Indian Journal of Ecology (2024) 51(6): 1278-1286
DOI: https://doi.org/10.55362/IJE/2024/4394

Manuscript Number: 4394
NAAS Rating: 5.38

Identification and Evaluation of Soil-Derived Microbial Strains for Thiamethoxam Biodegradation

Shivnam Rana, Kousik Mandal[1] and V.K. Gupta[1]

Department of Microbiology and [1]Department of Entomology
Punjab Agricultural University, Ludhiana-141 004, India
E-mail: shivnamrana@gmail.com

***Abstract*:** This study investigates the enrichment, isolation, and characterization of thiamethoxam-degrading bacterial species from cotton field soils. Soil previously exposed to xenobiotics was used to enrich microbial biomass in MSMT broth, leading to the isolation of nine distinct bacterial species based on colony morphology. These isolates were screened for their ability to degrade thiamethoxam in liquid cultures, revealing that bacterial isolate TY achieved the highest degradation rate of 35.52% over 15 days. Taxonomic identification through 16S rDNA sequencing identified isolate TY as a novel strain of *Salmonella enterica* subsp. *enterica* with a reduced sequence identity of 96% compared to reference strains. The bacterial isolate TY was non-pathogenic, as determined by Congo red agar assays. Optimal growth conditions for TY were identified at 37°C and pH 6.5, and its thiamethoxam degradation was influenced by the availability of carbon and nitrogen sources. This research highlights the potential of *S. enterica* strain TY for bioremediation of thiamethoxam and contributes to the understanding of pesticide degradation by non-traditional bacterial species.

***Keywords*:** Thiamethoxam, Biodegradation, *Salmonella enterica* subsp. *Enterica*, Pesticide metabolism, 16S rDNA sequencing

Over recent decades, various chemical compounds, including organochlorines, triazines, carbofuran, phenoxyacetates, and organophosphates have been extensively used in agriculture over the decades to protect crops from pests and enhance yield to meet the demands of a growing population (Fulton et al 2014). While these pesticides are effective in pest control, only a small fraction (about 1%) of the applied pesticide reaches the target organisms and the remainder contaminates the environment, adversely affecting public health and beneficial biota by contaminating soil, water, and the atmosphere (Elhamalawy et al 2024). In response to these environmental concerns, new classes of chemical compounds, such as neonicotinoids, were developed in the 1970s. These include imidacloprid and thiamethoxam, which were introduced in the 1990s (Fulton et al 2014, Silcox and Vittum 2008). Thiamethoxam is a second-generation neonicotinoid insecticide effective against a broad range of insects. It acts on the nicotinic acetylcholine receptors (nAChRs) of insects, leading to neuromuscular paralysis and death (Mohamed 2009). Despite its effectiveness, thiamethoxam has the potential to leach into groundwater due to its weak soil binding, thus contaminating the ecosystem and potentially causing issues such as "colony collapse disorder" in bees and liver, kidney, and brain damage in laboratory albino male rats (Henry et al 2012, Khaldoun-Oularbi et al 2017). This highlights the need for efficient, eco-friendly methods to degrade such compounds.

To mitigate these environmental risks, bioremediation via microbial degradation presents a viable solution, as certain microorganisms can metabolize these pesticides into less harmful substances. For instance, *E. adhaerens* TMX-23, *Pseudomonas* sp., *Acinetobacter* sp., *Enterobacter* sp., and *Bacillus* sp. have been identified as capable of degrading thiamethoxam through specific metabolic pathways (Zhou et al 2013, Hegde et al 2017). Since microbial degradation using selected bacterial species offers the most attractive, and cost-effective bioremediation approach. This study focuses on isolating bacterial species from agricultural soils with high thiamethoxam-degrading potential, and evaluating their effectiveness in thiamethoxam-fortified liquid media, paving the way for future bioremediation applications.It could contribute to the advancement of sustainable agricultural practices by enhancing our understanding of microbial capabilities for pesticide degradation and fostering the development of effective bioremediation strategies to address pesticide pollution.

MATERIAL AND METHODS

Bacteriological media: Two types of media were used: Luria-Bertani (LB) for general purposes and Mineral Salt Medium (MSM) for enriching thiamethoxam-degrading bacterial species. To solidify LB and MSM, 1.6% bacteriological agar (S. d. fine-chem Ltd, Mumbai) was added before autoclaving. MSM was supplemented with thiamethoxam stock (10 mg/ml in acetone) to achieve

concentrations of 20 or 50 µg/ml as the sole source of carbon, nitrogen, and energy.

Enrichment, isolation, and purification of bacterial species from soil: Soil samples (~100 g each) were collected from a cotton field with a history of thiamethoxam application at Punjab Agricultural University, Ludhiana (30.904751, 75.806932 https://maps.app.goo.gl/9Qug NGA6bMxPPR5j6). These samples were mixed, stored at 4°C, and processed in the lab. A 50 g aliquot of the mixed soil was suspended in 250 ml of 0.05 M potassium phosphate buffer (pH 7.0), filtered, and centrifuged to obtain bacterial biomass. The pellet was re-suspended in buffer, and inoculated into MSMT containing thiamethoxam (20 µg/ml). The culture was grown at 28±2°C with shaking. Enrichment was followed by plating on solid MSMT-agar to isolate colonies. Colony characteristics were examined under a stereomicroscope, and Gram staining was performed on LB broth cultures.

Screening of bacterial species for thiamethoxam degradation in liquid cultures: Thiamethoxam degradation potential was assessed in 15 ml MSM broth (50 µg/ml thiamethoxam) inoculated with 500 µl ofovernight grown bacterial culture (10^8 CFUml^{-1}). The cultures were incubated at 37±1°C with shaking (120 rpm) for 15 days. Samples (1 ml) were taken at 3, 8, and 15 days to measure thiamethoxam levels. Control flasks without bacterial inoculation were included for comparison. Culture broth samples were extracted using dichloromethane, concentrated, and reconstituted in acetone. Thiamethoxam content was quantified using GLC.The degradation rate, half-life and correlation coefficient (R^2) was calculated to assess the kinetics of degradation. The relative thiamethoxam degradation potential of the different bacterial species was interpreted in terms of the half-life ($t_{1/2}$) period of thiamethoxam in the presence of growth of specific bacterial species(Mandal et al 2014, Rana et al 2015).

