(2-Chloro-3,3,3-trifluoro-propenyl)-2,2-dimethyl-cyclopropanecarboxylic acid cyano-(3-phenoxy-phenyl)-methyl ester
15	42.72	$C_{21}H_{20}Cl_2O_3$	390	 3-(2,2-Dichloro-vinyl)-2,2-dimethyl-cyclopropanecarboxylic acid 3-phenoxy-benzyl ester

Table 2 *(Continued)*

S. No	Retention time	Molecular formula	Molecular weight	Identified molecular structure and compound name
16	44.25	$C_{22}H_{18}Cl_2FNO_3$	433	 3-(2,2-Dichloro-vinyl)-2,2-dimethyl-cyclopropanecarboxylic acid cyano-(4-methyl-3-phenoxy-phenyl)-methyl ester
17	44.56	$C_{22}H_{19}Cl_2NO_3$	415	 3-(2,2-Dichloro-vinyl)-2,2-dimethyl-cyclopropanecarboxylic acid cyano-(3-phenoxy-phenyl)-methyl ester
18.	44.66	$C_{22}H_{19}Cl_2NO_3$	415	 3-(2,2-Dichloro-vinyl)-2,2-dimethyl-cyclopropanecarboxylic acid cyano-(3-phenoxy-phenyl)-methyl ester
19	44.73	$C_{22}H_{19}Cl_2NO_3$	415	 3-(2,2-Dichloro-vinyl)-2,2-dimethyl-cyclopropanecarboxylic acid cyano-(3-phenoxy-phenyl)-methyl ester
20	45.02	$C_{22}H_{19}Br_2NO_3$	505	 3-(2,2-Dibromo-vinyl)-2,2-dimethyl-cyclopropanecarboxylic acid cyano-(3-phenoxy-phenyl)-methyl ester

of the *E. ludwigii*. [34]. The transient accumulation of intermediate compounds of pesticide on degradation such as 3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropanecarboxylic acid, 3,4-dihydroxybenzoate, 4,6-di-tert-butyl-m-cresol, benzene-1,2-diol, terephthalic acid demonstrated a progressive degradation of the pesticide through co-metabolism [10]. Detection of muconic acid, a breakdown product of phenol [22] and medium-chain aliphatic compounds (S.No. 5–10 in Table 2) evidenced the utmost degradation of pesticides as per Deng et al. [38]. The GC-MS analysis revealed the release of no toxic end product as a result of this biodegradation. Thus, all pyrethroid pesticides were utilized rapidly without any substrate inhibition or toxicity, which reveals that the halotolerant *E. ludwigii* may effectively be employed as a biological agent for the potential treatment of saline wastewater containing pesticides.

3.7. Enhancement of degradation by microbial enzymes

Bacteria have several mechanisms that allow them to tolerant or resist toxic pollutants and one among them was the release of different enzymes for metabolizing toxic compounds [39]. Enzyme activity was used to authenticate the biodegradation of pyrethroid pesticide and the main reaction to the co-metabolic degradation of toxic chemicals by microorganisms was relatively complex. The microbes can easily utilize the biodegradable organic contaminant by producing catabolic enzymes with wide substrate specificity. All four enzymes analyzed were required for the degradation of cypermethrin and other pyrethroid pesticides. In detail, the esterase hydrolyzes the fragile ester linkage to break the compound to yield 3-phenoxybenzaldehyde. This compound was further modified to 3-phenoxybenzoic acid with the action of enzyme 3-phenoxybenzaldehyde dehydrogenase, where 3-phenoxybenzoic acid was metabolized to protocatechuic acid and phenol through diphenyl ether cleavage [9] by the activity of 3-phenoxybenzoate dioxygenase. Protocatechuic acid was further metabolized through the ortho cleavage pathway and Phenol was oxidized to catechol, which was further metabolized through the ortho cleavage pathway by the activity of enzyme phenol-hydroxylase.

