Characterizations of novel pesticide-degrading bacterial strains from industrial wastes found in the industrial cities of Pakistan and their biodegradation potential

Noreen Asim/Noreen Azhar[1], Mahreen Ul Hassan[2,3], Farheen Shafique[4], Maham Ali[5], Hina Nayab[6], Nuzhat Shafi[7], Sundus Khawaja[8], Sadaf Manzoor[9]

[1]Division of Genomics and Bioinformatics, Institute of Biotechnology and Genetic Engineering, The University of Agriculture, Peshawar , Pakistan

[2]Department of Microbiology, Shaheed Benazir Bhutto Women University, Peshawar , Pakistan

[3]Department of Molecular Biology and Biotechnology, University of Sheffield, Sheffield, UK

[4]Department of Biomedical Science, University of Sheffield, Sheffield, UK

[5]Department of Zoology, University of Peshawar, Peshawar , Pakistan

[6]Institute of Biological Sciences, Sarhad University of Science and Information Technology, Peshawar , Pakistan

[7]Department of Zoology, University of Azad Jammu and Kashmir, Muzaffarabad , Pakistan

[8]University of Azad Jammu and Kashmir, Department of Biotechnology, Muzaffarabad , Pakistan

[9]Department of Statistics, Islamia college University, Peshawar, Khyber Pakhtunkhwa, Pakistan

Corresponding author: *Mahreen ul Hassan

                                          PhD Scholar

                                          Dept of Molecular Biology and Biotechnology

                                          University of Sheffield

                                          Firth Court,

                                          Western Bank

                                          Sheffield

30   United Kingdom

31   Email: muhassan1@sheffield.ac.uk

33 **Abstract**

34 **Background** Lack of infrastructure for disposal of effluents in industries leads to severe

35 pollution of natural resources in developing countries. These pollutants accompanied by

36 solid waste are equally hazardous to biological growth. Natural attenuation of these

37 pollutants was evidenced that involved degradation by native microbial communities. The

38 current study encompasses the isolation of pesticide-degrading bacteria from the vicinity

39 of pesticide manufacturing industries. **Methods** The isolation and identification of

40 biodegrading microbes was done. The pesticide-degrading bacteria were isolated by

41 using enrichment culture method from industrial waste. **Results** Around 20 different

42 strains were isolated, among which 6 isolates showed significant pesticide biodegrading

43 activity. After 16S rRNA analysis, two isolated bacteria were identified as *Acinetobacter*

44 *baumannii* (5B) and *Acidothiobacillus ferroxidans*, and the remaining four were identified

45 as different strains of *Pseudomonas aeruginosa* (1A, 2B, 3C, 4D). Phylogenetic analysis

46 confirmed their evolution from a common ancestor. All strains showed distinctive

47 degradation ability up to 36 hours. The *Pseudomonas aeruginosa* strains 1A and 4D

48 showed highest degradation percentage of about 80% for DDT, and *P. aeruginosa* strain

49 3C showed highest degradation percentage, i.e., 78% for aldrin whilst in the case of

50 malathion, *A. baumannii* and *A. ferroxidans* have shown considerable degradation

51 percentages of 53% and 54%, respectively. Overall, the degradation trend showed that

52 all the selected strains can utilize the given pesticides as sole carbon energy sources

53 even at a concentration of 50 mg/mL.

54 **Conclusion** This study provided strong evidence for utilizing these strains to remove

55 persistent residual pesticide; thus, it gives potential for soil treatment and restoration.

56

57 **Keywords**   organo-pesticides,   biodegradation,   *Acinetobacter*   *baumannii*,

58 *Acidothiobacillus ferroxidans*, *Pseudomonas aeruginosa*, 16S rRNA, malathion, DDT ,

59 aldrin

60

INTRODUCTION

Pesticides are hydrocarbons widely used in agriculture and households to control variety of pests (Bhatt *et al.,* 2021). Pesticides were introduced in Pakistan during the 1960s in the period of green revolution, and during the 1980s its applied quantity rose from 665 tons to 46,000 tons in the year 2000 (Syed *et al.,* 2014). Pesticides are categorized into four types: organochlorines, organophosphorus, carbamates and pyrethroids. Among these, organochlorine pesticides were the primary class with high toxicity, hydrophobicity, and persistent nature. Many organochlorine pesticides have been banned. However, pesticides like chlorinated hydrocarbons such as DDT, aldrin, and dieldrin are still ignorantly used, and a significant number of obsolete pesticides have been stockpiled in developing countries (Arrebola *et al.,* 2015). Among all the synthetic pesticides, organophosphate is the more stable and less toxic class. Therefore, their wide structural variety and diverse chemical properties enable them for multiple uses. Derivatives of these organo-chemicals are also controversial because they also have persistent effects on the surroundings when applied for a more extended period (Jayaraj R *et. al.,* 2016) and even within the organism after exposure. Organochlorine and organophosphate, even at low concentration, are toxic and carcinogenic to mammals. They can affect the nervous system, immune system, respiratory system, reproductive function, and hormonal balance, and cause serious illnesses in invertebrates and vertebrates. Accumulation of these pollutants in soil or water bodies has been the reason for the loss of many marine and sea animals. Dramatic consumption would lead to the integration of these pesticides in the food chain, which ultimately accumulate in the tissue of consumers, causing deleterious effects (Kim *et al.,* 2017). Additionally, wide ranges of water and terrestrial ecosystems have been polluted with these compounds. The eradication and safe removal of such pollutants from the environment is essential, and industries related to pesticides are the main culprits (Hattab and Ghaly, 2012). Advanced countries have treatment facilities for safe disposal of effluents, but these issues are rarely tackled in underdeveloped and developing countries, particularly in small scale industries due to their unaffordability (Mishra S, *et al.,* 2021). Biodegradation and detoxification with the help of native microbes are promising approaches for toxic waste removal and environmental remediation. Microbial communities on exposure to new compounds

92   generate new enzymes with the emergence of genetic mutations to survive. Hence native

93   microbes adapt to new surroundings.

94   Furthermore, it is easy to extract genes from microbes possessing degradation ability,

95   which can be expressed in other hosts by genetic engineering (Ye *et al.,* 2018; Regueiro

96   *et al.,* 2015; Bhatt P *et al.,* 2020). Many lower income countries do not have any facility

97   to regulate, manage, and dispose of the obsolete persistent organic pollutants. Thus,

98   implementation of cost-effective waste management procedures like bioremediation,

99   phytoremediation and bioaugmentation is needed to restore the natural resources

100   (UNEP, 2018).

101   Biodegradation of pollutants through natural attenuation is the most preferable, cost

102   effective and safest solution. But the availability, effectiveness, and popularization of

103   certain microbial communities for treatment of specific pesticides are a significant issue

104   and are under consideration by examining and isolating the native strains from such

105   contaminated (effluent) areas that combat these toxicities (Cycoń M *et al.,* 2017).

106   Biodegradation through microbes is the most effective way to make our environment

107   pesticide free. For their sustenance, microbes utilize toxic substances such as carbon,

108   nitrogen, and phosphorus, etc. (Bhatt *et al.,* 2021). Currently microbial degradation is

109   considered as most thorough and extensive method for biodegradation. For this purpose,

110   bacteria, fungi, and algae are all used in different ways, but the bacteria are very common.

111   In the past, many scientists have worked in search of microbes that could degrade

112   pollutants to teir non-toxic states (Huang *et al.,* 2021). Bacteria and fungi have been

113   utilized as whole-cell biocatalysts in a variety of biotechnological processes, not only for

114   manufacturing various products but for the development of biotransformation/

115   biodegradation reactions also (Birolli *et al.,* 2019). Many bacterial and fungal genera such

116   as *Aerobacter, Acinetobacter, Agrobacterium, Aspergillus, Bacillus, Clostridium,*

117   *Desulfuromonas, Enterobacter, Flavobacterium, Fusarium, Klebsiella, Lactobacillus,*

118   *Pasteurella, Pseudomonas, Pleurotus, Phlebia sp., Penicillium, Rhizopus,*

119   *Sedimentibacter*, *Streptococcus*, and *Staphylococcus* were identified in degradation of

120   popular pesticides. Although many biodegrading microbes have been extensively studied

121   for their pesticide degrading ability, many scientists are still searching for better microbes

122   with high and specific efficacy (Kumar *et al.,* 2018 and Bhatt P *et al.,* 2021). There are

123   many bacteria which can degrade a variety of xenobiotic compounds quite efficiently

124   under controlled laboratory conditions, but they failed to do so in a natural environment

125   (Arora *et al.,* 2018).

126   Although the use of most organo-pesticides is also banned in Pakistan, their residues still

127   exist and have accumulated in biotic and abiotic resources (Mazzon *et al.,* 2020).