Taxonomic identification of bacterial species via 16S ribosomal DNA (rDNA) Sequencing

Amplification of 16S ribosomal DNA from total bacterial DNA: Total DNA was isolated from bacterial cell pellets (~20 mg) obtained from a 48 hrs grown culture in LB, using a CTAB method. Using this DNA as template, 16S rDNA was amplified with QUGP Fn5: 5'-actcctacgggaggcagcag-3' and QUGP-Rn2: 5'-tgacgggcggtgtgtacaag-3' gold primers (Jariyal et al 2014). The size of amplified bands was ascertained by co-running a molecular weight standard (100 bp DNA ladder plus, Fermentas Life Sciences) along with the samples in the gel.

Purification of PCR product, cloning and sequence determination: The agarose block containing the specifically amplified 16S rDNA band (1073bp) was excised from the agarose gel and transferred to a 1.5 ml Eppendorf tube. DNA fragments from this gel band were purified using 'QIAquick Gel Extraction Kit' (Qiagen) as per manufacturer's protocol. The purified DNA fragments were ligated into a pTZ57R/T- a sequencing plasmid vector and the product was transformed into *Escherichia coli* DH5-alpha host cells using 'InsTAcloneTM PCR Product Cloning Kit' (Fermentas Life Sciences) as per manufacturer's protocol. The authenticity of the cloned insert as the target 16S rDNA fragment in this recombinant plasmid was ascertained by positive amplification of a PCR fragment of the desired size (1073 bp) with QUGP gold primers using this recombinant plasmid DNA as a template as described earlier. The information on the nucleotide sequence of the cloned 16S rDNA fragment was obtained for both strands through custom sequencing services of 'M/S Xcelris Labs, Ahmedabad, India' and the final sequence was edited using DNA software Chromaslite 2.01 (Technelysium Pty Ltd, Australia), and CLC Sequence Viewer 6.5.4 (CLC bio A/S).

Analysis of sequence data for taxonomic identification of the bacterial isolate: The 16S rDNA sequence corresponding to a bacterial isolate was blasted in the 'Nucleotide blast program' of the 'National Center for Biotechnology Information' (available at www.ncbi.nlm.nih .gov/Blast). Phylogenetic analysis of the 16S rDNA sequences of isolated bacterial species and bacterial species showing closer sequence homologies was derived using 'create tree' function of CLC Sequence Viewer 6.5.4 (CLC bio A/S) through 'nearest neighbor algorithm with 100 bootstrap replications'. Polymorphic nucleotides between the isolated bacterial species and species showing the closest nucleotide homology were identified using the 'create alignment' function of the above program.

Congo redagar assay for pathogenicity evaluation: Pathogenicity of isolated strains was screened using Congo red agar (CRA) assay *as per* Agenta et al (1997) which is a fast method. Briefly, a loopful of a bacterial culture (bacterial isolate TY) grown overnight in LB medium was streaked onto a Congo red agar plates (1% Bacto tryptone, 0.5% yeast extract, 40 µgml^{-1} Congo red dye, 1.5% agar) incubated at 28°C. The test was performed in triplicate. The plates were observed after 24 hrs for the appearance of red and white colonies as an indicator of potentially pathogenic, and non-pathogenic colonies, respectively (Malcova et al 2008). The red color colony formed on media containing the dye was due to the adsorption of Congo red on the surface of the bacterial cell, to the ions which predominate at the surface, and to the production of acid or alkali by the bacterium during growth (Yakupova et al 2019).

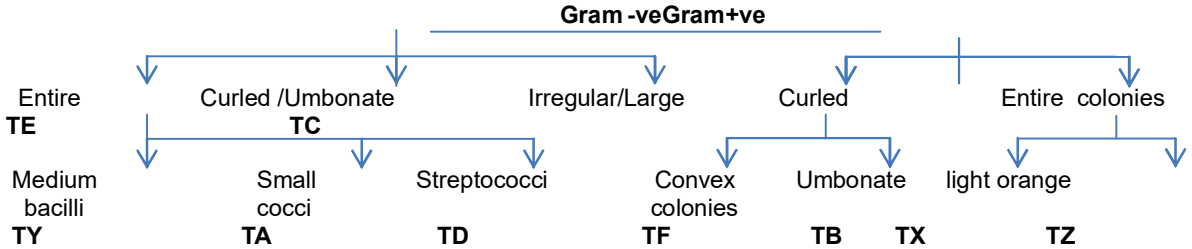
Effect of cultural parameters on bacterial growth: The ability of bacterial species to potentially metabolize different insecticides is directly related to their growth rate (Chishti and Arshad 2012). Therefore, the effect of different cultural parameters- initial medium pH (5.5, 6.0, 6.5, 7.0, 7.5, 8.0, 8.5 & 9.0), incubation temperature (32°, 37° and 42°C) and aeration (shaking vs. stationary conditions) on the growth of bacterial isolate (in terms of OD_{600}) was studied in 15 ml of MSM broth supplemented with glucose at 0.5% level (MSMG) as a sole source of carbon and energy. Each medium aliquot was inoculated with 300 µl of an overnight grown culture of a bacterial isolate to provide an initial OD_{600} of 0.030±0.005. Using one ml aliquots of culture broth drawn at regular intervals of 24 hrs, the growth of the bacterial isolate was recorded in terms of OD_{600} in an 'Eppendorf Biophotometer' up to 7 days.