Esterase activity showed a gradual decrease in absorbance and witnessed a significant change after 6 d of incubation due to the disappearance of the SP pesticides. 3-phenoxybenzaldehyde dehydrogenase activity was increased up to 6th day and started decreasing afterward (Fig. 4a). The decreasing level of esterase decipher the complete cleavage of ester linkage after 2nd day and gradual decrease of 3-phenoxy benzaldehyde dehydrogenase after 6th day evidenced the breakdown of the majority of SP pesticide. 3-phenoxybenzoic acid persists towards microbial degradation and limits the process because of its antimicrobial activity [14,38].

The increasing level of 3-phenoxybenzoate dioxygenase on time indicates the efficient degradation of pyrethroid intermediates into smaller and less toxic compounds. On the other hand, the activity of phenol-hydroxylase increases exponentially until the 8th day and subsides to one half on the 10th day (Fig. 4a). The progressive increase of enzymes 3-phenoxybenzoate dioxygenase and phenol hydroxylase showed the effective degradation of the SP pesticide into smaller molecules such as phenol [9]. The decrease in the level of phenol hydroxylase, observed on the 10th day might be due to substrate inhibition mechanism of phenol [22]. This evidenced the complete breakdown of pesticides into non-toxic compounds. The above results evidenced that the enzymes secreted by the *E. ludwigi* proceed the biodegradation of SP pesticides.

