



(RESEARCH ARTICLE)



ISOLATION, MOLECULAR IDENTIFICATION AND IMIDACLOPRID TOLERANCE OF HALOTOLERANT ENDOPHYTIC BACTERIA ASSOCIATED WITH NATIVE HALOPHYTES OF THE LITTLE RANN OF KUTCH, GUJARAT, INDIA

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ABSTRACT

Little is known about the bacteria living inside the plants of the Little Rann of Kutch, a hypersaline desert in western India, and its culturable microbiota has barely been described at all. Imidacloprid is sprayed heavily on the farmland around the Rann, it lingers in soil, and it turns up in groundwater, so bacteria that can both survive salt and act on the compound are worth looking for. We collected roots of nine halophyte species at four sites inside the Wild Ass Sanctuary, surface sterilized and macerated them, then enriched the macerates in yeast extract mannitol broth carrying 5% (w/v) sodium chloride at pH 9. Eleven isolates came out of this. Each was described morphologically and biochemically, and partial 16S rRNA gene sequences were matched against public databases. Tolerance was tested on agar amended with a commercial imidacloprid formulation, and supernatants from the four best growers went through reversed-phase high-performance liquid chromatography. The isolates fell into *Bacillus*, *Brevibacillus* and *Pseudomonas*. Eight of the eleven grew on the amended medium; *Bacillus aryabhatai* 10AY and *Bacillus nealsonii* 5 grew best of all. In every one of the four treated cultures the peak that dominates the untreated control had gone, and peaks that were absent or minor in the control had appeared, which points to transformation of the parent compound. These salt-tolerant endophytes deserve a proper quantitative look as candidates for clearing neonicotinoid residues from saline farmland.

Keywords: Endophytic Bacteria; Halotolerance; Imidacloprid; Little Rann of Kutch; Biotransformation; *Bacillus aryabhatai*

1 INTRODUCTION

Imidacloprid was the first neonicotinoid sold at scale, and the class it opened has since become the most widely used group of insecticides in the world [1]. Its selectivity comes from the receptor it targets: the compound binds the insect nicotinic acetylcholine receptor far more tightly than it binds the vertebrate one. It also travels in the plant vascular system. Those two properties together explain its appeal as a seed dressing, a soil drench and a foliar spray on cotton, cereals, vegetables and horticultural crops.

The molecule is 1-(6-chloro-3-pyridylmethyl)-N-nitroimidazolidin-2-ylideneamine, a chloronicotinyl nitroguanidine of molecular weight 255.7. High water solubility and a low octanol-water partition coefficient let it move in the xylem and spread through treated tissue. Because it acts as an agonist at the receptor rather than as a cholinesterase inhibitor, pest populations that have shrugged off organophosphate and carbamate chemistry remain susceptible to it, and it works at low rates on sucking and soil-dwelling insects alike. Mammalian toxicity is comparatively low. That combination pushed the compound into veterinary and household products as well as field agriculture, and consumption has stayed heavy for three decades.

What makes imidacloprid useful in the field also makes it awkward once it is there. It dissolves readily and breaks down slowly in the dark, so it moves with soil water, and field dissipation half-lives past 100 days have been recorded [2,3]. Residues have been found in surface water and in shallow groundwater under treated land, and a recent survey of the literature documents persistence over much longer periods in some soils [4]. The non-target reach is correspondingly wide, taking in aquatic invertebrates, pollinators and soil microbial communities [5].

Stripping these residues out by physicochemical means is expensive and awkward in the field, which is why attention has moved to microorganisms. *Pseudomonas*, *Mycobacterium*, *Bacillus*, *Rhizobium*, *Rhodococcus*, *Stenotrophomonas* and *Pseudoxanthomonas* have all been shown to act on the compound [6-11]. Most of the reported chemistry runs through nitro reduction, hydroxylation of the imidazolidine ring and cleavage of the methylene bridge, giving imidacloprid-guanidine, the olefin derivative, 5-hydroxy-imidacloprid and 6-chloronicotinic acid [12]. Nearly all of these strains, though, were pulled from ordinary agricultural or contaminated soil and studied near neutral pH at low ionic strength.

That leaves a gap worth minding. A large share of the world's irrigated land is salt-affected, and inocula raised on ordinary soil tend to do badly there. Bacteria from naturally saline habitats are the better bet. Endophytes, which live inside plant tissue without causing disease [13-15], look especially promising: imidacloprid is systemic, so it accumulates in precisely the compartment an endophyte occupies, and an organism that acts on it there might lower residues in the plant as well as in the soil around it. Endophytes have already been put to work on a range of other xenobiotics [16,17]. On neonicotinoids the literature is thin.