Effect of supplemented carbon and nitrogen sources on thiamethoxam degradation: The effect of glucose and ammonium sulfate as added carbon, and nitrogen sources on thiamethoxam degradation by selected bacterial species was studied in thiamethoxam supplemented (@ 50 μgml^{-1}) MSM broth and raised with optimum pH. Glucose was used at a constant level of 0.1% to provide 0.04% carbon. However, ammonium sulfate as a nitrogen source was studied at two different levels (0.1 and 0.2%) to provide a C:N ratio of 1:1.34 and 1:1.67. So, abbreviations C_0N_0, C_0N_1, C_1N_1, and C_1N_2 represented C_0- No glucose; C_1- 0.1% glucose, N_0- No Nitrogen, N_1, and N_2- 0.1%, and 0.2% ammonium sulfate, respectively. Fifteen ml of so prepared media were inoculated with the bacterial inoculums to provide initial OD_{600} of 0.030±0.005 and growth allowed at optimum temperature in an orbital shaking incubator (120 rpm). One set of conical flasks containing thiamethoxam but without inoculum served as control. After 15 days of growth, the whole medium was analyzed for bacterial growth (OD_{600}) and contents of thiamethoxam through GLC as described earlier.

RESULTS AND DISCUSSION

Enrichment and isolation of thiamethoxam-metabolizing bacterial species: Soil samples previously exposed to xenobiotics, such as those from cotton fields treated with thiamethoxam, typically harbor higher microbial diversity and degradation capabilities compared to untreated soils. Enrichment of microbial biomass from these soils in MSMT broth led to observable increases in turbidity, indicative of active microbial growth. Pour plating of the enriched broth on MSMT-agar yielded several distinct bacterial colonies, which were morphologically categorized into nine types. These bacterial isolates formed circular or irregular colonies, but differed in their colony color (creamy, gray, light orange), colony size (small, medium, large) and colony margin (entire, curled, rhizoid). Three species (TC, TE & TY) produced single rod-shaped cells; while in the case of six other species, cells were either single-celled cocci (TA, TB, TF, TX, TZ) or short-chain streptococci (TD). These isolates varied in colony shape, color, size, and Gram staining characteristics, with four of these species (TB, TF, TX, TZ) were gram-positive, and others were gram-negative in reaction (Fig. 1).

Screening of isolated bacterial species for thiamethoxam degradation: The thiamethoxam degradation potential of the isolated bacterial species was assessed in liquid cultures over a 15-day period. The control cultures, which were uninoculated, exhibited a minimal degradation rate of 8.80%. Among the bacterial isolates, TF showed no thiamethoxam degradation, while the remaining eight isolates demonstrated varying degrees of degradation. The highest degradation was achieved by isolate TY, which reduced thiamethoxam by 35.52%, followed by TD, TX, TB, TZ (Table 1). Notably, degradation increased progressively with incubation time (up to 15 days). Isolate TY exhibited the most efficient degradation, with a half-life of 28.13 days, significantly lower than the control's half-life of 103.79 days, showed that degradation of thiamethoxam in control without microorganism is slow or negligible (Table 2). Regression analysis estimates the relationships among variables whereas half-life ($t_{1/2}$) or DT_{50} find out the time taken for the disappearance of given material from its initial concentration



* Colony characteristics were recorded visually under 10X magnification of a stereomicroscope. Colony size was rated as small: 1-2 mm, medium: 2-3 mm and large: more than 3 mm in diameter

** Cell characteristics were recorded on 48 hr grown broth cultures in LB at 100X magnification under a phase contrast microscope

Fig. 1. Differentiation amongst isolated bacterial species based upon gram's reaction and morphological characteristics

to half of its value (Rana and Gupta 2019). The correlation coefficient of control (0.96) along with other sample was less than 0.99, so thiamethoxam degradation in all samples did not follow first-order reaction (Rana and Gupta 2019). Similarly, Zhou et al (2013) reported *E. adhaerens* TMX-23, Jariyalet al (2014) *Pseudomonas* sp., Myresiotis et al (2011) reported *Bacillus amyloliquefaciens* IN937a, *B. pumilus* SE34, and *B. subtilis* FZB24, Wang et al (2011) reported *Acinetobacter* sp. TW, and *Sphingomonas* sp. TY in liquid medium that have ability to degrade insecticide as discussed in the above section.

Taxonomic identification of bacterial species ty based on 16S rDNA sequencing: The 16S rDNA gene sequence is a universally accepted genetic marker for the taxonomic identification of bacterial species (Schmalenberger et al 2001). Thus, a series of 'Al-Quds University General & Golden Primers' (QUGP) has become available, which enables the amplification of the 16S rDNA from most of the known and unknown bacterial species. However, no single QUGP primer set could amplify the target 16S rDNA region from bacterial specie TY. Therefore, the primer sets for amplification of this region from TY isolate were identified through multiple amplifications using all the different QUGP primer sets. As a result, the 16S rDNA region of the TY bacterial specie was amplified using QUGP golden primer set, QUGPFn5- QUGP-Rn2 to result in amplification of 16S rDNA fragments of ~1073 bp (Fig. 2). The edited nucleotide sequence of the amplified 16S rDNA from bacterial specie TY was submitted to the GenBank database and is available as GenBank Acc # KF 527575. Blasting of the 16S rDNA sequence of bacterial isolate TY with the GenBank database using the 'Nucleotide Blastn tool' of 'NCBI" resulted in >100 homology hits out of which 10 hits with 100% query coverage, maximum score and lowest e-value (0.00) (Table 3). The data showed that bacterial specie TY holds maximum 16S rDNA sequence homology score (1768) with three different strains of *Salmonella enterica* subsp. enterica serovar Heidelberg (GenBank Acc #, CP003416.1, CP001120.1, and CP004086.1) but with the reduced identity of 96%. Figure 3 illustrates the phylogenetic tree constructed from the 16S rDNA sequences of *Salmonella enterica* subsp. *enterica* strain TY alongside ten other bacterial species. This phylogenetic distribution distinctly placed the bacterial isolate TY nearest to two strains (B182- CP003416.1 & SL476- CP001120.1) of *S. enterica* subsp. enterica serovar Heidelberg. Thus, based upon phylogenetic distribution and