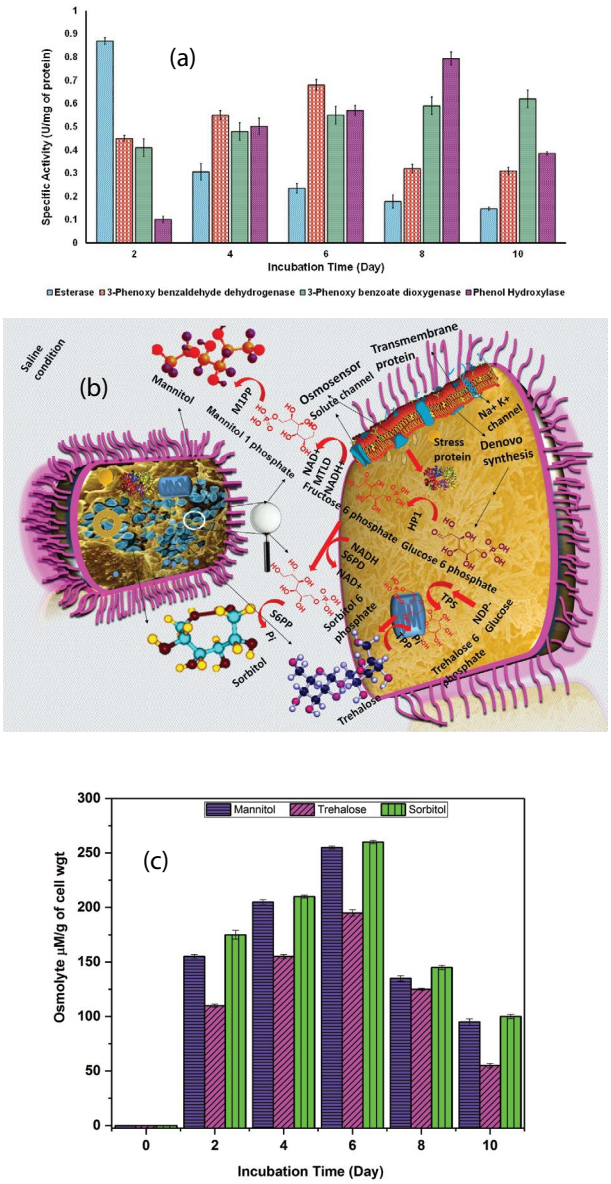


Fig. 4. (a) Specific enzyme activity by *Enterobacter ludwigii* strain. (b) Mechanism of osmolyte accumulation by halotolerant bacteria. HPI-Hexose Phosphate Isomerase, MTLD-Mannitol-1-Phosphate Dehydrogenase, M1PP-Mannitol-1-Phosphate Phosphatase, S6PD-Sorbitol-6-Phosphate Dehydrogenase, S6PP-Sorbitol-6-Phosphate Phosphatase, TPS-Trehalose-6-Phosphate Synthase, TPP-Trehalose-6-Phosphate phosphatase. (c) Varying osmolyte concentrations (µM/g of cell wt.) of *Enterobacter ludwigii*. The growth conditions was 100 ppm pesticide concentration, 3% NaCl at pH 7.0. The sampling time was 2 d. The values are mean of three replicates and error bar represent the standard deviation.

3.8. Osmolytes on salt tolerance maintenance

The ability of the halotolerant bacteria to produce osmolyte is one of the adaptation mechanisms to improve the suitable condition for the growth of bacteria in osmotic condition, which then positively influence the efficiency of enzymes or metabolites production. The bacterium was believed to withstand the higher concentrations, acclimatizing to the extreme toxic condition, by secreting inducers called osmolytes through activation of specific genes (Fig. 4b). Halotolerant bacteria usually synthesize or accumulate compatible solutes to maintain the osmotic equilibrium in response to the high-salt external environment. These are compatible with the intracellular machinery even at molar concentrations and maintain the cell volume, turgor and electrolyte concentrations within the cell system (Fig. 4b). Accumulation of osmolyte was an important responsibility for the halotolerant bacterium to adjust the osmotic stress when developed in elevated saline conditions. As a result, an appropriate hydration level of the cytoplasm is achieved and cell growth can proceed under osmotically unfavorable conditions [40].

In general, osmolytes are known to alter the chemical potential of proteins in the native and the unfolded states to different extents. NMR was employed to qualitatively analyze the osmolytes synthesized in the cytoplasm of *E. ludwigii* under saline condition. The results revealed that mannitol, sorbitol, and trehalose were accumulated in the cytoplasm of *E. ludwigii* during the degradation of pyrethroid pesticides. Denovo synthesis and mechanism of the compatible solute strategy was described by Nissen et al. [41] (Fig. 4b). Trehalose is essential to withstand the salinity and acts as a cryopreservant for biological molecules in the absence of exogenous osmolytes. Mannitol was accumulated by yeasts and sorbitol plays a role in the enhancement of protein synthesis and turn-over rate [42]. Being chemical chaperons, these compatible solutes exhibit protein-stabilizing property which helps in the proper folding of polypeptide chains. These compatible solutes, rather than changing the structure of the protein, exert their effect through changing the solvent structure and/or subtle changes in the dynamic properties of the protein [40].

On the other hand, the osmolytes produced in the cytoplasm of the halotolerant bacterial strain *E. ludwigii* was quantitated using HPLC. Concentration of osmolytes gradually increases till 250 µM/g of cell weight till the 6 d of biodegradation and decreases their after up to 100 µM/g on the subsequent days (Fig. 4c). The concentration of Sorbitol and Mannitol was high compare to trehalose in all the samples analyzed. Sorbitol precedes mannitol in all the samples analyzed (Fig. 4c). This is well correlated with the previous

findings by Roberts et al. [42], where halotolerant bacteria such as *Sulfolobus solfataricus* and *S. ambivalens*; *Thermoproteus tenax*; *Thermoplasma acidophilum* and *Pyrobaculum aerophilum* synthesized trehalose as an osmolyte to tolerate the higher saline condition. Osmolytes may undergo unfavorable interactions with the peptide backbone in the unfolded state of the protein which leads to strengthening its secondary structure (an intermediate structure in the folding pathway) before attaining the folded conformation [43].