128   The current study aims to isolate and identify novel bacterial strains from the vicinity of

129   pesticide manufacturing industrial sites that could use pesticides like DDT, aldrin and

130   malathion as their nutritional source.

131   **Contribution**

132   Our study's uniqueness was to collect samples specifically from the unexplored points of

133   pesticide wastes to isolate the possible native bacterial strains. We also observed the

134   extent of bacterial chemical utilization capacity, especially for DDT, aldrin and malathion.

135   The study gives an insight into the soil that was chemically polluted with these pesticides

136   and could be restored for further cultivation purposes. After confirming their presence,

137   further chemical tests and morphological studies were conducted to improve our

138   understanding of these bacterial strains that can further be utilized to treat chemically

139   polluted land and water.

140

141   **Materials & Methods**

142   **Sampling and management**

143   Samples were obtained from effluents of five industrial sites, mainly located at Faisalabad

144   and Lahore in Pakistan. Water and soil samples were randomly collected from industrial

145   drainage sources and garbage/disposal points, respectively, where the possibility of the

146   presence of effluents was higher. The soil and water samples were collected with the

147   consent of the landowner. No animal or plant species were harmed during the collection

148   process. Standard procedures were used to obtain sludge samples (Ghanem *et al.,*

149   2007), from wastewater near industrial area carrying effluents from the pesticide

150   manufacturing plants (Farhan *et al.,* 2012). All the reagents and chemicals were analytical

151   grade purchased from Merck, Germany, and Sigma-Aldrich USA. The pesticides

152   (malathion, DDT and aldrin) with > 99% purity were obtained from Sigma-Aldrich, USA.

153   Synthetic oligonucleotides were provided by the Integrated DNA Technologies (IDT), USA

154   for this study. Physical samples of microbial isolates used in this study were permanently

155   deposited at National Institute of Biotechnology and Genetic Engineering (NIBGE)

156   Faisalabad, Pakistan

157

158   **Sample processing and enrichment**

159   To maintain the biological activity of the soil microflora, the samples were obtained from

160   contaminated areas in sterile polythene bags kept at 4°C. On the day of collection, the

161   soil samples were further treated by mixing 10 g of soil sample in a 100 mL of autoclaved

162   water. The mixture was then kept at room temperature (30°C) in an orbital shaker for 24

163   hours at 100 rpm. The enrichment culture method was acquired to isolate pesticide

164   biodegrading bacterial strains. To improve the likelihood of detecting pesticide-degrading

165   native microflora, samples were first supplemented with commercial-grade pesticides

166   (Khan et al., 2016). From each water and soil sample, 10 mL were inoculated into 100

167   mL of fresh MSM broth in 250 mL Erlenmeyer flasks containing 20 mg/mL selected

168   pesticides as the sole source of carbon and phosphorus. The flasks were incubated for

169   seven days at 150 rpm under ambient conditions. The cultures were gradually acclimated

170   to increase the three applied pesticides ranging from 20-50 mg/mL at weekly intervals. At

171   about a final concentration of 50 mg/mL of different pesticides, the pesticide tolerant

172   cultures were subjected to further degradation study. Media without inoculation was run

173   as a control. The experiments were performed in triplicate for authentication (Jiang et al.,

174   2019).

175   **Isolation of biodegrading strains**

176   For the isolation of pesticide degrading bacteria, the nutrient agar media plates were first

177   inoculated with 1mL of each enriched culture and then incubated at 37°C till colonies

178   appeared. Lone colonies were picked and streaked aseptically on solid media plates

179   followed by incubation at 37°C for 48 hours. Separate colonies were sub-cultured onto

180   nutrient agar plates till pure cultures were obtained. The strains from pure culture were

181   then inoculated into 100 mL MSM broth which contained 50 mg/mL of selected pesticides

182   added as the sole source of carbon or phosphorus, or both carbon and phosphorus

183   sources to determine their degradation ability at different time intervals (2, 6, 8, 12, 16,

184   20, 24, 30, and 36 hours) at 30°C. Negative controls were always run to ensure that

185   mentioned sources in the media were not contaminated. The measurement of the

186   absorbance was done by using an Agilent Cary 5000 Ultraviolet-to-Visible to-Near-

187   Infrared (UV-Vis-NIR) spectrophotometer (Agilent Technologies, Petaling Jaya, Malaysia)

188   (Roy *et al.,* 2018). The cultures with the highest degradation ability were stored in glycerol

189   as nutrient-agar slants at 4°C.

190   Degradation Rate %= $\dfrac{\text{Initial OD} - \text{Final OD}}{\text{Initial OD}} \times 100$

191 Identification and characterization of isolates

192   The identification and characterization of biodegrading isolates were carried out using

193   morphological, biochemical, and molecular tests. The preserved isolates showing

194   maximum degradation activity were picked and grown on the nutrient medium through

195   spread plate method followed by culturing at 37°C for 48 hours as described by Jiang *et*

196   *al.,* 2019. A phase-contrast microscope Axiovert by (Zeiss model MC-80). was used to

197   examine the colony features as well as the morphological structure of the cells. Then,

198   strains capable of degradation were further tested through gram staining for the

199   identification. The biochemical tests included catalase test, gelatinase test, starch

200   hydrolysis test (amylase test), casein hydrolysis test, oxidase test, Indole production test,

201   Methyl red test, Citrate utilization test, Nitrate reduction test and Urease test.

202

203 Molecular characterization

204   The isolated strains' DNA was extracted using the Cetyltrimethylammonium Bromide

205   (CTAB) technique (Orek, 2018). The RT PCR (QIAGEN) was used to conduct the 16S

206   rDNA amplification process and phylogenetic analysis. The DNA obtained from the

207   isolated strains was utilized as a template for PCR amplification using 16S rDNA primers.

208   The forward primer for the bacterium was FD1 (AGAGTTTGATCCTGGCTCAG 20bp),

209   and the reverse primer was rP1 (ACGG(ACT)TACCTTGTTACGACTT, 23bp). The PCR

210   products were sequenced by Macrogen Inc., Seoul, Korea. The Basic Local Alignment

211   Search Tool (BLAST) was run to analyze sequences (Altschul *et al.,* 1990). The 16S

212   rRNA gene sequences of selected strains were acquired from GenBank and matched

213   with the gene sequences of our isolates using CLUSTALX. A distance matrix was

214   constructed using the aligned sequences (Jukes and Cantor 1969). Following the

215 generation of 500 bootstrap sets, MEGA X version 10.2.6 was used to construct a
216 phylogenetic tree using the neighbour-joining technique (Stecher et al., 2020).
217

218 **Growth Rate Study**
219 To check the efficacy of growth of pesticide degrading microbes in MSM broth, the growth
220 curve was observed.
221 In a sterile conical flask holding 100 mL of MSM broth, an aliquot (1mL) of bacterial
222 suspension was added (Ghanem, Khaled M *et al*., 2012). The MSM composition (w/v)
223 was 0.1% NH_4NO_3, 0.1% NaCl, 0.15% K_2HPO_4, 0.05% KH_2PO_4, 0.01% $MgSO_4.7H2O$,
224 0.0025% FeSO4, 1% glucose and incubated in a shaking incubator at 150 rpm using
225 WiseCube Fuzzy System (model WIS-20) (Al-Thukair & Malik, 2016) at various
226 temperatures (15 °C, 30 °C and 45 °C). The wavelength was set at 600 nm (OD600) to
227 check cell density at different time intervals (2, 6, 8, 12, 16, 20, 24, 30 and 36 hours) using
228 an   Agilent   Cary   5000   Ultraviolet-to-Visible   to-Near-Infrared   (UV-Vis-NIR)
229 spectrophotometer (Agilent Technologies, Petaling Jaya, Malaysia). The optimum pH of
230 the growth media was adjusted to pH 7. The growth curve was constructed by determining
231 the optimum culture time of each biodegrading strain at different temperature. All the
232 experiments were done in triplicate, and a negative control with no microbial inoculation
233 was carried under the identical circumstances.

234 **Statistical Analysis**
235 The normality of the data was tested using the Pearson's correlation tests for all of the
236 data obtained. Statistical analysis was done using MS Excel 16.0.
237 **Approval of field permit**
238 Field experiments were approved by Research committee of the University of Agriculture
239 Peshawar, Pakistan (Project number :27/FCPS/AUP) and National institute of
240 biotechnology and genetic engineering (NIBGE), Faisalabad-Pakistan.
241
242

243 **RESULTS**
244 **Isolation of pesticide-degrading bacteria**

245   Through enrichment, many indigenous pesticide-tolerant bacteria with pesticides as their
246   only carbon source were identified from contaminated soil and industrial effluents. From
247   these enrichment cultures, 20 morphologically diverse strains were isolated on nutrient
248   agar plates containing DDT and aldrin and malathion pesticides, respectively. These
249   strains' pesticide degradation ability was checked on MSM agar plates containing
250   pesticides (50 mg/mL). The six bacterial isolates used in the degradation experiments.
251   Isolates 1A, 2B, and 3C came from soil contaminated with DDT (from Industrial city
252   Faisalabad). While isolates 4D, 5E and 6F were from the industrial area of Lahore,
253   Pakistan, with DDT, aldrin and malathion contamination. Of the two locations
254   investigated, samples from the Lahore industrial area had the highest contamination.
255   Pesticide contamination was found in all the soils and effluents utilized to isolate
256   microorganisms. All six isolates were correctly identified using 16S rRNA sequencing.
257   The strain *Pseudomonas* was found to be the most common among the isolates.