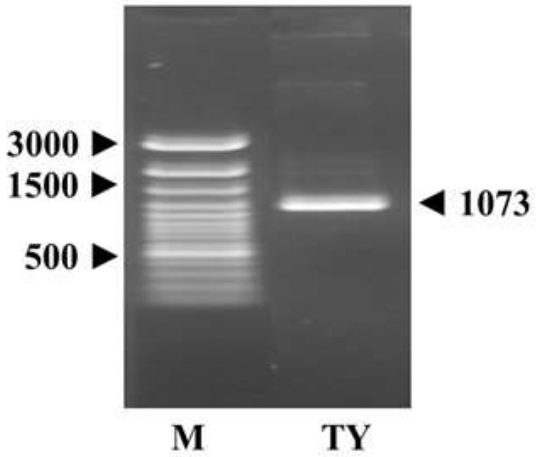


Fig. 2. PCR amplification of 16S rDNA fragments (1073 bp) from total DNA of bacterial specie TY using QUGP golden primer set (QUGP-Fn5 - QUGP-Rn2). M is 100bp DNA ladder (Fermentas Life Sciences). Sizes of DNA bands are given in base pairs (bp)

Table 1. Thiamethoxam degradation by isolated bacterial species after different periods of growth in the MSMT liquid culture

Bacterial species	3 day		8 day		15 day	
	1	2	1	2	1	2
TA	45.36±0.65	7.76	43.22±0.76	12.11	41.00±0.72	16.63
TB	41.16±0.54	16.31	40.65±0.65	17.34	36.45±0.59	25.88
TC	43.46±0.76	11.63	40.93±0.86	16.77	39.12±0.89	20.45
TD	45.06±0.86	8.37	43.32±0.78	11.91	34.47±0.69	29.91
TE	46.09±0.73	6.28	43.77±0.82	11.00	39.02±0.71	20.65
TF	47.73±0.83	2.95	45.54±0.71	7.40	45.06±0.76	8.37
TX	45.06±0.63	8.37	36.63±0.58	25.52	35.79±0.65	27.22
TY	36.45±0.68	25.88	34.49±0.71	29.87	31.71±0.61	35.52
TZ	44.39±0.85	9.74	42.63±0.90	13.32	38.19±0.82	22.35
Control	48.74±0.78	0.89	46.95±0.83	4.53	44.85±0.64	8.80

Data is mean±SE of triplicate values each. Mean thiamethoxam concentration under all treatments at 0 day was estimated to be 49.18±0.0.64 µg ml^{-1}
1- Total thiamethoxam residue (µg ml^{-1}), 2 Percent thiamethoxam reduction

maximum score but with reduced homology, the bacterial species TY was identified as a new strain of *S. enterica* subsp. enterica serovar Heidelberg and was named as *S. enterica* subsp. enterica strain TY. The alignment of 16S rDNA regions of bacterial species TY, and the reference strain (*S. enterica* subsp. enterica serovar Heidelberg strain B182) resulted in the identification of 38 polymorphic nucleotides that differentiated between the newly isolated *S. enterica* subsp. enterica strain TY from the reference strain (Fig. 4). Besides, though bacterial species TY has been assigned strains of *S. enterica* subsp. enterica serovar Heidelberg, but the reduced identity of 96% between these two species/strains, the former demands taxonomic revision, pending availability of 16S rDNA data on more new types of

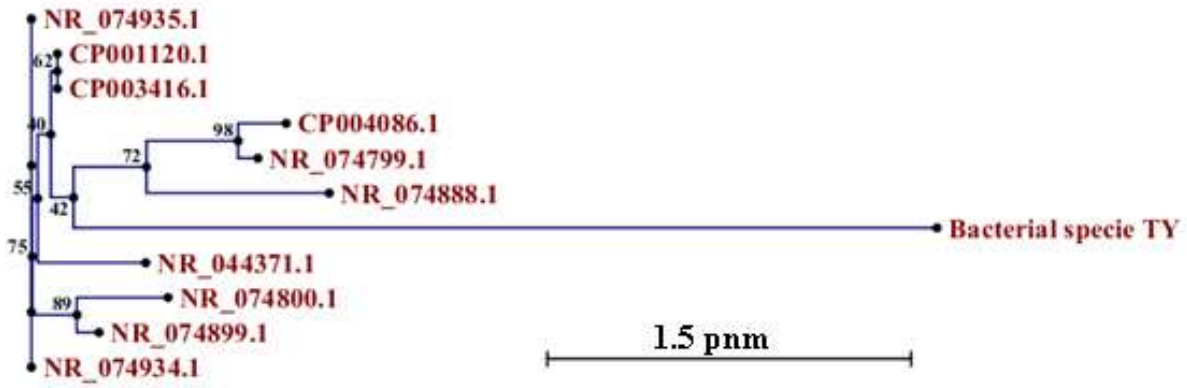


Fig. 3. Bootstrap dendrogram of 16S rDNA nucleotide sequences of ten different subspecies of *S. enterica* showing closest homology with bacterial specie TY, generated using distance based neighbor joining algorithm and 100 bootstrap resembling steps. Bacterial species are indicated by their respective GenBank Accession numbers as given in Table 3. Bar represents sequence deviation in term of percent nucleotide mutations (indicator of % substitutions). The frequency of each group in dendrograms is presented behind the respective node