The Little Rann of Kutch covers roughly 4900 square kilometres of western Gujarat. It floods seasonally, bakes in summer, and supplies a large fraction of India's salt. Its bacteria are beginning to be described: haloalkaliphiles from bare soil there include lineages new to the *Bacillaceae* and secrete enzymes of industrial interest [18], and halophiles from the wider Rann have been formally named [19]. Nobody, so far as we can tell, has looked inside the halophytes themselves, and nothing is known about what the bacteria in them might do to agrochemicals.

We set out with three aims: to recover culturable endophytes from halophyte roots under saline and alkaline conditions; to identify them by partial 16S rRNA gene sequencing, backed by morphology and biochemistry; and to find out which of them tolerate a commercial imidacloprid formulation and show chromatographic signs of acting on it.

2 MATERIAL AND METHODS

2.1 Study site and sampling

Roots came from four spots inside the Wild Ass Sanctuary in the Little Rann of Kutch: Pungbet, Vithroi, Kodadha and Dhutbet. The sanctuary is saline desert, and its flora is sparse but distinctive, adapted to salty ground and seasonal flooding. Nine species across eight families were sampled: *Cynodon dactylon* and *Aeluropus lagopoides* (Poaceae), *Alysicarpus monilifer* (Fabaceae), *Salvadora persica* (Salvadoraceae), *Echinops echinatus* and *Jacobaea vulgaris* (Asteraceae), *Balanites aegyptiaca* (Zygophyllaceae), *Capparis decidua* (Capparaceae) and *Cressa cretica* (Convolvulaceae). Whole root systems were lifted, bagged in sterile polythene and brought to the laboratory the same day.

2.2 Surface sterilization and isolation

Adhering soil was rinsed away under running sterile water. Roots then went into 70% (v/v) ethanol for 30 s, into 0.1% (w/v) mercuric chloride for 3 min, and through ten changes of sterile water. Sterilized roots were macerated aseptically with mortar and pestle and the macerate suspended in sterile water. A 100 μ L aliquot of each suspension went into yeast extract mannitol (YEM) broth carrying 5% (w/v) NaCl at pH 9, held at 37 °C and 100 rpm for 72 h. Once the broths turned turbid, a loopful of each was streaked onto YEM agar of matching salinity and pH; well-separated colonies were picked and cleaned up by repeated streaking. Stocks were kept on YEM slants with 5% (w/v) NaCl at pH 9 at 4 °C.

2.3 Morphological and biochemical characterization

Colony form, margin, elevation and texture were read off YEM agar at 48 h and 37 °C. Gram reaction, cell shape and motility followed standard procedures. Biochemistry combined commercial kits (ENTEROtest 24N, VP, INDOLE and OXI; Transasia Bio-Medicals, Mumbai, India) with conventional tests for oxidation-fermentation of carbohydrates, nitrate reduction, hydrogen sulfide, catalase, indole, methyl red, Voges-Proskauer, urease and citrate. Acid and gas from glucose, lactose, xylose, mannitol, maltose and sucrose were scored at 48 h and 37 °C. To examine phenetic structure, simple matching coefficients were calculated across the twenty-one binary characters and clustered by the unweighted pair-group method with arithmetic averages.

2.4 Molecular identification

Genomic DNA was extracted from overnight cultures, and the 16S rRNA gene was amplified with the universal primers 27F (5'-AGAGTTTGATCMTGGCTCAG-3') and 1492R (5'-TACGGYTACCTTGTTACGACTT-3') [20]. Reactions of 100 μ L contained 0.5 μ g of each primer, 2 U Taq polymerase, 10 μ L of 10 $\times$ reaction buffer, 4 μ L of 50 mM MgCl₂, and 200 μ M of each deoxynucleoside triphosphate. Amplification comprised an initial denaturation step at 95 °C for 4 min, followed by 30 cycles at 95 °C for 1 min, 55 °C for 1 min, and 72 °C for 90 s, with a final extension step at 72 °C for 5 min. The products were purified using a commercial column-based kit and sequenced at Chromous Biotech, Bengaluru, India. Partial sequences were compared with the non-redundant nucleotide collection using BLASTn [21], aligned with CLUSTAL W [22], and phylogenetic relationships were inferred using the neighbor-joining method in MEGA version 4.0 [23]. Identities were further verified using the Ribosomal Database Project classifier.

2.5 Insecticide and tolerance screening

Every experiment used one commercial formulation, imidacloprid 30.5% SC (Greenland Crop Science Pvt. Ltd., India). For the first screen, 50 μ L of it was dissolved in 10 mL acetone and 2 mL of that solution stirred into molten YEM agar before pouring. Isolates were streaked out, read at 72 h and 37 °C, and scored on a four-point scale running from no growth to confluent growth. Plates carrying acetone alone served as solvent controls.