4. Conclusions

This is the novel report of the halotolerant bacterium *E. ludwigii* isolated from pyrethroid pesticide manufacturing industry wastewater. The adaptability of the strain to the saline environment through compatible solute strategy and enzyme activity showed its excellent stability in degrading pyrethroid pesticide. This is the first evidence of mannitol, sorbitol and trehalose synthesis by halotolerant strain, *E. ludwigii* which degrade different combinations of pyrethroid pesticide as the sole carbon source under saline condition. The maximum degradation of pesticide and its metabolites was achieved without the accumulation of any toxic products. This result will expand the application of *E. ludwigii* in the treatment of pesticide-contaminated wastewater

The study revealed that the strain exhibited an appreciable rate of growth at an optimal concentration of 3% NaCl and 100 ppm substrate with 90% of SPs degradation before the 8th day of treatment. An utmost degradation of SPs and their metabolites was achieved via the production of various metabolic enzymes and osmolytes which accumulates inside the cytoplasm. To the best of our knowledge, this is the first-ever report on the efficient degradation of pyrethroids by adapting hypersaline condition of SP pesticide industrial wastewater by the novel *E. ludwigii* strain.

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Reference

[1] C. Zhang, S.H. Wang, Y.C. Yan, Isomerization and biodegradation of betacypermethrin by *Pseudomonas aeruginosa* CH7 with biosurfactant production, Bioresour. Technol., 102 (2011) 7139–7146.
[2] M. Cycon, Z. Piotrowska-Seget, Pyrethroid-degrading microorganisms and their potential for the bioremediation of contaminated soils: a review, Front. Microbiol., 7 (2016) 1463.
[3] R. McKinlay, J.A. Plant, J.N. Bell, N. Voulvoulis, Endocrine disrupting pesticides: implications for risk assessment, Environ. Int., 34 (2008) 168–183.
[4] J. Fei, J.H. Qua, X.L. Ding, K. Xue, C.C. Lu, J.F. Chen, L. Song, Y.K. Xia, S.L. Wang, X.R. Wang, Fenvalerate inhibits the growth of primary cultured rat preantral ovarian follicles, Toxicology, 267 (2010) 1–6.
[5] A.H. Gu, X.G. Shi, C. Yuan, G.C. Ji, Y. Zhou, Y. Long, L. Song, S.L. Wang, X.R. Wang, Exposure to fenvalerate causes brain impairment during zebrafish development, Toxicol. Lett., 197 (2010) 188–192.
[6] E. Aksakal, S.B. Ceyhun, O. Erdogan, D. Ekinci, Acute and long-term genotoxicity of deltamethrin to insulin-like growth factors and growth hormone in rainbow trout, Comp. Biochem. Physiol. C: Toxicol. Pharmacol., 152 (2010) 451–455.
[7] A. Agrawal, R.S. Pandey, B. Sharma, Water pollution with special reference to pesticide contamination in India, J. Water Resour. Prot., 2 (2010) 432–448.
[8] Y. Gao, S.H. Chen, M.Y. Hu, Q.B. Hu, J.J. Luo, Y.N. Li, Purification and characterization of a novel chlorpyrifos hydrolase from *Cladosporium cladosporioides* Hu-01, PLoS One, 7 (2012) 1–7.
[9] P.N. Tallur, V.B. Megadi, H.Z. Ninnekar, Biodegradation of cypermethrin by Micrococcus sp. strain CPN 1, Biodegradation, 28 (2008) 77–82.