258   **Pesticide degradation activity**

259   Out of 20 bacterial strains from the industrial wastes, only six (1A, 2B, 3C, 4D, 5E and
260   6F) showed significant degradation against all three pesticides at 50 mg/mL, which was
261   tested. The plates were marked into small segments. The zone of degradation was plotted
262   against the periodic time intervals shown in Figures 1, 2 and 3 for the pesticides.

263   The percentage degradation of DDT pesticide was plotted against time. The isolates 1A,
264   2B, 3C and 4D showed highest degradation activity of 70-80% during the 36 hours while
265   5E and 6F degraded 28-36% of the same DDT concentration (Figure 1 and Table S1).

266

267   **Figure 1.** Graphical representation of percent DDT degradation shown by each bacterial
268   isolate plotted against time. The experiment was done in triplicate, and errors bars
269   indicate the standard deviation.

270

271   In the case of aldrin, a little variation in percentage values of degradation was observed
272   when plotted against time (Figure 2 and Table S2). The isolate 3C showed the highest
273   degradation (78%) against aldrin, followed by 2B and 4D with 72% and 68%, respectively.
274   In contrast, isolate 1A showed 58% degradation. The degradation values for the isolates

275 5E and 6F were 30% and 40%, respectively. However, 5E and 6F values were observed
276 to be lower than 1A, 2B, 3C and 4D.

277

278 **Figure 2.** Graphical representation of percent aldrin degradation shown by each bacterial
279 isolate plotted against time. The experiment was done in triplicate, and errors bars
280 indicate the standard deviation.

281

282 In the case of malathion, a reverse trend was observed, and highest values of 54% and
283 53% were recorded from F6 and E5 isolates over 1A, 2B, 3C, 4D, where degradation rate
284 was recorded between 30-40%, as shown in Figure 3 and Table S3.

285

286 **Figure 3.** Graphical representation of percent malathion degradation shown by each
287 bacterial isolate plotted against time. The experiment was done in triplicate, and errors
288 bars indicate the standard deviation.

289
290 Overall degradation of the selected bacterial isolates showed that selected locally
291 collected bacteria could easily degrade organophosphates and organo-chlorines.
292 However, the degradation rate can depend on both the type of bacteria and the pesticide
293 composition, which needs to be explored further. These strains could play a useful role in
294 achieving the safe recovery of polluted sites, particularly in a situation where pesticides,
295 chemicals and other undesired toxic substances containing organochlorine and organo-
296 phosphates and their variety of derivatives might persist. These isolates might also be
297 helpful to produce beneficial microbes sensitive to such toxicities. However, the isolated
298 strains had varying effectiveness for specific pesticides. This trend showed that strains
299 might be applied according to the nature of pollutant present in a particular environment
300 for eradicating toxic chemicals.

301

302 **Identification and characterization of isolates**

303 The bacterial strains were selected by analyzing their morphology and colony
304 characteristics, indicating the microbial diversity present in the respective collected

305 (environmental) samples. Based on the isolates' physical appearance, the distinguishing

306 yellow, cream, and white pigmented colonies (Fig. 4), a colonial diversity (raised and flat)

307 was observed. When viewed under a phase-contrast microscope, the isolates were

308 appeared with different cellular morphologies. All isolates were mostly rod-shaped, while

309 the isolate 4D exhibited coccus shape as well. Similarly, all isolates were gram-negative

310 and motile except 4D, which is non-motile. None of the isolates contained endospores,

311 and none of the isolates indicated exospores. The isolates 4D, 5E and 6F were circular,

312 while the rest are irregular in shape. The isolates 2B, 3C, and 4D have undulated colony

313 margins whereas 1A, 5E and 6F had entire margins (Table 1). The selected isolates' cell

314 size was found to be 1.5 µm for 1A and 4D, while the rest of the isolates' cell size ranges

315 from 2.0 to 3µm, as presented in Table 1.

316

317

318 **Figure 4.** The appearance of colonies of the bacterial isolates **1A**, **2B, 3C, 4D, 5E** and
319 **6F** on nutrient agar.

320

321 **Table 1.**   Cellular characteristics of biodegrading pesticide isolates

322

PCR method for 16S rRNA amplification and Sequencing

324 Before sequencing all the DNA samples were amplified under optimized conditions. To

325 obtain a PCR product of 1, 500 base pairs, universal eubacterial rDNA primers fD1 and

326 rP1 were used (Figure S1). Purified PCR product was sequenced commercially. A partial

327 sequence of the 16S rRNA showed that four isolates had 97-99 % homology with

328 *Pseudomonas spp*. Simultaneously, the other two isolates were 97 and 96 % identical to

329 *Actinobacter baumannii* and *Acidothiobacillus ferroxidans*, respectively (Table 2).

330 All these sequences are deposited in the National Center for Biotechnology Information

331 (NCBI) (https://www.ncbi.nlm.nih.gov/).

332 **Table 2.** Sequence identity (%) of the partial 16S rRNA gene sequence of isolates
333 obtained from environmental samples with related microbes

334

Phylogenetic Analysis

336   Partial 16S rRNA were obtained from the NCBI (www.ncbi.nlm.nih.gov) web site, and they

337   were aligned with our isolates to generate a phylogenetic tree (Figure 5). Phylogenetic

338   analysis showed that all five isolates have evolved from a common ancestor. They have

339   a close relationship among themselves except 5E which shares a common ancestor but

340   is far off from the other isolates in the tree. This showed the diversity among the isolates

341   persists that can tolerate pesticides even at high concentration.

342   **Figure 5.** Phylogenetic tree showing evolutionary relationships of various strains**.** The
343   Neighbor-Joining technique was used to infer the evolutionary history. MEGA X version
344   10.2.6 was used to undertake the evolutionary analyses (Kumar S., 2018; Stecher et al.,
345   2020).

346
347

Estimation of bacterial growth rate

349   At 600nm, optical density of each isolate was recorded at various intervals i.e., 2, 8, 12,

350   16, 20, 24, 30 and 36 hours, on MSM medium. All the isolates studied in the experiment

351   attained an OD of around 0.7 in broth at 30 °C after 8 hours, while it took more than 10

352   hours to reach the OD of 0.7 at other temperatures, so the optimal temperature for these

353   strains was 30°C, as shown in Figure 6 and Table S1. 6F showed maximum growth at

354   different temperatures among all the isolates, while A1 showed the least. Similar results

355   were reported by Kaya *et al.,* in 2014 and Murad et al. in 2007.

356

357   **Figure 6.** Growth curve of isolated strains 1A, 2B,3 C,4 D, 5E and 6F at 15°C, 30°C and
358   45°C. The black bars represent standard error.

359

Discussion

361   The study addresses the isolation and identification of bacteria residing in pesticide-

362   polluted sites. We also observed the extent of bacterial chemical utilization capacity

363   for DDT, aldrin, and malathion. The identification of isolated bacteria was governed

364   by analyzing both phenotypic and genotypic traits. From the current study, microbial

365   biodiversity present in industrial waste was assessed using 16S rRNA analysis which

366   is by far the best genetic marker that could exquisitely identify and illustrate

367   the evolutionary relationship of mixed cultures of environmental and clinical bacterial

368   isolates (Jovel *et. al.,* 2016 and Lagier *et al.,* 2015).

369   In this study, six novel strains were found to have distinct degradation abilities for the

370   three pesticides DDT, aldrin, and malathion. All the isolates used these pesticides as the

371   sole carbon and energy source (Rani et al., 2018). The optical density was relatively

372   increasing at the same rate as within the nutrient medium. (Figure 1).