2.6 Batch incubation and chromatographic analysis

The four isolates with the strongest tolerance were grown in 10 mL of complete medium at 37 °C and 100 rpm for 24 h. Biomass was harvested by centrifugation, washed twice in sterile normal saline to remove residual organic matter, and then resuspended. Ten milliliters of the washed cell suspension was transferred to mineral salt medium containing 50 μ L of the imidacloprid formulation, equivalent to approximately 15.3 mg of the active ingredient, in a 100 mL Erlenmeyer flask, and incubated at 37 °C and 100 rpm for seven days. The mineral salt medium contained (g L⁻¹): K₂HPO₄ 1.5, KH₂PO₄ 0.5, NH₄NO₃ 1.0, MgSO₄·7H₂O 0.2, NaCl 0.1, CaCl₂·2H₂O 0.02, and FeSO₄·7H₂O 0.01, adjusted to pH 7.0 before autoclaving. The cultures were then centrifuged, and the supernatant was extracted twice with an equal volume of hexane in a separating funnel for 20 min. The pooled upper phases were air-dried, and the residue was redissolved in 2 mL of acetonitrile. Analysis was performed on an Elico HD 469 system (Elico Ltd, Hyderabad, India) fitted with a C18 column and an ultraviolet detector. A 20 μ L sample was injected and eluted isocratically with acetonitrile and water (20:80, v/v) at 1.5 mL min⁻¹ at ambient temperature, with detection at 222 nm. An uninoculated preparation of the formulation processed in the same manner served as the reference chromatogram.

3 RESULTS AND DISCUSSION

3.1 Isolation and identification of endophytes

Enrichment of the sterilized macerates in saline alkaline YEM broth gave eleven isolates from the nine hosts (Table 1). Yield was uneven. *Aeluropus lagopoides* and *Jacobaea vulgaris* gave two apiece; every other species gave one. All eleven grew at 5% (w/v) NaCl and pH 9, which marks them as halotolerant and alkalitolerant. That fits both the chemistry of the ground their hosts grow in and what has been reported of bacteria elsewhere in this desert [18,19].

Sequence comparison put the isolates in three genera. *Bacillus* took seven: *B. subtilis* (2Y), *B. licheniformis* (3Y), *B. pumilus* (7Y and 8Y), *B. aryabhattai* (10AY) and *B. nealsonii* (5 and 13B). *Brevibacillus* took one, *B. brevis* (1Y).

Pseudomonas took the remaining three, as *P. stutzeri* (5AY and 13A) and *P. mendocina* (11). Gram reactions and cell shapes recorded separately agree with all of this (Table 2), the three *Pseudomonas* isolates being the only Gram-negative short rods in the set. That *Bacillus* dominates is no surprise. Endospores are useful in ground that dries out, heats up and stays osmotically hostile, and *Bacillus* has been reported again and again as the dominant culturable genus in saline soil of the Rann [18]. *B. aryabhattai* and *B. nealsonii* are both recent descriptions, one from the upper atmosphere and the other from a spacecraft assembly facility [24,25], and both are known for shrugging off stress.

The spread of isolates across hosts deserves a word. The two grasses, *Cynodon dactylon* and *Aeluropus lagopoides*, between them gave three isolates from two genera, while the woody perennials *Salvadora persica*, *Balanites aegyptiaca* and *Capparis decidua* gave one each. All three *Pseudomonas* isolates came from hosts at Vithroi and Dhutbet; the two most imidacloprid-tolerant isolates came from Kodadha and Dhutbet. With one isolate from most hosts and no replicate sampling, none of this supports any claim about how host identity or site shapes the community. It is offered only as a description of what was collected.

Eight isolates were Gram-positive large rods and three Gram-negative short rods; all eleven were motile (Table 2). Most colonies were circular, entire-margined and smooth, though 7Y, 10AY and 13B threw irregular colonies with uneven margins. Biochemically the set was uniform in places: catalase, nitrate reduction and citrate utilization were positive in every isolate, and urease negative in every one (Table 3). Indole, methyl red, Voges-Proskauer and hydrogen sulfide varied more. Carbohydrate profiles told them apart better (Table 4), with 2Y, 3Y and 10AY fermenting all six sugars while 13B produced gas from none.