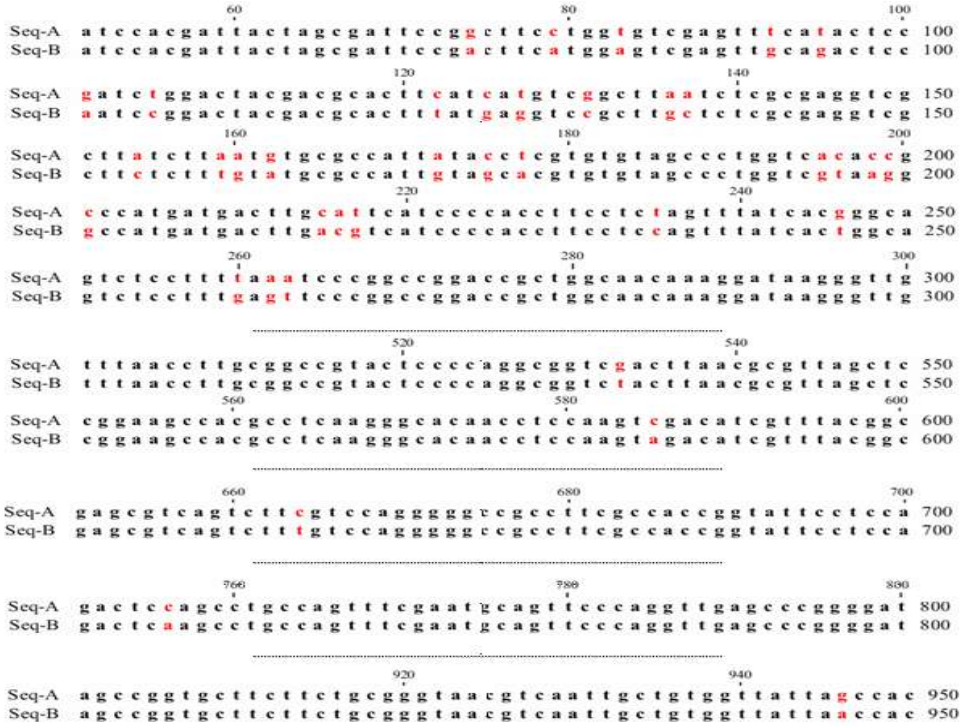


Fig. 4. Five regions of aligned 16S rDNA sequences of bacterial specie TY (Seq-A) and *S. enterica* subsp. enterica serovar Heidelberg str. B182 (Seq-B) showing 38 polymorphic nucleotides (in red colour)

bacterial species for which sequence data is not yet available in the GenBank database.

Screening of bacterial species ty for pathogenicity:

Bacterial species TY was found to relate closely with two different strains (B 182 & SL 476) of *S. enterica* subsp. enterica serovar Heidelberg strain B182, both of which are established to be pathogenic towards mammals (Bars et al *2012, Hoffmann* et al 2013). Congo red agar assay was performed to know whether the isolated specie was pathogenic or not. However, Bacterial specie TY produced only white colonies on Congo red agar medium (Fig. 5), which was suggestive of possible non-pathogenic nature of this bacterial species which indicate bacterial isolate *S. enterica* subsp. enterica strain TY was non-pathogenic thiamethoxam degrading bacteria. Besides this, biodegradation of different insecticides molecules by various species of *Bacillus* and *Pseudomonas* genera are well established but this is the first report on similar metabolization of any insecticide by species of *Salmonella*. Although, there are some other reports on *Salmonella* species which showed that this species can degrade such compounds which are explosive and toxic. Joseph *et al* (2006) observed that *Salmonella enterica* degrade ethanolamine (EA) is a sole source of carbon and nitrogen under aerobic, and anaerobic conditions. Acetaldehyde, an intermediate in ethanolamine degradation, is both volatile and toxic. Evidence had been presented that the primary function of the carboxysome (in *Salmonella*) encoded by *eut* is to minimize acetaldehyde loss and improve the efficiency of growth on EA. There is another report by Ederer et al (1997), according to which facultative enteric bacteria *Escherichia coli,* and *Salmonella typhimurium*, produced 4-amino-2,6-dinitrotoluene (ADNT), and accumulated less than stoichiometric amounts of 2,4-diamino-6-nitrotoluremovalene (DANT) from explosive 2,4,6-Trinitrotoluene (TNT). These previous studies have shown that *Salmonella* species can degrade toxic compounds, suggesting the broader metabolic capability of the specie.

Fig. 5. Screening of bacterial species TY (*S. enterica* strain TY) for pathogenicity produced only white colonies on Congo red agar medium

Effect of various cultural parameters on bacterial growth

Salmonella enterica subsp. *enterica* strain TY demonstrated optimal growth at 37°C, with maximum growth occurring between 2-4 days under shaking conditions (Fig. 6A and 6B). The strain exhibited effective growth over a pH range of 5.5-7.5, with the optimal pH at 6.5 (Fig. 6C). This suggests that TY is somewhat alkalophilic but can tolerate a range of pH levels. Therefore, further experiment was performed according to optimum parameters i.e at 37ºC in a medium of initial pH of 6.5 under shaking conditions.