373   The *P. aeruginosa* strains 1A and 4D showed the highest degradation percentage

374   of about 80% for DDT. Strains 3C and 4D of *P. aeruginosa* also successfully

375   degraded up to 70% of 50 mg/mL DDT as shown in Figure 1. The *P. aeruginosa* strain

376   has good potential for biodegradation of DDT, also observed in the study done by

377   Sariwati *et al.*, (2018), where *P. aeruginosa* degraded 90% of 5 mM DDT (**1.7 mg/mL**),

378   a smaller amount as compared to the current study. In another degradation study, Wu

379   et al., 2018 (Wu et al., 2018) identified *P. aeruginosa* NBRC 3080 and *Bacillus subtilis*

380   NBRC 3009 degraded about 65.97% and 86.44% respectively of 5 mM DDT in 7

381   days, which was quite similar to our findings. Moreover, other microbes like *Serratia*

382   *marcescens* NCIM 2919 and *Fomitopsis pinicola* also degraded 42% DDT in 10

383   and 7 days of incubation while *P. aeruginosa* addressed in this study utilized pesticide

384   and degraded it in 36 hours of incubation (Grewal, J. and Bhattacharya 2016; Sariwati

385   *et al.,* 2017; Sariwati and Purnomo, 2018; Rizqi *et al.,* 2021). This indicates that the

386   strains reported here have a rapid rate of DDT degradation, which could be increased

387   further by the synergic effect of two similar or different species.

388   For aldrin, all the *P. aeruginosa* strains showed significant activity. The

389   degradation percentage given by *P. aeruginosa* strains 1A, 2B, 3C, 4D, i.e., 58%, 72%,

390   78% and 68%, respectively, is shown in Figure 2. Lately, no evidence of aldrin

391   degradation was observed by *P. aeruginosa*. However, other microbes like *P.*

392   *fluorescens*, White-Rot Fungus (*Pleurotus ostreatus),* a n d *Paenibacillus sp.*

393   *IITISM08* have shown the best degradation ability with percentages of 94.8 % (conc.

394   10 mg/L), 100% (conc. 5 mM) and 79% (50 µg/mL), respectively but utilized less

395   concentration as compared to recent isolates that explain the decent activity of our

396   strains (Purnomo *et al.,* 2017, Rani *et al.,* 2018).

397 Aldrin and DDT both are organochlorine pesticides, and it was noticed that all the *P.*
398 *aeruginosa* strains had got reductive de-chlorination ability; therefore, they efficiently
399 degraded these difficult compounds (Chen *et al.,* 2013). It is also stated that *P. aeruginosa*
400 possesses hydroxylase enzymes, like oxygenases and reductases, which play their part
401 during the process of degradation (Hamdan *et al.,* 2021). DDT is normally metabolized to
402 DDD, DDE, alkane and cycloalkene. But the recent study could not provide the
403 metabolites of degradation. Pseudomonas species are also known for the production of
404 biosurfactant, which apparently caused its increased rate of degradation as compared to
405 other isolates (Sariwati *et al.,* 2018). That might imply their role in malathion degradation
406 to the extent of 30-40%. These observations indicate that *P. aeruginosa* maintains genetic
407 diversity and metabolic flexibility, a phenomenon also addressed by Chanika et al., 2011
408 (Chanika *et al.,* 2011).

409 Malathion is an organophosphate pesticide and was degraded efficiently by isolates
410 *A. baumannii* (53%) and *A. ferroxidans* (54%), as shown in Figure 3. A similar study
411 was done by Kadhim et al., (2015) where *Pseudomonas putida* degraded 47.18%
412 a n d *Staphylococcus vitulinus* 44.24% in ten days. In contrast, our findings were
413 somewhat similar but were prime in terms of degradation rate. In 2016, Khan et al.
414 identified *Bacillus licheniformis,* which utilized malathion as the sole carbon source
415 and degraded up to 78% within 5 days efficiently (Kadhim *et al.,* 2015, Khan *et al.,* 2016).
416 Whereas in our study, the best degradation percentage was about 54% which is less than
417 the preceding study, but if the time of degradation is exceeded it might show a better
418 result. Malathion, being an organophosphate pesticide, contains phosphorus-ester bonds
419 (P-S bond), which usually proves to be hydrolyzed by esterases and organophosphorus
420 hydrolases. It is observed that esterase is of great importance when microbes use
421 malathion as a sole carbon source. Synthetic pyrethroids like permethrin and malathion
422 are normally used against garden pests. In a previous study, *A. baumannii* strain ZH-14
423 promotes 100% (50 mg/mL) degradation of permethrin in 72 hours by using a similar
424 method (Zhan, H et al. 2018). Co-resistance against both malathion and permethrin is
425 shown by many pests which control unique esterases(Francis *et al.,* 2017).

426 The current study showed that *A. baumannii and Acidothiobacillus ferroxidans* utilized
427 malathion as a sole carbon source, which indicates that both the strains may contain

428 enzyme esterases which could break it down to acceptable metabolites. Furthermore, it
429 is noteworthy that *A. ferroxidans* is mainly involved in bioleaching of elements like sulphur,
430 iron, copper, and heavy metals from waste material. The effective biodegradation of
431 malathion by the strain also implies its capability to utilize phosphate-esters (Pankaj *et*
432 *al.,* 2021). *A. baumannii* and *A. ferroxidans* also degraded DDT and aldrin to the extent of
433 28-40%. Thus, we infer dynamic catalytic activity in all the isolates with variable degradation
434 rate depending upon pesticide composition, which needs to be explored further.

435 The current study provides six microbes with varying degradation ability. Almost all the
436 preceding studies highlighted showed degradation at 30°C in about 7 to 10 days of
437 incubation, whereas the current study identified microorganisms with a higher degradation
438 ability at the same temperature in 36 hours. This could be because these organisms were
439 isolated from pesticide polluted lands and waters, therefore, were more thoroughly
440 adapted. This trend showed that particular strains might be applied according to the
441 nature of pollutant present in a specified environment for eradicating toxic chemicals.
442 **Limitations of the study**
443 Our findings' scope can be further improved by considering the other factors such as
444 organic matter, pH, temperature etc., that might help to pace the specific microbes'
445 availability for biodegradation and their interactive association with chemicals.
446 Moreover, the study fails to address the extent of degradation of DDT, aldrin, and
447 malathion: whether to their intermediate compounds or a more complete degradation.
448 Furthermore, the enzymes contributing to the degradation ability should be isolated and
449 studied. These deficiencies provide a topic for further investigators in this regard.
450
451 **Conclusion**
452 Current study found that among all the six strains isolated from polluted pesticide
453 environment, two strains of *P. aeruginosa* (1A and 4D) have shown higher potential ability
454 to degrade 80% of DDT, and *P. aeruginosa* 3C showed the highest degradation
455 percentage, i.e., 78 % for aldrin whilst in the case of malathion *A. baumannii* and *A.*
456 *ferroxidans* have shown considerable degradation percentages of 53% and 54%
457 respectively.
458

459

Conflict of interest

461 The authors note no conflicts of interest for the editorial process, the corresponding author
462 is the only contact person and is responsible for communicating with the other authors
463 about progress, submissions of revisions and final approval of proofs.

464

Supplementary Data

466 The data show the statistical analysis of biodegrading microbes against three pesticides
467 (DDT, aldrin and malathion). Table S1 shows the biodegradation analysis of microbes
468 against DDT; Table S2 shows the biodegradation analysis of microbes against aldrin;
469 Table S3 shows the biodegradation analysis of microbes against malathion; and Table
470 S4 shows growth curves of the biodegrading bacteria in MSM broth.

Funding Statement

472 The research was supported by University of Agriculture Peshawar; KPK-Pakistan and
473 samples were provided by the National Institute of Biotechnology and Genetic
474 Engineering (NIBGE) Faisalabad-Pakistan.

475

Author Contribution

477 NA devised methodology, did all the experiments, and wrote original draft preparations.
478 MH contributed to concept development and data curation, FS has done the phylogenetic
479 analysis, analyzed the data statistically and prepared the figures. HN contributed to the
480 final writing of the manuscript. and NS contributed to the data collections. SK contributed
481 to the manuscript preparation and finalization. The final paper has been reviewed and
482 approved by all the co-authors.

Acknowledgements

484 This work was supported by the University of Azad Jammu and Kashmir Muzaffarabad,
485 Pakistan and Shaheed Benazir Bhutto Women University, Peshawar Pakistan. The
486 research was supported by University of Agriculture Peshawar; KPK-Pakistan and
487 samples were provided by the National Institute of Biotechnology and Genetic
488 Engineering (NIBGE) Faisalabad-Pakistan, University of Sheffield UK, and Islamia

489 College University Peshawar Pakistan. The manuscript was proof-read by Prof Mike P

490 Williamson from the University of Sheffield UK.

491

492

493 **REFERENCES**

494 **Altschul, S. F.; Gish, W.; Miller, W.; Myers, E. W.; Lipman, D. J.** Basic Local Alignment

495   Search Tool. *J. Mol. Biol.* **1990**, *215*, 403–410. https://doi.org/10.1016/S0022-

496 2836(05)80360-2.