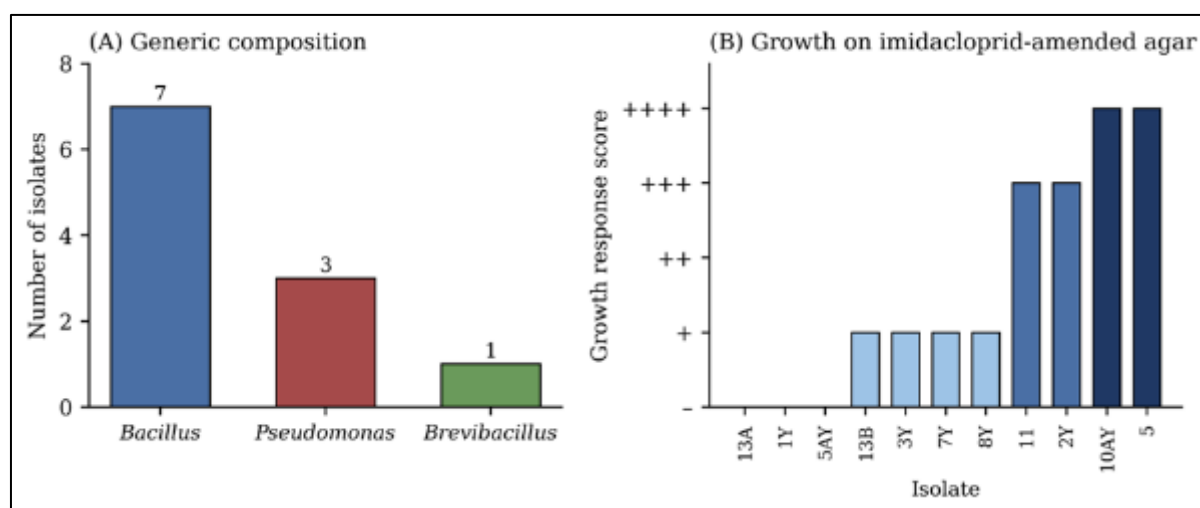


Figure 1: (A) Generic composition of the eleven endophytic isolates recovered from native halophytes of the Little Rann of Kutch, India. (B) Growth response of each isolate on YEM agar amended with the imidacloprid formulation, scored after 72 h at 37 °C.

3.2 Phenetic relationships among the isolates

Numerical analysis of the twenty-one characters in Tables 3 and 4 gave the phenogram in Figure 2. Two principal groups separate at roughly 65% similarity. The upper one holds every *Bacillus* isolate along with *Brevibacillus brevis* 1Y; the lower one holds the three *Pseudomonas*. The two *B. pumilus* strains, 7Y and 8Y, came from different hosts at the same site and are phenotypically identical at 100% similarity, which sits well with their identical 16S assignment. Phenetic and molecular groupings therefore agree for ten of the eleven.

The exception is worth dwelling on. Isolate 13B is *Bacillus nealsonii* on 16S evidence and Gram-positive in the stained preparation, yet it lands in the *Pseudomonas* cluster. Its carbohydrate profile drives this: it was the only isolate that failed to acidify glucose, and it gave gas from none of the six sugars. Phenetic and phylogenetic placements have parted company before among haloalkaliphiles from this same desert, where strains of one species varied widely in phenotype [18]. Characters scored at high salt and high pH are evidently a poor stand-in for phylogeny, and where the two disagree the sequence should win.

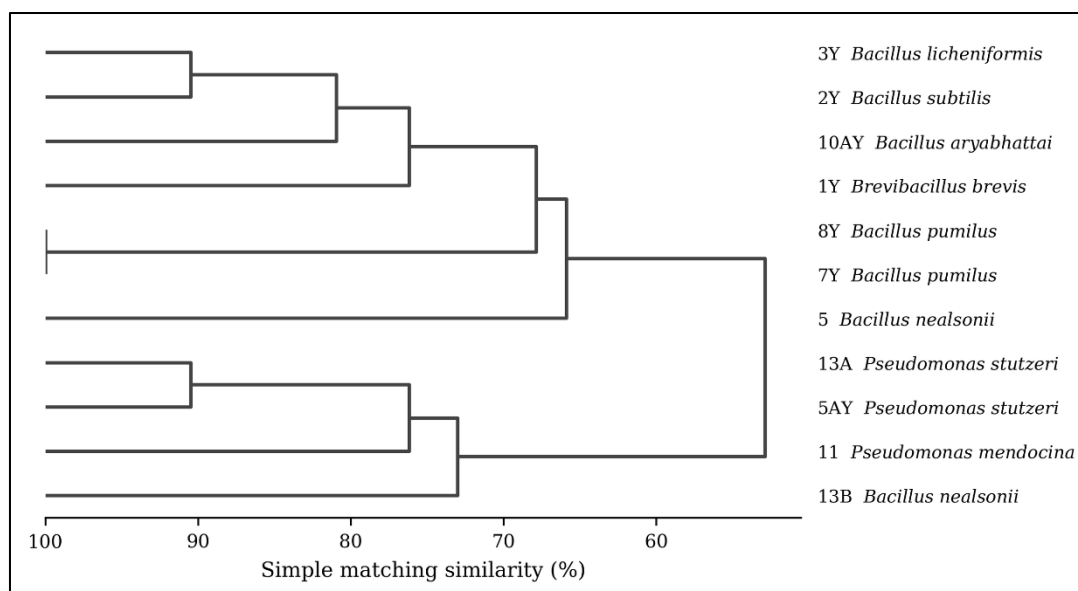


Figure 2: Phenogram of the eleven endophytic isolates constructed by unweighted pair-group clustering of simple matching similarity coefficients derived from twenty-one phenotypic characters (nine biochemical tests and acid and gas production from six carbohydrates).