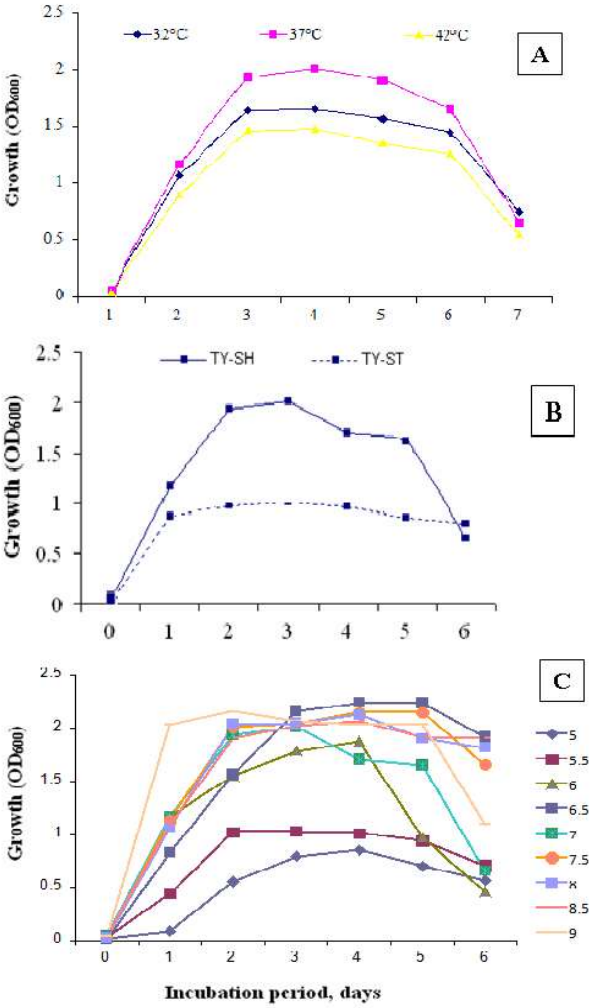


Fig. 6. Cell growth (OD_{600}) of different bacterial species as influenced by A- Incubation temperature, B- Aeration (shaking vs. stationary culturing) and C- Medium pH . TY- *S. enterica* strain TY, SH- Shaking conditions, ST- Stationary conditions

Effect of supplemented carbon and nitrogen sources on thiamethoxam degradation:

The prepared MSMT media raised with an optimum pH of 6.5 was inoculated with the bacterial inoculum and incubated @ 37ºC as described in material and methods. After 15 days of incubation, thiamethoxam degradation in uninoculated control treatments remained low (6.02-6.93%). Under unsupplemented conditions (C_0N_0), the cell growth of *S. enterica* subsp. enterica strain TY, remained low (OD_{600}= 0.484 to 0.510) but was associated with a maximum reduction in thiamethoxam (41.68%). The availability of additional nitrogen (C_0N_1) as well as carbon source (C_1N_1, C_1N_2) in the amended MSMT resulted in improved growth of the bacterial species (Table 4). However, this increase in growth due to the additional presence of carbon and nitrogen sources (C_0N_1, C_1N_1, and C_1N_2) was associated with a parallel decrease in thiamethoxam reduction by the bacterial isolate. Thus decline in thiamethoxam degradation as compared to unamended MSMT was enhanced in the presence of additional nitrogen ($C_0N_0 \rightarrow C_0N_1$) and further with the increase in the level of nitrogen in the presence of additional carbon ($C_1N_1 \rightarrow C_1N_2$). Though the study did not include analysis for degradation products of thiamethoxam, GLC chromatograms of culture extracts always showed only one peak specific to thiamethoxam only with a complete absence of peaks due to any possible degradation product of the same suggesting complete metabolization of this insecticide to metabolites that are not detectable by GLC. Thiamethoxam degradation by *S. enterica* subsp. enterica strain TY is repressed by the presence of easily available carbon and nitrogen sources and is stimulated by the absence of the same from the culture medium. It is also possible that enzymes involved in thiamethoxam metabolization are induced in the presence of thiamethoxam as the sole source of carbon and nitrogen causing higher degradation of the same in unamended media. A similar pattern of biodegradation in the presence of nitrogen and carbon followed by *B. aerophilus* strain IMBL 4.1, and *P. putida* strain IMBL 5.2 (Rana *et al* 2015). This phenomenon is typical of repressible metabolic pathways, reactions of which are inhibited by the presence of easily available nutrients for growth. The strain's ability to degrade thiamethoxam effectively in nutrient-limited conditions highlights its potential utility in contaminated environments.

Table 2. Statistical data on regression analysis and half-life for the dissipation of total thiamethoxam by isolated bacterial species in liquid cultures

Bacterial species	Regression equation *Y=a + bx (Y)*	Half-life (days)	Correlation coefficient *(R2)*
TA	3.68 + (-0.005x)	60.20	0.96
TB	3.67 + (-0.0073x)	41.23	0.80
TC	3.67 + (-0.0062x)	48.55	0.86
TD	3.69 + (-0.0094x)	32.02	0.93
TE	3.69 + (-0.0064x)	47.03	0.98
TF	3.69 + (-0.0027x)	91.48	0.95
TX	3.68 + (-0.0097x)	31.03	0.85
TY	3.64 + (-0.0027x)	28.13	0.93
TZ	3.68 + (-0.0069x)	43.62	0.97
Control	3.69 + (-0.0029x)	103.79	0.96

Table 3. Bacterial species producing maximum 16S rDNA homology with bacterial isolate TY as characterized by 100% query coverage, maximum score and lowest e-value (0.00)

Seq #	Species description	Max score	Identity	Gen Bank Acc. #
1.	*Salmonella. enterica* subsp. enterica serovar Heidelberg str. B182	1768	96%	CP003416.1
2.	*Salmonella enterica* subsp. enterica serovar Heidelberg str. SL476	1768	96%	CP001120.1
3.	*Salmonella. enterica* subsp. enterica serovar Heidelberg str. 41578	1768	96%	CP004086.1
4.	*Salmonella enterica* subsp. enterica serovar Paratyphi A str. AKU_12601 strain AKU12601 16S ribosomal RNA, complete sequence	1762	96%	NR_074935.1
5.	*Salmonella enterica* subsp. enterica serovar Paratyphi A str. ATCC 9150 strain ATCC 9150 16S ribosomal RNA, complete sequence	1762	96%	NR_074934.1
6.	*Salmonella enterica* subsp. enterica serovar Paratyphi C strain RKS4594 strain RKS4594 16S ribosomal RNA, complete sequence	1746	96%	NR_074899.1
7.	*Salmonella bongori*NCTC 12419 strain NCTC 12419 16S ribosomal RNA, complete sequence	1735	96%	NR_074888.1
8.	*Salmonella enterica* subsp. houtenae strain DSM 9221 16S ribosomal RNA, partial sequence	1735	96%	NR_044371.1
9.	*Salmonella enterica* subsp. enterica serovar Choleraesuis str. SC-B67 strain SC-B67 16S ribosomal RNA, complete sequence	1729	96%	NR_074800.1
10.	*Salmonella enterica* subsp. enterica serovar Typhi str. Ty2 strain Ty2 16S ribosomal RNA, complete sequence	1724	96%	NR_074799.1