497 **Arrebola, J. P.; Belhassen, H.; Artacho-Cordón, F.; Ghali, R.; Ghorbel, H.; Boussen,**

498   **H.; Perez-Carrascosa, F. M.; Expósito, J.; Hedhili, A.; Olea, N.** Risk of Female

499   Breast Cancer and Serum Concentrations of Organochlorine Pesticides and

500   Polychlorinated Biphenyls: A Case-Control Study in Tunisia. *Sci. Total Environ*.

501   **2015**, 520, 106–113. https://doi.org/10.1016/j.scitotenv.2015.03.045.

502 **Arora, P. K.; Srivastava, A.; Garg, S. K.; Singh, V. P.** Recent advances in

503   degradation of chloronitrophenols. *Bioresource Technology*, 250 (2017), 902–909.

504   https://doi:10.1016/j.biortech

505 **Al-Thukair.; Assad Ahmed.; Karim Malik.** Pyrene metabolism by the novel bacterial

506   strains *Burkholderia fungorum* (T3A13001) and *Caulobacte*r sp (T2A12002) isolated

507   from an oil-polluted site in the Arabian Gulf. *International Biodeterioration &*

508   *Biodegradation*, **2016**,110, 32-37. https://doi.org/10.1016/j.ibiod.2016.02.005.

509 **Bhatt, P.; Bhatt, K.; Sharma, A.; Zhang, W.; Mishra, S.; Chen, S.;** Biotechnological

510   basis of microbial consortia for the removal of pesticides from the

511   environment. *Critical Reviews in Biotechnology*, **2020**, 1-22.

512 **Bhatt P.; Gangola S.; Bhandari G.; Zhang W.; Maithani D.; Mishra S.; Chen S.** New

513   insights into the degradation of synthetic pollutants in contaminated environments.

514   *Chemosphere,* **2020**, 30:128827.

515 **Bhatt, P.; Joshi, T.; Bhatt, K.; Zhang, W.; Huang, Y.; Chen, S.** Binding interaction of

516   glyphosate with glyphosate oxidoreductase and C–P lyase: Molecular docking and

517   molecular dynamics simulation studies. Journal of Hazardous Materials, 409,

518   **2020**, 124927. https://doi.org/10.1016/j.jhazmat.2020.124927

519   **Bhatt, P.; Verma, A.; Gangola, S.; Bhandari, G.; Chen, S.** Microbial glycoconjugates in

520   organic pollutant bioremediation: recent advances and applications. *Microbial Cell*

521   *Factories*, **2021,** 20(1), 1–18. https://doi.org/10.1186/s12934-021-01556-9

522   **Birolli, W. G.; Lima, R. N.; & Porto, A. L. M.** Applications of marine-derived

523   microorganisms and their enzymes in biocatalysis and biotransformation, the

524   underexplored potentials. *Frontiers in Microbiology*, **2019**, 10(AUG).

525   https://doi.org/10.3389/fmicb.2019.01453

526   **Chanika, E.; Georgiadou, D.; Soueref, E.; Karas, P.; Karanasios, E.; Tsiropoulos, N.**

527   **G.; Tzortzakakis, E. A.; Karpouzas, D. G.** Isolation of Soil Bacteria Able to

528   Hydrolyze Both Organophosphate and Carbamate Pesticides. *Bioresour. Technol.*

529   **2011**, *102* (3), 3184–3192. https://doi.org/10.1016/j.biortech.2010.10.145.

530   **Chen, M.; Cao, F.; Li, F.; Liu, C.; Tong, H.; Wu, W.; Hu, M.** Anaerobic Transformation

531   of DDT Related to Iron (III) Reduction and Microbial Community Structure in Paddy

532   Soils. *J. Agric. Food Chem.* **2013**, *61* (9), 2224–2233.

533   https://doi.org/10.1021/jf305029p.

534   **Cycoń M.; Mrozik A.; Piotrowska-Seget Z**.; Bioaugmentation as a strategy for the

535   remediation of pesticide-polluted soil: A review. Chemosphere. 2017 Apr 1;

536   172:52-71.

537   **Farhan, M.; Khan, A. U.; Wahid, A.; Ahmad, M.; Ahmad, F.** Biodegradation of

538   Chlorpyrifos Using Indigenous *Pseudomonas* Sp. Isolated from Industrial Drain.

539   *Pakistan J. Nutr.* **2012,** 11 (12), 1183–1189.

540   https://doi.org/10.3923/pjn.2012.1183.1189.

541   **Francis, S.; Saavedra-Rodriguez, K.; Perera, R.; Paine, M.; Black IV, W.C.; Delgoda,**

542   **R.** Insecticide resistance to permethrin and malathion and associated mechanisms

543   in *Aedes aegypti* mosquitoes from St. Andrew Jamaica. PLoS One, **2017**. 12(8),

544   p.e0184387.

545   **Ghanem, I.; Orfi, M.; Shamma, M.** Biodegradation of Chlorpyrifos by *Klebsiella* Sp.

546   Isolated from an Activated Sludge Sample of Waste Water Treatment Plant in

547   Damascus. *Folia Microbiol. (Praha).* **2007**, *52* (4), 423–427.
548   https://doi.org/10.1007/BF02932098.

549   **Ghanem, Khaled M.; Fahad A. Al-Fassi; Abdulghafoor K. Biag.** Optimization of methyl
550   orange decolorization by mono and mixed bacterial culture techniques using
551   statistical designs. *African Journal of Microbiology Research*, **2012**, 6(2): 436-446.
552   https://academicjournals.org/journal/AJMR/article-abstract/1E0A67440355

553   **Grewal, J.; Bhattacharya, A.; Kumar, S.; Singh, D. K.; Khare, S. K.** Biodegradation of
554   1,1,1-Trichloro-2,2- Bis (4-Chlorophenyl) Ethane (DDT) by Using *Serratia*
555   *Marcescens* NCIM 2919. *J. Environ. Sci. Heal. Part B* **2016,** 51 (12), 809–816.
556   https://doi.org/10.1080/03601234.2016.1208455.

557   **Huang, Y.; Zhang, W.; Pang, S.; Chen, J.; Bhatt, P.; Mishra, S.; Chen, S.** Insights into
558   the microbial degradation and catalytic mechanisms of chlorpyrifos. *Environmental*
559   *Research*, *194*, **2020**, 110660. https://doi.org/10.1016/j.envres.2020.110660.

560   **Jayaraj R.; Megha P.; Sreedev P.** Organochlorine pesticides, their toxic effects on living
561   organisms and their fate in the environment. Interdiscip Toxicol. **2016**, 9(3-4):90.
562   https://doi.org/10.1515/intox-2016-0012.

563   **Jiang, J.; Tang, M.; Chen, J.; Yang, Y.** Identification and Degradation Characteristics of
564   *Bacillus Cereus* Strain WD-2 Isolated from Prochloraz-Manganese-Contaminated
565   Soils. *PLoS One,* **2019,** 14 (8), e0220975.
566   https://doi.org/10.1371/journal.pone.0220975.

567   **Jovel, J.; Patterson, J.; Wang, W.; Hotte, N.; O'Keefe, S.; Mitchel, T.; Perry, T.; Kao,**
568   **D.; Mason, A. L.; Madsen, K. L.; Wong, G. K. S.** Characterization of the Gut
569   Microbiome Using 16S or Shotgun Metagenomics. *Front. Microbiol.* **2016,** 7.
570   https://doi.org/10.3389/fmicb.2016.00459.

571   **Jukes, T. H.; Cantor, C.R.** Evolution of protein molecules. Mammalian protein
572   metabolism, **1969**; 3, 21-132.

573   **Kadhim, F.; Rabee, A. M.; Abdalraheem, E.** Biodegradation of Malathion by Selected
574   Bacterial Isolates. *J. Int. Environ. Appl. Sci.* **2015**, *10* (3), 361–366.

575   **Kaya, T.; Aslim, B.; Kariptaş, E.** Production of Biosurfactant by *Pseudomonas* Spp.
576   Isolated from Industrial Waste in Turkey. *Turkish J. Biol.* **2014**, 38 (3), 307–317.
577   https://doi.org/10.3906/biy-1303.

578 **Khan, S.; Zaffar, H.; Irshad, U.; Ahmad, R.; Khan, A. R.; Shah, M. M.; Bilal, M.; Iqbal,**
579   **M.; Naqvi, T.** Biodegradation of Malathion by *Bacillus Licheniformis* Strain ML-1.
580   *Arch. Biol. Sci.* **2016**, *68* (1), 51–59. https://doi.org/10.2298/ABS141218007K.

581 **Kim, K. H.; Kabir, E.; Jahan, S. A.** Exposure to Pesticides and the Associated Human
582   Health Effects. *Sci. Total Environ.* **2017**, *575*, 525–535.
583   https://doi.org/10.1016/j.scitotenv.2016.09.009.