A note on names is needed here. Both of the most tolerant isolates carry combinations that have since changed. In the phylogenomic reappraisal of *Bacillus* by Gupta et al. [26], *B. aryabhattai* moved to *Priestia* as *Priestia aryabhattai* and *B. nealsonii* moved to *Niallia* as *Niallia nealsonii*. We have kept the original names throughout, for consistency with the database records the sequences were matched against.

3.3 Tolerance to imidacloprid

Eight of the eleven isolates grew on imidacloprid-amended YEM agar (Table 1, Figure 1B). Three did not grow at all, and it is worth noting which three: the *P. stutzeri* strains 5AY and 13A, plus *Brevibacillus brevis* 1Y. Tolerance is clearly a property of the strain, not of the habitat the strain came from. Four isolates grew weakly and two strongly, with the best responses from *B. aryabhattai* 10AY and *B. nealsonii* 5. Tolerating a compound is necessary for transforming it but not sufficient, so the screen was used for one purpose only: choosing isolates to put through chromatography. Those four were 10AY, 5, 2Y (*B. subtilis*) and 11 (*P. mendocina*).

3.4 Chromatographic evidence of transformation

One peak dominated the reference chromatogram of the untreated formulation, at 5.825 min and 58.8% of total area, with a second at 6.383 min contributing 11.2% (Figures 3A and 4). Seven days of incubation changed all four profiles markedly. The consistent finding was the loss of that 5.825 min peak from every treated sample, together with peaks that were absent from the reference or minor in it.

The *B. subtilis* 2Y culture resolved into two peaks, 6.422 min (51.1%) and 8.593 min (48.9%). *B. aryabhattai* 10AY gave much the same two-peak shape at 8.548 min (45.4%) and 6.380 min (44.6%). *Bacillus nealsonii* 5 was messier, with peaks at 7.113 min (28.2%), 1.772 min (11.8%) and 3.163 min (11.4%), while *P. mendocina* 11 gave 2.083 min (19.3%) and 7.325 min (18.3%) inside a chromatogram full of minor components. A peak near 6.4 min is shared by the two *Bacillus* cultures and by the reference, which hints that one product may accumulate as the major fraction across isolates while the minor products differ. Several early-eluting, more polar components turned up in the *B. nealsonii* and *P. mendocina* cultures. That would fit the polar metabolites usually reported from imidacloprid transformation, among them the guanidine and 5-hydroxy derivatives and 6-chloronicotinic acid [8,12], but the present data cannot assign any peak to any compound.