[10] P. Guo, B. Wang, B. Hang, L. Li, S.W. Ali, J. He, S. Li, Pyrethroid degrading Sphingobium sp. JZ-2 and the purification and characterization of a novel pyrethroid hydrolase, Int. Biodeterior. Biodegrad., 63 (2009) 1107–1112.
[11] S. Chen, M. Hu, J. Liu, G. Zhong, L. Yang, M. Rizwan-ul-Haq, H. Han, Biodegradation of beta-cypermethrin and 3-phenoxybenzoic acid by a novel *Ochrobactrum lupini* DG-S-01, J. Hazard. Mater., 187 (2011a) 433–440.
[12] S. Chen, K. Lai, Y. Li, M. Hu, Y. Zhang, Y. Zeng, Biodegradation of deltamethrin and its hydrolysis product 3-phenoxybenzaldehyde by a newly isolated Streptomyces aureus strain HP-S-01, Appl. Microbiol. Biotechnol., 90 (2011b) 1471–1483.
[13] S.H. Chen, Q.B. Hu, M.Y. Hu, J.J. Luo, Q.F. Weng, K.P. Lai, Isolation and characterization of a fungus able to degrade pyrethroids and 3-phenoxybenzaldehyde, Bioresour. Technol., 102 (2011c) 8110–8116.
[14] W.J. Xie, J.M. Zhou, H.Y. Wang, X.Q. Chen, Effect of nitrogen on the degradation of cypermethrin and its metabolite 3-phenoxybenzoic acid in soil, Pedosphere, 18 (2008) 638–644.
[15] C. Yuan, C. Wang, S.Q. Gao, T.T. Kong, L. Chen, X.F. Li, L. Song, Y.B. Wang, Effects of permethrin, cypermethrin and 3-phenoxybenzoic acid on rat sperm motility in vitro evaluated with computer-assisted sperm analysis, Toxicol. in Vitro, 24 (2010) 382–386.
[16] K. Veenagayathri, N. Vasudevan, Degradation of 4-chlorophenol by a moderately halophilic bacterial consortium under saline conditions, Br Microbiol. Res. J., 3 (2013) 513–524.
[17] S. Silambarasan, J. Abraham, Halophilic bacterium JAS4 in biomineralisation of endosulfan and its metabolites isolated from *Gossypium herbaceum* rhizosphere soil, J. Taiwan Inst. Chem. Eng., 45 (2014) 1748–1756.
[18] T. Oncescu, P. Oancea, M. Enache, G. Popescu, L. Dumitru, M. Kamekura, *Halophilic bacteria* are able to decontaminate dichlorvos, a pesticide, from saline environments, Cent. Eur. J. Biol., 2 (2007) 563–573.
[19] S.I. Abou-Elela, M.M. Kamel, M.E. Fawzy, Biological treatment of saline wastewater using a salt-tolerant microorganism, Desalination, 250 (2010) 1–5.
[20] L.C. Castillo-Carvajal, J.L. Sanz-Martín, B.E. Barragán-Huerta, Biodegradation of organic pollutants in saline wastewater by halophilic microorganisms: a review, Environ. Sci. Pollut. Res., 21 (2014) 9578–9588.
[21] Y. Sharifi, A.A. Pourbabaei, A. Javadi, M.H. Abdolmohammad, M. Saffari, A. Morovvati, Biodegradation of glyphosate herbicide by *Salinicoccus* spp. isolated from Qom Hoze-soltan lake, Iran, Environ. Health Eng. Manag. J., 2 (2015) 31–36.
[22] S.C. Tsai, L. Tsai, Y. Li, An isolated *Candida albicans* TL3 capable of degrading phenol at large concentration, Biosci. Biotechnol., Biochem., 69 (2005) 2358–2367.
[23] M.M. Bradford, A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding, Anal. Biochem., 72 (1976) 248–254.
[24] D. Desmarais, P.E. Jablonski, N.S. Fedarko, M.F. Roberts, 2-Sulfotrehalose, a novel osmolyte in Haloalkaliphili, Archaea, J. Bacteriol., 179 (1997) 3146–3153.