Table 4. Effect of carbon and nitrogen sources on thiamethoxam degradation (%) by different bacterial specie after 15 days of incubation

Bacterial specie	CN level	Growth (OD_{600})	Thiamethoxam	
			Contents, µg ml^{-1}	% Reduction
Control	C_0N_0	-	45.89± 1.02	6.57
	C_0N_1	-	45.80±0.95	6.93
	C_1N_1	-	46.17±0.89	6.18
	C_1N_2	-	46.25±1.10	6.02
S. enterica	C_0N_0	0.484±0.012	28.70±0.75	41.68
	C_0N_1	0.867±0.019	29.62±0.79	39.81
	C_1N_1	1.210±0.029	29.97±0.65	39.10
	C_1N_2	1.284±0.032	31.35±0.78	36.29

Data is mean± SE of three replications each. Mean thiamethoxam concentration in uninoculated control at 0 day irrespective of treatment was estimated to be 49.21±0.86 µg ml^{-1}

Irrespective of treatment, mean OD_{600} was kept constant at 0.030±0.005 through use of uniform inoculation conditions.

Abbreviations are: C_0- No glucose; C_1- 0.1% glucose, N_0- No Nitrogen, N_1 and N_2- 0.1% and 0.2% ammonium sulphate, respectively. All media also contained ammonium ferric citrate at 0.001% level to serve as source of iron.

The number of microorganisms reported to degrade different pesticides but in this paper only thiamethoxam degrading microorganism discussed. Rana et al (2015) reported twelve bacterial strains of *Bacillus* and *Pseudomonas* which cause thiamethoxam reduction over a wide range of 20.88 to 45.28% after 15 days. Among them, two bacterial species *B. aerophilus* strain IMBL 4.1 (45.28%) and *P. putida* strain IMBL 5.2 (38.23%) caused the highest thiamethoxam biodegradation during 15 days periods of growth in the MSMT liquid culture. Rana and Gupta (2019) studied the persistence of thiamethoxam in clay loam soil for 56 days in laboratory conditions. They reported active biodegradation of thiamethoxam by *Bacillus aerophilus* strain IMBL 4.1. They also studied the half-life ($t_{1/2}$) in clay loam soil at different concentration (@ 25, 50, and 100 $mgkg^{-1}$) of thiamethoxam and found a reduction in half-life ($t_{1/2}$) (11.15, 11.58. and 12.54) as compared to half-life ($t_{1/2}$) in control (33.44, 37.63, and 37.63). Oanh and Duc (2023) showed that mix culture of *Phanerochaete* sp. Th1 and *Ensifer* sp. Th2 effectively degrade thiamethoxam in liquid media, maize straw, and soil, with the highest degradation rate observed in their mixed culture (*Phanerochaete* sp. Th1, *Ensifer* sp. Th2, and mixed culture has 0.53 ± 0.05, 0.74 ± 0.07, and 0.81 ± 0.08 mg/day degradation rates, respectively). They showed that *Phanerochaete* sp. Th1 excels in decomposing maize straw components, while *Ensifer* sp. Th2 efficiently handles Cl^- removal after initial dechlorination by the fungus. Xiang et al (2023) also demonstrates that immobilizing *Chryseobacterium* sp. H5 in polyvinyl alcohol (PVA)/sodium alginate (SA)/biochar beads significantly enhances the biodegradation of thiamethoxam, achieving up to 90.47% removal and 68.03% degradation rates. The approach also improves microbial tolerance to extreme conditions and provides valuable insights into the degradation pathways of thiamethoxam.Similarly, this study results also contribute to understanding the broader metabolic capabilities of *Salmonella* species. While *Salmonella* is not typically associated with pesticide degradation, strain TY's ability to metabolize thiamethoxam, combined with its non-pathogenic nature, opens new avenues for bioremediation research. The study demonstrates that *Salmonella enterica* subsp. *enterica* strain TY effectively degrades thiamethoxam under specific nutrient-limited conditions. The strain exhibited optimal growth at 37°C and pH 6.5, with significant degradation observed in unsupplemented media. Notably, the presence of easily available nitrogen and carbon sources suppressed thiamethoxam degradation, indicating a repressible metabolic pathway, while nutrient-limited conditions stimulated higher degradation rates. GLC analysis showed complete metabolization of thiamethoxam to undetectable products, suggesting that degradation pathways may produce metabolites not easily measured by conventional methods. These findings highlight the potential of strain TY for bioremediation applications in environments contaminated with thiamethoxam, particularly in scenarios where nutrient levels are low, allowing for the maximization of its degradation capabilities. Overall, this research contributes valuable insights into the microbial metabolism of insecticides and the conditions that optimize such processes for environmental remediation efforts.

CONCLUSIONS

This study has successfully isolated, and characterized a novel thiamethoxam-degrading bacterial strain, *S. enterica* subsp. *enterica* strain TY. This strain shows significant

potential for bioremediation of thiamethoxam in contaminated environments. Further research into the specific metabolic pathways involved, and the development of optimized conditions for large-scale application could enhance the practical utility of this strain in environmental cleanup efforts. Biodegradation is the best way as it uses specific microorganism which has capabilities to degrade harmful compound along with this some of them have a broad spectrum of action on different pesticides. Thus, there is a need to know more about microbe's physiology, genetic behavior, and mechanism of action to enhance its biodegradation in less time and rapidly.

REFERENCES

Agenta K, Alison N and Bretifinal Y 1997. Marine Salmonellosis studied by confocal microscopy *Salmonella Typhimurium* and cytotoxic effect on phagocytes in vivo. *Journal of Experimental Medicine* **18:** 569-580.