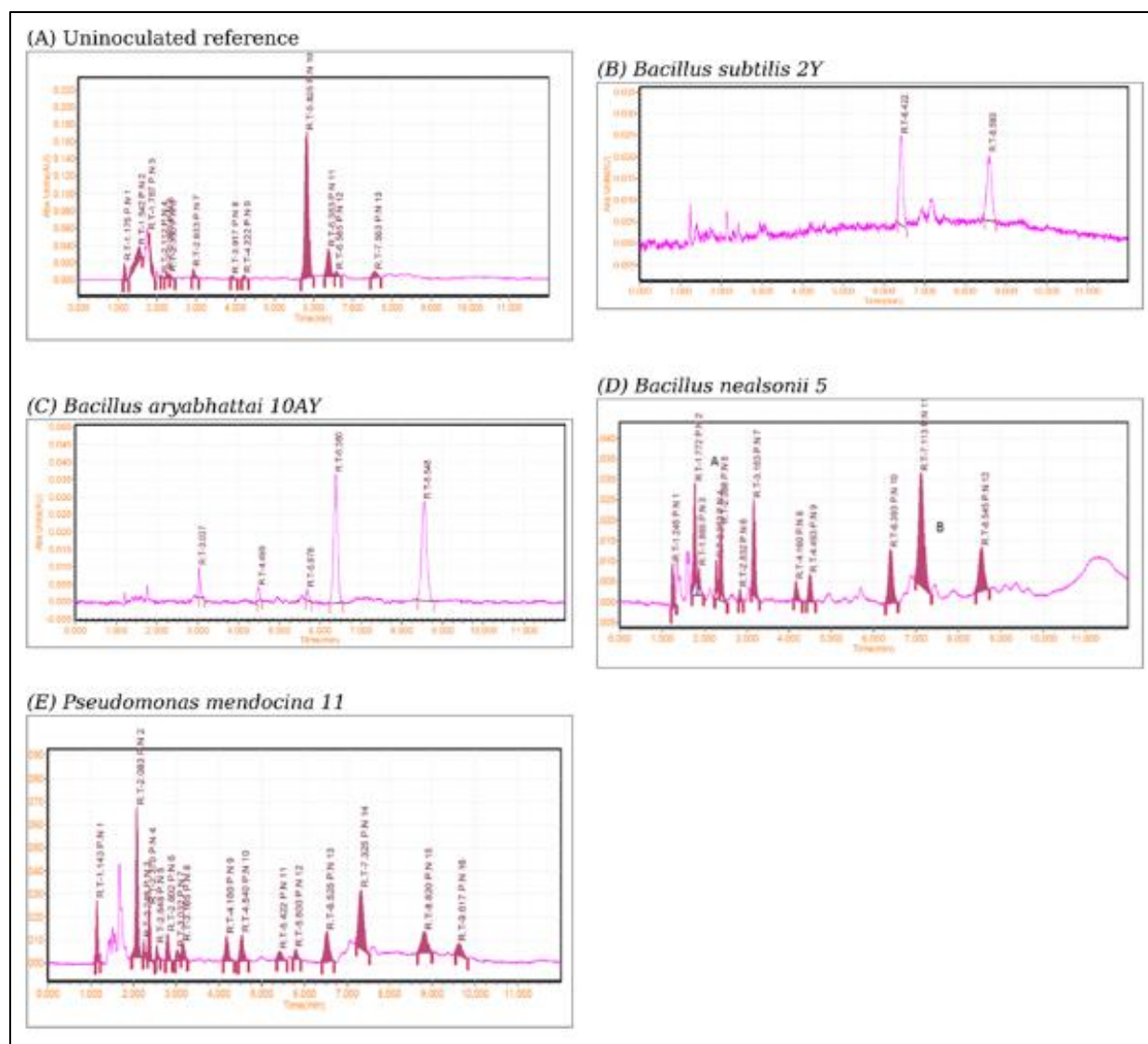


Figure 3: Reversed-phase HPLC chromatograms of hexane-extracted culture supernatants after seven days of incubation at 37 °C, detection at 222 nm. (A) Uninoculated reference; (B) *Bacillus subtilis* 2Y; (C) *Bacillus aryabhattai* 10AY; (D) *Bacillus nealsonii* 5; (E) *Pseudomonas mendocina* 11. Panels were reproduced from the original instrument output and differed in the absorbance scale.

None of this is out of step with the wider literature, where *Bacillus* and *Pseudomonas* are prominent among the genera that act on neonicotinoids [6,11,27] and where endophytic and plant-associated bacteria have been shown to transform members of the class [28]. What sets these isolates apart is where they came from. They were pulled out of halophyte roots in a hypersaline, alkaline desert, and they grow without difficulty at 5% NaCl and pH 9. An inoculum meant for salt-affected farmland has to work under exactly those conditions, and strains raised on ordinary soil often will not.

That is the practical point. Salinization is spreading across irrigated land in western India, and the same ground routinely receives neonicotinoid seed treatments and soil drenches. A bioremediation inoculum for it must stay metabolically active at high ionic strength and alkaline pH, which is where organisms selected on ordinary soil tend to fail. Endophytic colonization brings a second advantage: an organism established inside root tissue faces less competition and predation than a free-living inoculant in bulk soil, and it sits where a systemic insecticide collects.