584 **Kumar, S.; Kaushik, G.; Dar, M. A.; Nimesh, S.; López-Chuken, U. J.; Villarreal-Chiu,**
585   **J. F.** Microbial Degradation of Organophosphate Pesticides: A Review.
586   *Pedosphere* **2018,** 28 (2), 190–208. https://doi.org/10.1016/S1002-
587   0160(18)60017-7.

588 **Lagier, J. C.; Hugon, P.; Khelaifia, S.; Fournier, P. E.; La Scola, B.; Raoult, D.** The
589   Rebirth of Culture in Microbiology through the Example of Culturomics To Study
590   Human Gut Microbiota. *Clin. Microbiol. Rev.* **2015**, *28* (1), 237–264.
591   https://doi.org/10.1128/CMR.00014-14.

592

593 **Mazzoni, M.; Ferrario, C.; Bettinetti, R.; Piscia, R.; Cicala, D.; Volta, P.; Borgå, K.;**
594   **Valsecchi, S.; Polesello, S.** Trophic Magnification of Legacy (PCB, DDT and Hg)
595   and Emerging Pollutants (PFAS) in the Fish Community of a Small Protected
596   Southern Alpine Lake (Lake Mergozzo, Northern Italy). Water **2020**, 12 (6), 1591.
597   https://doi.org/10.3390/w12061591.

598 **Mishra S.; Lin Z.; Pang S.; Zhang W.; Bhatt P.; Chen S.** Recent advanced technologies
599   for the characterization of xenobiotic-degrading microorganisms and microbial
600   communities. Frontiers in Bioengineering and Biotechnology. **2021** Feb 10;9:31.

601 **Murad, S.; Hasan, F.; Ali Shah, A.; Hameed, A.; Ahmed, S.** Isolation of Phthalic Acid
602   Degrading *Pseudomonas* SP. P1 from Soil. *Pakistan J. Bot.* **2007**, *39* (5), 1833–
603   1841.

604 **Orek, C. O.** An Optimised Cetyltrimethylammonium Bromide (CTAB)-Based Protocol for
605   Extracting RNA from Young and Old Cassava Leaves. Journal of Advances in
606   Biology & Biotechnology, **2018**, 19(4), 1–7.
607   https://doi.org/10.9734/jabb/2018/45370

608

609 **Purnomo, A. S.; Nawfa, R.; Martak, F.; Shimizu, K.; Kamei, I.** Biodegradation of Aldrin
610 and Dieldrin by the White-Rot Fungus *Pleurotus Ostreatus*. *Curr. Microbiol.* **2017**,
611 *74* (3), 320–324. https://doi.org/10.1007/s00284-016-1184-8.

612 **Rani, R.; Usmani, Z.; Gupta, P.; Chandra, A.; Das, A.; Kumar, V.** Effects of
613 Organochlorine Pesticides on Plant Growth-Promoting Traits of Phosphate-
614 Solubilizing *Rhizobacterium, Paenibacillus* Sp. IITISM08. *Environ. Sci. Pollut. Res*.
615 **2018,** 25 (6), 5668–5680. https://doi.org/10.1007/s11356-017-0940-z.

616 **Regueiro, J.; López-Fernández, O.; Rial-Otero, R.; Cancho-Grande, B.; Simal-**
617 **Gándara, J.** A Review on the Fermentation of Foods and the Residues of
618 Pesticides—Biotransformation of Pesticides and Effects on Fermentation and
619 Food Quality. *Crit. Rev. Food Sci. Nutr.* **2015,** 55 (6), 839–863.
620 https://doi.org/10.1080/10408398.2012.677872.

621 **Rizqi, H. D.; Purnomo, A. S.; Kamei, I.** Interaction and Effects of Bacteria Addition on
622 Dichlorodiphenyltrichloroethane Biodegradation by *Daedalea Dickinsii*. *Curr.*
623 *Microbiol.* **2021.** https://doi.org/10.1007/s00284-020-02305-8.

624 **Roy, D. C.; Biswas, S. K.; Saha, A. K.; Sikdar, B.; Rahman, M.; Roy, A. K.; Prodhan,**
625 **Z. H.; Tang, S. S.** Biodegradation of Crystal Violet dye by bacteria isolated from
626 textile industry effluents. *PeerJ*, **2018** (6), 1–15. https://doi.org/10.7717/peerj.5015

627 **Sariwati, A.; Purnomo, A. S.** The Effect of Pseudomonas Aeruginosa Addition on 1,1,1-
628 Trichloro-2,2-Bis(4-Chlorophenyl)Ethane (DDT) Biodegradation by Brown-Rot
629 Fungus *Fomitopsis Pinicola*. *Indones. J. Chem.* **2018**, 18 (1), 75.
630 https://doi.org/10.22146/ijc.25158.

631 **Sariwati, A.; Purnomo, A. S.; Kamei, I.** Abilities of Co-Cultures of Brown-Rot Fungus
632 *Fomitopsis Pinicola* and *Bacillus Subtilis* on Biodegradation of DDT. *Curr.*
633 *Microbiol.* **2017**, *74* (9), 1068–1075. https://doi.org/10.1007/s00284-017-1286-y.

634 **Stecher, G.; Tamura, K.; Kumar, S.** Molecular Evolutionary Genetics Analysis (MEGA)
635 for MacOS. *Mol. Biol. Evol.* **2020**, 37 (4), 1237–1239.
636 https://doi.org/10.1093/molbev/msz312.

637 **Syed, J. H.; Alamdar, A.; Mohammad, A.; Ahad, K.; Shabir, Z.; Ahmed, H.; Ali, S. M.;**
638 **Sani, S. G. A. S.; Bokhari, H.; Gallagher, K. D.; Ahmad, I.; Eqani, S. A. M. A.**
639 **S.** Pesticide Residues in Fruits and Vegetables from Pakistan: A Review of the
640 Occurrence and Associated Human Health Risks. *Environ. Sci. Pollut. Res.* **2014**,
641 *21* (23), 13367–13393. https://doi.org/10.1007/s11356-014-3117-z.

642 **T. Al Hattab, M.; E. Ghaly, A.** Disposal and Treatment Methods for Pesticide Containing
643 Wastewaters: Critical Review and Comparative Analysis. *J. Environ. Prot. (Irvine,.*
644 *Calif).* **2012**, 03 (05), 431–453. https://doi.org/10.4236/jep.2012.35054.

645

646 **UNEP** Listing of POPs in the Stockholm Convention.
647 http://chm.pops.int/TheConvention/ThePOPs/AllPOPs/tabid/2509/Default.aspx.
648 Accessed 10 Apr 2018.

649 **Wu, T.; Xu, J.; Xie, W.; Yao, Z.; Yang, H.; Sun, C.; Li, X.** *Pseudomonas aeruginosa*
650 L10: A hydrocarbon-degrading, biosurfactant-producing, and plant-growth-
651 promoting endophytic bacterium isolated from a Reed (*Phragmites australis*).
652 Frontiers in Microbiology, **2018**, 9 (1), 1–12.
653 https://doi.org/10.3389/fmicb.2018.01087.

654 **Ye, X.; Dong, F.; Lei, X.** Microbial Resources and Ecology - Microbial Degradation of
655 Pesticides. Nat. Resour. Conserv. Res**.** **2018**, 1 (1).
656 https://doi.org/10.24294/nrcr.v1i1.242.

657 **Zhan, H.; Wang, H.; Liao, L.; Feng, Y.; Fan, X.; Zhang, L.; and Chen, S**. Kinetics and
658 novel degradation pathway of permethrin in *Acinetobacter baumannii* ZH-
659 14. *Frontiers in microbiology*, 2018,*9*, 98.

Figure 1

Graphical representation of percent DDT degradation showed by each bacterial isolate plotted against time.

The experiment was done in triplicate, and errors bars indicates the standard deviation.

Figure 2

Graphical representation of percent aldrin degradation shown by each bacterial isolate plotted against time.

The experiment was done in triplicate, and errors bars indicates the standard deviation.