Bars HL, Bousarghin L, Bonnaure-Mallet M, Jolivet-Gougeon A and Barloy-Hubler F 2012. Complete genome sequence of the strong mutator *Salmonella enterica* subsp. *enterica* serotype Heidelberg strain B182. *Bacteriology* **194:** 3537-3538.

Chishti Z and Arshad M 2012. Growth linked biodegradation of chlorpyrifos by *Agrobacterium* and *Enterobacter* spp. *International Journal of Agriculture and Biology* **15:** 19-26.

Ederer MM, Lewis TA and Crawford RL 1997. 2,4,6-Trinitrotoluene (TNT) transformation by clostridia `isolatedfrom a munition-fed bioreactor: comparison with non-adapted bacteria. *Journal of Industrial Microbiology and Biotechnology* **18:** 82-88.

Elhamalawy O, Bakr A and Eissa F 2024. Impact of pesticides on non-target invertebrates in agricultural ecosystems. *Pesticide Biochemistry and Physiology* **202:** 105974

Fulton MH, Key PB and Delorenzo ME 2014. Insecticide toxicity in fish. Organic Chemical ToxicologyFishes, *Fish Physiology* **33:** 309-368.

Hegde DR, Manoharan T and Sridar R 2017. Identification and characterization of bacterial isolates and their role in the degradation of neonicotinoid insecticide thiamethoxam. *Journal of Pure and Applied Microbiology* **11:** 393-400.

Henry M, Be´guin M, Requier F, Rollin O, Odoux JF, Aupinel P, Aptel J, Tchamitchian S and Decourtye A 2012. A common pesticide decreases foraging success and survival in honey bees. *Sciences* **336:** 348-350.

Hoffmann M, Luo Y, Lafon PC, Timme R, Allard MW, McDermott PF, Brown EW and Zhaoa S 2013. Genome sequences of *Salmonella enterica* Serovar Heidelberg isolates isolated in the United States from a multistate outbreak of human *Salmonella* infections. *Genome Announcement* **1:** 1-2.

Jariyal M, Gupta VK, Mandal K, Jindal V, Banta G and Singh B 2014. Isolation and characterization of novel phorate-degrading bacterial species from agricultural soil. *Environmental Science and Pollution Research* **21:** 2214-2222.

Joseph T, Penrod, John R and Roth 2006. Conserving a volatile metabolite: A role for Carboxysome-Like Organelles in *Salmonella enterica. Journal of Bacteriology* **188**(8): 2865-2874.

Khaldoun-Oularbi H, Bouzid N, Boukreta S, Makhlouf C, Derriche F and Djennas N 2017. Thiamethoxam Actara® induced alterations in kidney liver cerebellum and hippocampus of male rats. *Journal of Xenobiotics* **7:** 7149.

Malcova M, Hradecka H, Karpiskova R and Rychlik I 2008. Biofilm formation in field strains of *Salmonella enterica serovar Typhimurium*: identification of a new colony morphology type and the role of SGI1 in biofilm formation. *Veterinary Microbiology* **129:** 360-366.

Mandal K, Singh B, Jariyal M and Gupta VK 2014. Bioremediation of fipronil by a *Bacillus firmus* isolate from soil. *Chemosphere* **101:** 55-60.

Mohamed MS 2009. Degradation of methomyl by novel bacterial strain *Stenotrophomonas maltophilia* M1. *Electronic Journal of Biotechnology* **12:** 57-62.

Myresiotis C, Vryzas Z and Papadopoulou-Mourkidou E2011. Biodegradation of soil-applied pesticides by selected strains of plant growth-promoting rhizobacteria (PGPR) and their effects on bacterial growth. *Biodegradation* **23:** 297-210.

Oanh NT and Duc HD 2023. Degradation of Thiamethoxam by mixed culture of *Phanerochaete* sp. Th1 and *Ensifer* sp. Th2. *Applied Biochemistry and Microbiology* **59:** 858-866.

Rana S and Gupta VK 2019. Microbial degradation of second generation Neonicotinoid: Thiamethoxam in clay loam soils. *Journal of Pharmacognosy and Phytochemistry* **SP1:** 294-298

Rana S, Jindal V, Mandal K, Kaur G and Gupta VK 2015. Thiamethoxam degradation by *Pseudomonas* and *Bacillus* strains isolated from agricultural soils. *Environmental Monitoring and Assessment* **187:** 300.

Schmalenberger A, Schwieger F andTebbeCC2001. Effect of primers hybridizing to different evolutionarily conserved regions of the small-subunit rRNA gene in PCR-based microbial community analyses and genetic profiling. *Applied and Environmental Microbiology* **67:** 3557-3563.

Wang M, Yang G, Wang X, Yao Y, Min H and Lu Z2011. Nicotine degradation by two novel bacterial isolates of *Acinetobacter* sp. TW and *Sphingomonas* sp. TY and their responses in the presence of neonicotinoid insecticides. *World Journal of Microbiology and Biotechnology* **27:** 1633-1640.

Xiang X, Yi X, Zheng W, Li Y, Zhang C, Wang X, Chen Z, Huang M and Ying G-G 2023. Enhanced biodegradation of thiamethoxam with a novel polyvinyl alcohol (PVA)/sodium alginate (SA)/biochar immobilized *Chryseobacterium* sp H5. *Journal of Hazardous Mate*rials **443:** 130247.

Yakupova EI, Bobyleva LG, Vikhlyantsev IM and Bobylev AG 2019. Congo red and amyloids: History and relationship. *Bioscience Report* **39:** BSR20181415.

Zhou G, Wang Y, Zhai S, Ge F, Liu Z, DaiY, Yuan S and Hou J2013. Biodegradation of the neonicotinoid insecticide thiamethoxam by the nitrogen-fixing and plant-growth-promoting rhizobacterium *Ensifer adhaerens* strain TMX-23. *Applied Microbiology and Biotechnology* **97:** 4065-4074.

Received 14 September, 2024; Accepted 29 October, 2024