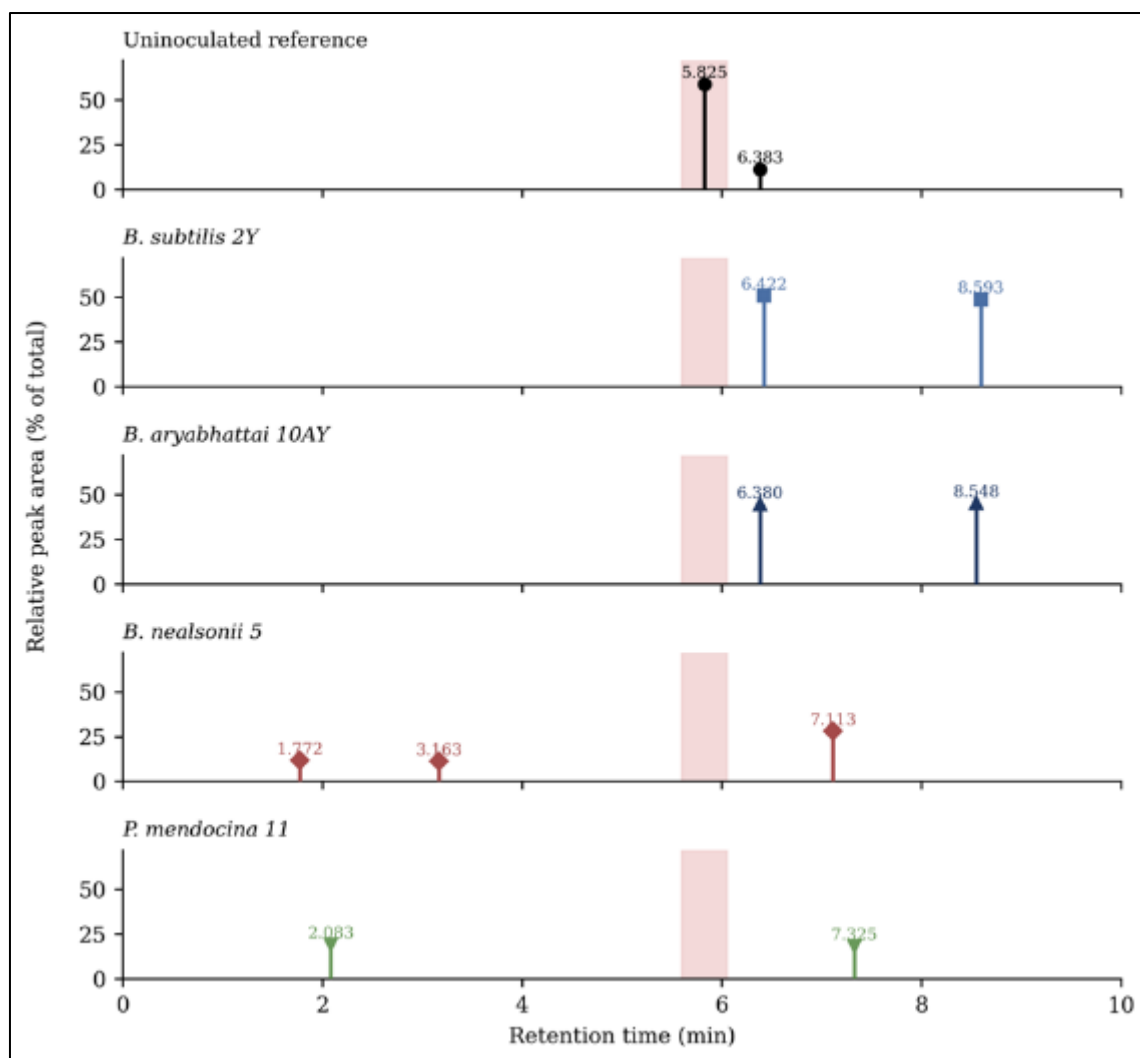


Figure 4: Relative peak areas of the major chromatographic components plotted against retention time for the uninoculated reference and the four inoculated cultures. The retention times are annotated above each peak.

The shaded band marks the position of the component dominating the reference chromatogram, which was not detected in any of the inoculated cultures. The areas represent the percentage of the total integrated peak area and are not absolute concentrations.

3.5 Limitations of the present study

Several things need saying plainly. Quantification here was relative, not absolute. Peak area percentages describe the make-up of a chromatogram; turning them into residual concentrations needs a calibration curve built from an analytical standard, which we did not run, so no percentage degradation is claimed. No abiotic control was incubated alongside for the full seven days, so hydrolysis and photolysis cannot be formally ruled out as contributors, although the size of the change and its variation between isolates argue against a purely abiotic account. Peaks were not confirmed by co-injection with authentic standards or by mass spectrometry, so the metabolites stay unidentified. Because a commercial formulation was used rather than analytical-grade material, the co-formulants rule out any claim that imidacloprid served as sole carbon or nitrogen source. Incubations were not replicated, which leaves no room for statistics. Finally, the sterilization protocol carried no sterility check on the final rinse water, so the endophytic status of these isolates, while likely after ten washes, is not formally demonstrated. Read what follows as a preliminary sign of biotransformation potential, and as a case for calibrated time-course work with abiotic and heat-killed controls, replication, and metabolite identification by liquid chromatography-tandem mass spectrometry.

Table 1: Sampling sites, host halophytes, isolate codes, closest 16S rRNA gene relatives, and growth response on imidacloprid-amended YEM agar.

Site	Host plant (family)	Isolate	Closest 16S relative	Growth on imidacloprid
Pungbet	<i>Cynodon dactylon</i> (Poaceae)	1Y	<i>Brevibacillus brevis</i>	-
Pungbet	<i>Alysicarpus monilifer</i> (Fabaceae)	2Y	<i>Bacillus subtilis</i>	+++
Vithroi	<i>Salvadora persica</i> (Salvadoraceae)	3Y	<i>Bacillus licheniformis</i>	+
Vithroi	<i>Aeluropus lagopoides</i> (Poaceae)	13A	<i>Pseudomonas stutzeri</i>	-
Vithroi	<i>Aeluropus lagopoides</i> (Poaceae)	13B	<i>Bacillus nealsonii</i>	+
Kodadha	<i>Echinops echinatus</i> (Asteraceae)	7Y	<i>Bacillus pumilus</i>	+
Kodadha	<i>Balanites aegyptiaca</i> (Zygophyllaceae)	8Y	<i>Bacillus pumilus</i>	+
Kodadha	<i>Capparis decidua</i> (Capparaceae)	10AY	<i>Bacillus aryabhatai</i>	++++
Dhutbet	<i>Jacobaea vulgaris</i> (Asteraceae)	5AY	<i>Pseudomonas stutzeri</i>	-
Dhutbet	<i>Jacobaea vulgaris</i> (Asteraceae)	5	<i>Bacillus nealsonii</i>	++++
Dhutbet	<i>Cressa cretica</i> (Convolvulaceae)	11	<i>Pseudomonas mendocina</i>	+++

- no growth; + weak growth; +++ strong growth; ++++ confluent growth after 72 h at 37 °C.