Lorem ipsum

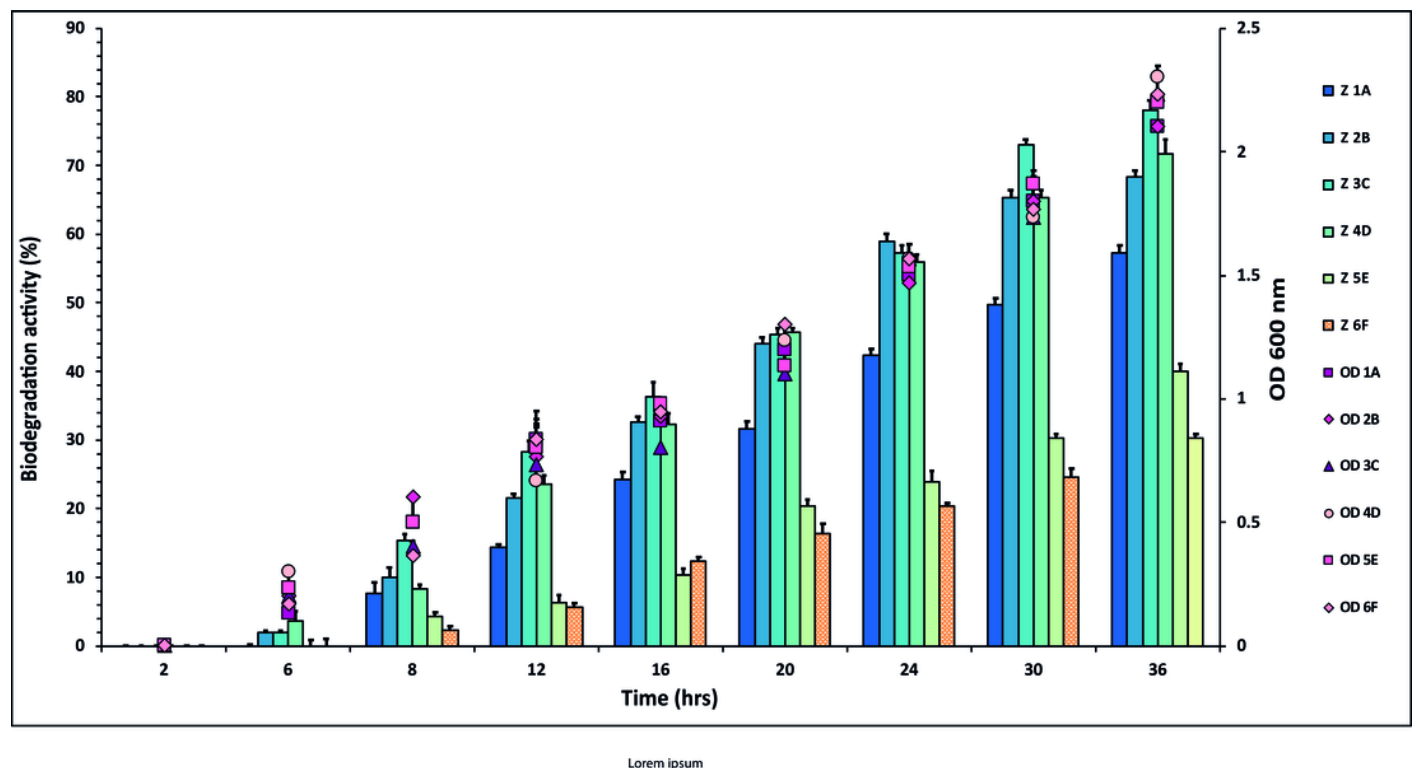



Figure 3

Graphical representation of percent malathion degradation shown by each bacterial isolate plotted against time.

The experiment was done in triplicate, and errors bars indicate the standard deviation.

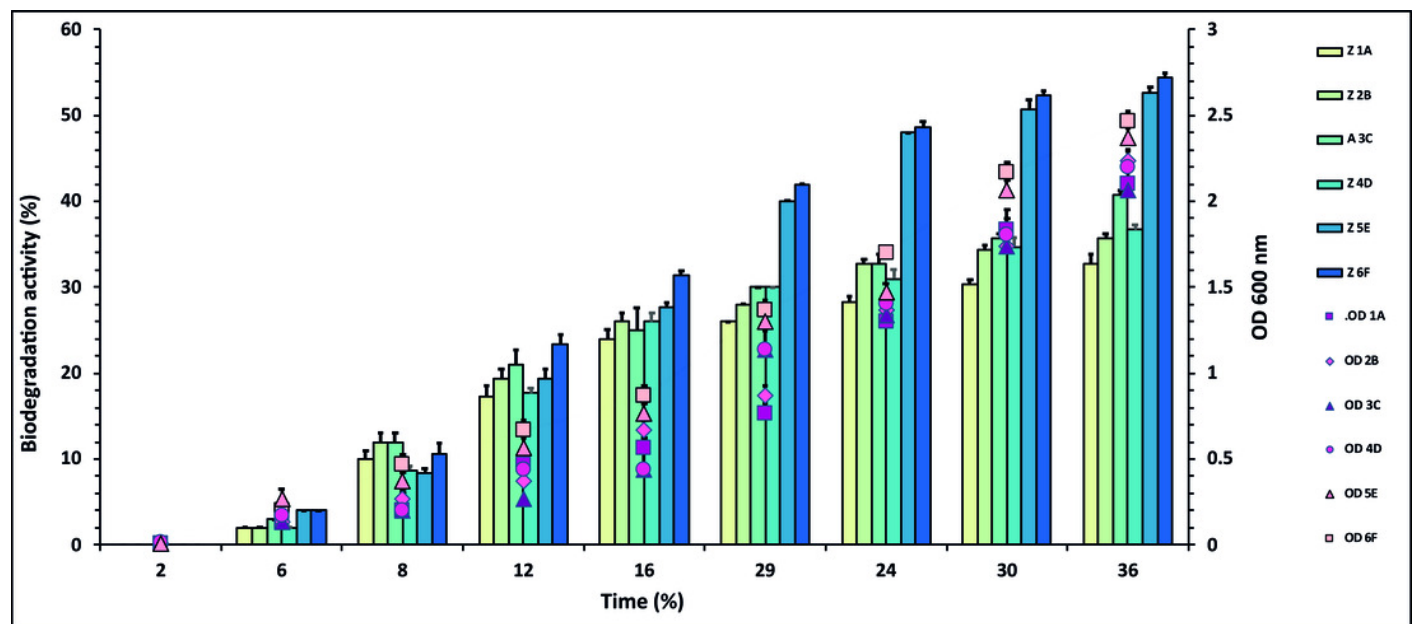



Figure 4

The appearance of colonies of the bacterial isolates 1A, 2B, 3C, 4D, 5E and 6F on nutrient agar.

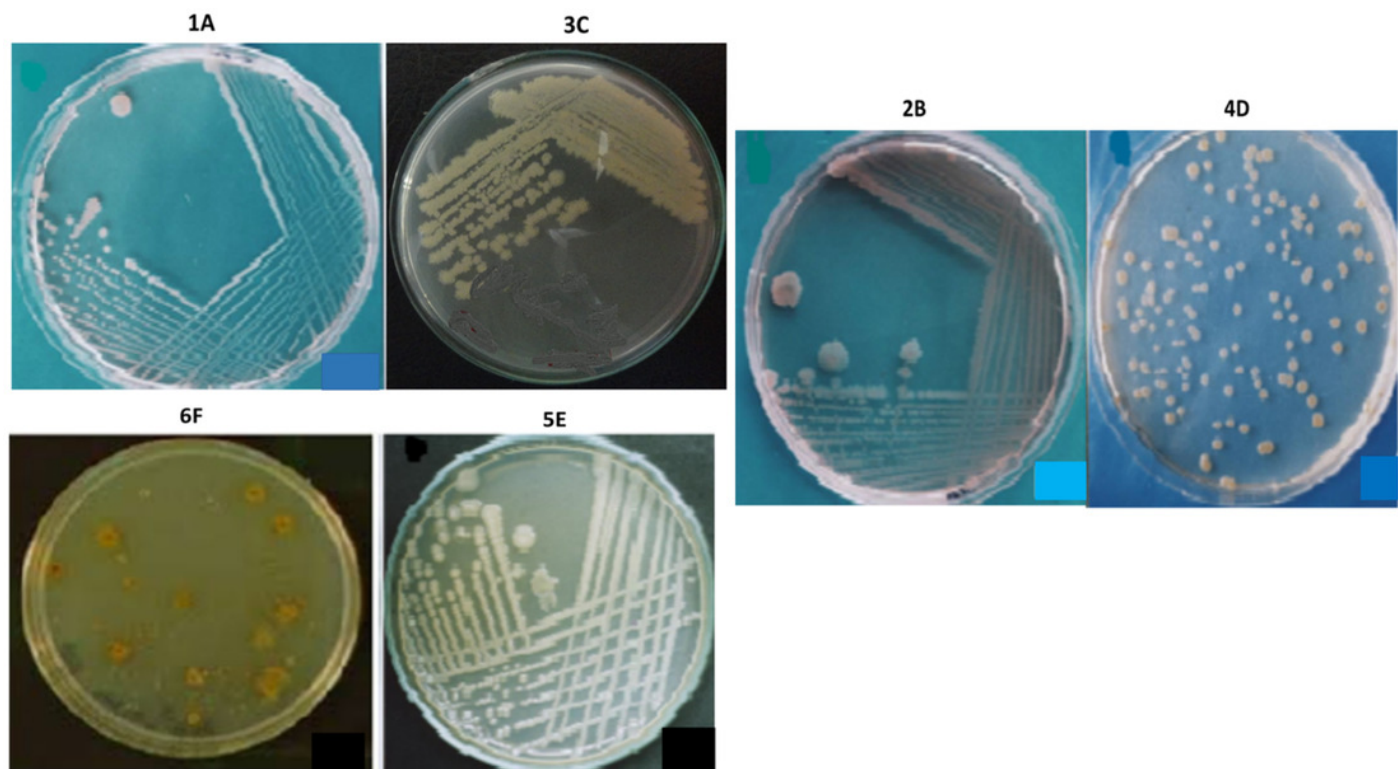



Figure 5

Evolutionary relationships of taxa -The evolutionary history was inferred using the
Neighbor-Joining method (Saitou and Nei, 1987).