[25] L.T. Smith, Role of osmolytes in adaptation of osmotically stressed and chill-stressed listeria monocytogenes grown in liquid media and on processed meat surfaces, Appl. Environ. Microbiol., 62 (1996) 3088–3093.
[26] Y. Zhao, P. Zhao, Y. Wang, W. Qi, Isolation, identification, and characterization of an organophosphorous pesticide degrading bacterium, *Enterobacter ludwigii* M2, Adv. Mater. Res., 1051 (2014) 398–403.
[27] F. Kafilzadeh, A. Khezri, Biodegradation of aniline by *Enterobacter ludwigii* kh-a5 isolated from the soil around shiraz refinery, Iran, Global Nest J., 18 (2016) 697–707.
[28] T. Li, X.P. Deng, J.J. Wang, H. Zhao, L. Wang, K. Qian, Biodegradation of 3,4-dichloroaniline by a novel *Myroides odoratimimus* strain LWD09 with moderate salinity tolerance, Water Air Soil Pollut., 223 (2012) 3271–3279.
[29] A. Roy, Comparative study of three rhizospheric bacteria belonging to different genera, co-infecting a leguminous plant, J. Invest. Genomics, 3 (2016) 1–12.
[30] K. Veenagayathri, N. Vasudevan, Effect of pH, nitrogen sources and salts on the degradation of phenol by the bacterial consortium under saline conditions, Int. J. Biotechnol. Biochem., 6 (2010) 783–971.
[31] N. Shapir, R.T. Mandelbaum, H. Gottlieb, Atrazine degradation in saline wastewater by Pseudomonas sp. strain ADP, J. Ind. Microbiol. Biotechnol., 20 (1998) 153–159.
[32] L.F. Martins, R.S. Peixoto, Biodegradation of petroleum hydrocarbons in hypersaline environments, Braz. J. Microbiol., 43 (2012) 865–872.
[33] T.E. Radwan, A.M. Reyad, A.M. Essa, Bioremediation of the nematicide oxamyl by *Enterobacter ludwigii* isolated from agricultural wastewater, Egypt. J. Exp. Biol., 13 (2017) 19–30.
[34] O. Gur, M. Ozdal, O.F. Algur, Biodegradation of the synthetic pyrethroid insecticide α-cypermethrin by *Stenotrophomonas maltophilia* OG2, Turk. J. Biol., 38 (2014) 684–689.
[35] M. Cycon, M. Wojcik, Z. Piotrowska-Seget, Biodegradation kinetics of the benzimidazole fungicide thiophanate-methyl by bacteria isolated from loamy sand soil, Biodegradation, 22 (2011) 573–583.
[36] W. Hu, Q. Lu, G. Zhong, M. Hu, X. Yi, Biodegradation of pyrethroids by a hydrolyzing carboxylesterase EstA from *Bacillus cereus* BCC01, Appl. Sci., 9 (2019) 477–491.
[37] S. Chen, Y. Deng, C. Chang, J. Lee, Y. Cheng, Z. Cui, J. Zhou, F. He, M. Hu, L. Zhang, Pathway and kinetics of cyhalothrin biodegradation by *Bacillus thuringiensis* strain ZS-19, Sci. Rep., 5 (2015) 1–10.
[38] W. Deng, D. Lin, K. Yao, H. Yuan, Z. Wang, J. Li, L. Zou, X. Han, K. Zhou, L. He, X. Hu, Characterization of a novel β-cypermethrin-degrading Aspergillus niger YAT strain and the biochemical degradation pathway of β-cypermethrin, Appl. Microbiol. Biotechnol., 99 (2015) 8187–8198.
[39] K.P. Talaro, Foundation in Microbiology: Basic Principles, 7th ed., McGraw-Hill Higher Education, New York, USA, 2008, pp. 534.
[40] P. Shivanand, G. Mugeraya, Halophilic bacteria and their compatible solutes – osmoregulation and potential applications, Curr. Sci., 10 (2011) 1516–1521.
[41] L. Nissen, G.P. Martinez, M.J. Yebra, Sorbitol synthesis by an engineered *Lactobacillus casei* strain expressing a sorbitol-6-phosphate dehydrogenase gene within the lactose operon, FEMS Microbiol. Lett., 249 (2005) 177–183.
[42] M.F. Roberts, Organic compatible solutes of halotolerant and halophilic microorganisms, Saline Syst., 1 (2005) 5.
[43] E. Judy, N. Kishore, Biological wonders of osmolytes: the need to know more, Biochem. Anal. Biochem., 5 (2016) 304.