Table 2: Morphological and microscopic characteristics of the endophytic isolates.

Isolate	Gram reaction	Cell shape	Motility	Form	Margin	Elevation / texture
1Y	+	Large rod	+	Circular	Entire	Convex, smooth
2Y	+	Large rod	+	Circular	Uneven	Flat, smooth
3Y	+	Large rod	+	Circular	Entire	Flat, smooth
5AY	-	Short rod	+	Circular	Entire	Convex, smooth
5	+	Large rod	+	Circular	Entire	Convex, smooth
7Y	+	Large rod	+	Irregular	Entire	Flat, smooth
8Y	+	Large rod	+	Circular	Entire	Convex, smooth
10AY	+	Large rod	+	Irregular	Uneven	Flat, smooth
11	-	Short rod	+	Circular	Undulate	Convex, smooth
13A	-	Short rod	+	Circular	Entire	Convex, smooth
13B	+	Large rod	+	Irregular	Uneven	Flat, smooth

+ positive; - negative.

Table 3: Biochemical characteristics of the endophytic isolates.

Isolate	OF	NR	H2S	CAT	IND	MR	VP	CIT	URE
1Y	-	+	-	+	-	-	-	+	-
2Y	-	+	+	+	+	+	+	+	-
3Y	-	+	+	+	-	-	+	+	-
5AY	+	+	-	+	+	+	-	+	-
5	-	+	-	+	+	-	-	+	-
7Y	-	+	+	+	-	+	+	+	-
8Y	-	+	+	+	-	+	+	+	-
10AY	+	+	+	+	-	-	-	+	-
11	+	+	-	+	-	+	-	+	-
13A	+	+	-	+	+	+	-	+	-
13B	-	+	+	+	+	+	-	+	-

OF, oxidation-fermentation of carbohydrates; NR, nitrate reduction; H2S, hydrogen sulfide production; CAT, catalase; IND, indole production; MR, methyl red; VP, Voges-Proskauer; CIT, citrate utilization; URE, urease. + positive; - negative.

Table 4: Utilization of carbon sources by the endophytic isolates, expressed as acid/gas production.

Isolate	Glucose	Lactose	Xylose	Mannitol	Maltose	Sucrose
1Y	+/+	+/+	-/-	+/+	+/+	+/+
2Y	+/+	+/+	+/+	+/+	+/+	+/+
3Y	+/+	+/+	+/+	+/+	+/+	+/+
5AY	+/+	-/-	-/-	-/-	+/-	-/-
5	+/-	+/+	+/+	+/-	+/-	+/-
7Y	+/+	+/-	+/-	+/-	+/-	+/-
8Y	+/+	+/-	+/-	+/-	+/-	+/-
10AY	+/+	+/-	+/+	+/+	+/+	+/+
11	+/-	+/-	+/-	-/-	-/-	+/-
13A	+/-	-/-	+/-	-/-	+/-	-/-
13B	-/-	+/-	+/-	-/-	-/-	-/-

+ positive; - negative.

4 CONCLUSION

Eleven halotolerant, alkalitolerant endophytes belonging to *Bacillus*, *Brevibacillus* and *Pseudomonas* were recovered from the roots of native halophytes in the Little Rann of Kutch, a saline desert whose endophytic microbiota had not been described before. Eight of them tolerated a commercial imidacloprid formulation, and chromatograms from four showed the parent peak gone and new components in its place, which indicates biotransformation. *Bacillus aryabhattai* 10AY and *Bacillus nealsonii* 5 responded most strongly. Since these organisms grow at 5% sodium chloride and pH 9, they are of particular interest for clearing neonicotinoid residues from salt-affected farmland, where inocula raised on ordinary soil commonly fail. Calibrated time-course studies with proper controls are the next step.

STATEMENTS ON COMPLIANCE WITH ETHICAL STANDARDS

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Disclosure of conflict of interest

Dhaval Prajapati declares that he has no conflict of interest. Akshay Suthar declares that he has no conflict of interest. Vijay Parmar declares that he has no conflict of interest. Ronak Patel declares that he has no conflict of interest. Rajesh Patel declares that he has no conflict of interest.

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