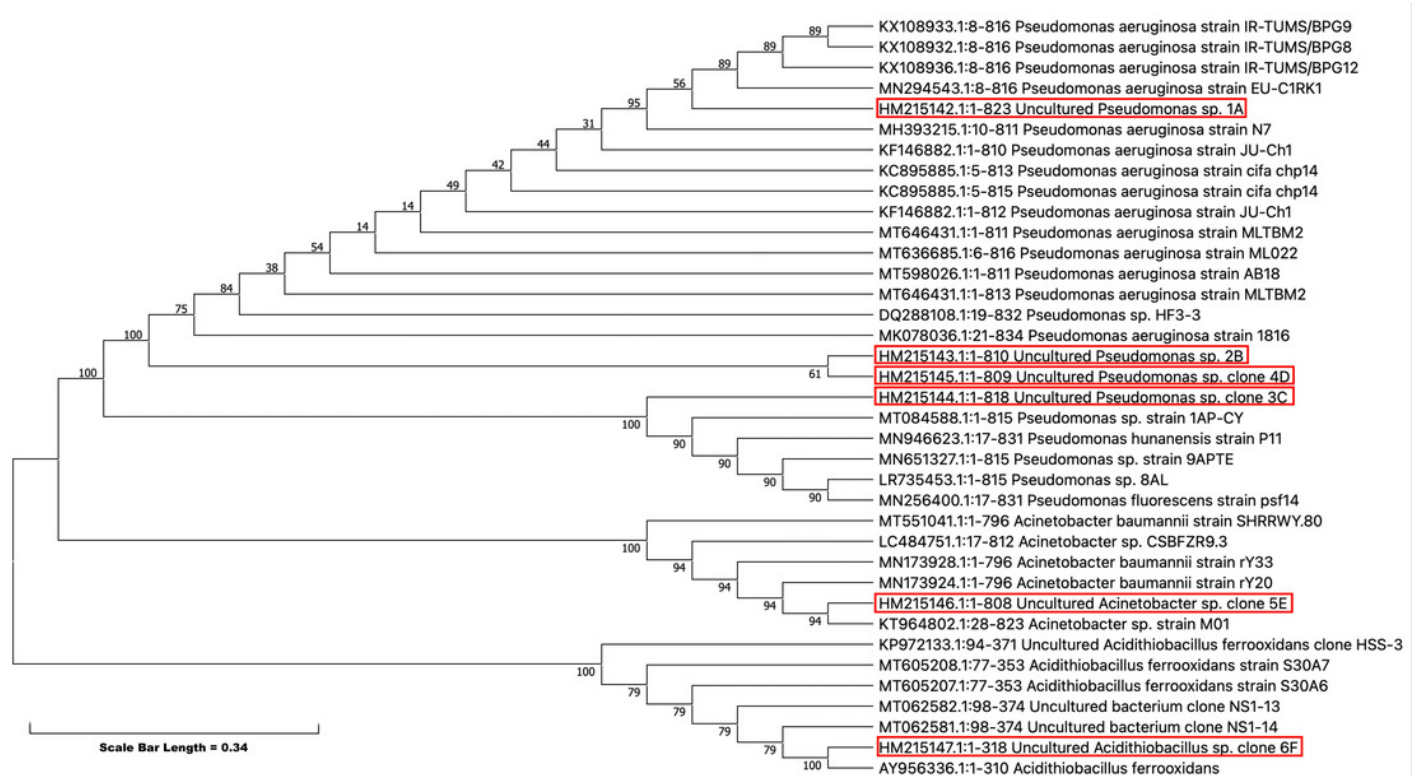
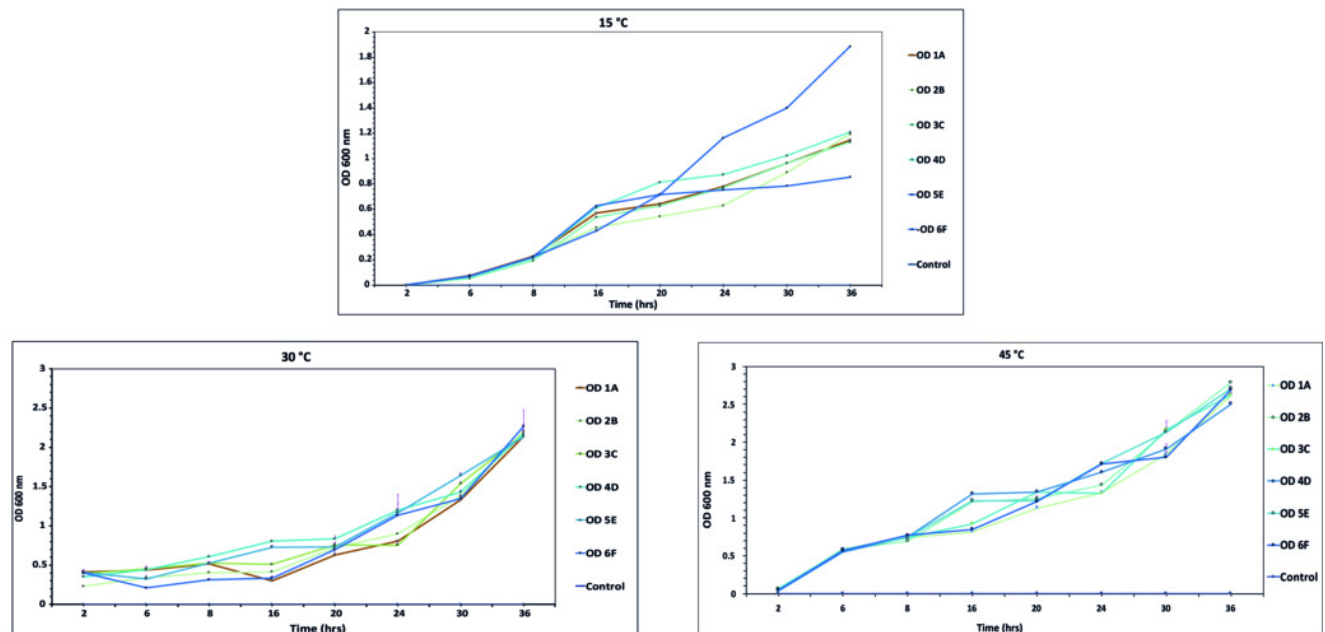



Figure 6

Growth curve of isolated strains 1A, 2B,3 C,4 D, 5E and 6F at 15°C, 30°C and 45°C.

The black bars represent standard error.

Cellular characteristics of biodegrading pesticide isolates

NA

2
3   **Table 1.** Cellular characteristics of biodegrading pesticide isolates.

Isolate code	Media used	Shape of cells	Cell size (µm)	Motility	Gram staining	Endo-spores
1A	Nutrient Agar	Rods	1.5	Motile	negative	Negative
2B	-do-	Rods	2.0	Motile	-do-	-do-
3C	-do-	Rods	2.5	Motile	-do-	-do-
4D	-do-	Coccus/rod	1.5	Non motile	-do-	-do-
5E	-do-	Rods	1.5-3.0	Motile	-do-	-do-
6F	-do-	Rods	2.2	Mitile	-do-	-do-

Sequence identity (%) of the partial 16S rRNA gene sequence of isolates obtained from environmental samples with related microbes

NA

1 **Table 1.** Sequence identity (%) of the partial 16S rRNA gene sequence of isolates obtained from
2 environmental samples with related microbes

Isolate with codes	Base pairs	Top Hit with	% Identity	Gap %	GenBank Accession No.
1A	797	*Pseudomonas aeruginosa strain N7* (MH393215.1)	99.13	0	HM215142.1
2B	796	*Pseudomonas aeruginosa strain 1816* (MK078036.1)	97.67	0	HM215143.1
3C	808	*Pseudomonas sp. strain 1AP-CY* (MT084588.1)	99.02	0	HM215144.1
4D	798	*Pseudomonas aeruginosa strain JU-Ch1* (KF146882.1)	98.40	0	HM215145.1
5E	780	*Acinetobacter baumannii strain SHRRWY.80* (MT551041.1)	97.99	0	HM215146.1
6F	307	*Acidothiobacillus ferroxidans* (AY956336.1)	95.94	3	HM215147.1