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Identification and pesticide degradation test of bacterial consortium of contaminated soil

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ABSTRACT

Excessive use of pesticides in shallot cultivation leads to soil contamination. Native bacteria in soils contaminated with suspected chlorpyrifos pesticides can be used to bioremediate soils contaminated with chlorpyrifos pesticides. This study aimed to identify and obtain a consortium of bacteria capable of degrading pesticides on shallot fields contaminated with the pesticide chlorpyrifos. The method used in this research is bacteria isolation using soil extract, morphological identification, molecular identification using Next Generation Sequencing (NGS), analysis of bacterial diversity index, and consortium bacterial degradation test to reduce levels of the chlorpyrifos pesticide. This study found 16 isolates at each study site resistant to 100 ppm of the pesticide chlorpyrifos, so these isolates were chosen to be used as consortium bacteria. Molecular identification of a consortium of bacteria has shown that there are 10 genera consisting of *Cutibacterium, Streptomyces, Staphylococcus, Ensifer, Ochrobactrum, Achromobacter, Escherichia Shigella, Klebsiella, Acinetobacter* and *Pseudomonas*. The bacterial diversity index in shallot soils reached 2,040 and 1,467 in forest soils. The reduction efficiency of the bacterial consortium using the method of growing cells and supernatant was 94.48% and 98.88%, respectively.

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1. INTRODUCTION

Shallots are one of the most competitive horticultural commodities. Brebes is known as the center of shallot producers in Indonesia. Generally, farmers use insecticides to control pests. Asian countries that use pesticides a lot are China, India, and Indonesia. The largest pesticide users in Indonesia are shallot farmers in Brebes Regency, Central Java. Over 30% of shallot farmers have been contaminated with pesticides due to inappropriate use. The increasing use of pesticides for pest control in agriculture affects soil, water, and crop quality. Especially in cultivating onions, insecticides with active ingredients such as Chlorpyrifos are intensively used. Chlorpyrifos's maximum soil residue limit is 0.10 ppm (Harsanti et al., 2015). Brebes Regency ranks first in Southeast Asia in the usage of pesticides—the most widely used pesticide on shallot plants. From 3200 pesticide brands registered at the Ministry of Agriculture, 1,300 pesticide brands are circulating in Brebes Regency (Lestari et al., 2019).

Agricultural systems with high input energy, such as chemical pesticides, can cause environmental pollution, especially in the farm environment. Pesticides are polluting agents that enter the environment through air, water, or soil. It can affect organisms and the environment through ecosystem destabilization, the presence of residues on crops, and pollution. It is toxic to humans and can even cause death. High use of pesticides results in high residues in the soil. Pesticides are highly persistent and cause soil degradation (Puspitasari & Khairuddin, 2016). Pesticides can react with other compounds into compounds that are more complex and difficult to detect. If the new mixture becomes a more toxic compound, it will potentially harm the environment, including humans.

Insecticides are substances used to control and reduce pests to reduce their harmful effects. Organophosphate compounds consist of highly toxic chemicals. Excessive use of these compounds can harm agricultural lands, ecosystems, and human health. Chlorpyrifos can affect the nervous system by inhibiting cholinesterase. Cholinesterase is an enzyme that is an indicator of the ability of the liver to function correctly. When the level of cholinesterase decreases, liver dysfunction occurs. Chlorpyrifos is very persistent in soil. Chlorpyrifos is

generally retained in the soil for 60-120 days and is affected by pH, climate, and soil type (Jaiswal et al., 2017). When the soil pH is high, chlorpyrifos degrades more quickly. TCP (3,5,6-trichloro-2-pyridinyl) is a product of hydrolysis of the pesticide chlorpyrifos, which is persistent and can be absorbed by soil particles.

Soil microorganisms can degrade pesticides by converting toxic compounds into less toxic ones (Alizadeh et al., 2018). Microorganisms break down pesticides or their derivatives in the environment. However, persistent xenobiotic compounds, including pesticides and their products, will be stored in the soil and affect the food chain cycle (Ma. Laura et al., 2013). The bacterial consortium formed from agricultural soil with selective enrichment techniques has the potential to degrade Chlorpyrifos (Sasikala et al., 2012). Microorganisms are one part of the biosphere that can utilize organophosphates to support their growth. Microorganisms use organophosphate pesticides as an energy source. The reduction of organophosphate pesticides such as chlorpyrifos by bacteria can be carried out by metabolic means. The removal of the chlorpyrifos pesticide begins by breaking the P-S or P-O bonds through enzymes owned by bacteria (Zheng et al., 2013).

A bacterial consortium is several bacteria that are united to complement each other in carrying out metabolism. Bacterial consortiums, both natural and artificial, have the advantage that they have complementary metabolic functions in an ecosystem (Sahlan et al., 2014). Consortiums are generally more resistant to environmental changes and complexity to perform complex tasks than single microbes. Reduction of a pesticide-polluted environment by a single microbe often results in less than optimal reduction efficiency. The pesticide reduction ability possessed by each of the different bacteria combined into a consortium resulted in a higher pesticide reduction ability than the single microbe. In 10 days in the mud system media, the bacterial consortium consisting of *Pseudomonas sp., Klebsiella sp., Stenotrophomonas sp., Bacillus sp.*, and *Ochrobacterum sp.* can reduce Chlorpyrifos up to 82% (Huang et al., 2021).

The soil analysis results at Wanasari Brebes showed that chlorpyrifos concentration was 0.9542 mg kg^{-1} and increased to 1.921 mg kg^{-1} in 2022. Joko et al. (2017) stated that the residual active ingredient chlorpyrifos was detected at approximately 0.01–0.06 mg kg^{-1} in samples from all sites in Wanasari. Excessive use of pesticides in shallot production centers causes elevated levels of chlorpyrifos pesticide residues in soil, exceeding the Maximum Residue Limit (MRL). Need to investigate the ability of a consortium of bacteria to reduce chlorpyrifos pesticides from contaminated soil. No studies on bioremediation of pesticide-infested soils were conducted in Wanasari. One of the technologies that can be applied to restore the chlorpyrifos-polluted environment is bioremediation. Bioremediation is an alternative to the decomposition of pesticides into simpler compounds so that they are not too dangerous through the utilization of the metabolic potential of microorganisms. Bioremediation is one method to overcome chemical pollution through enzymatic activity by utilizing pollutants as a source of nutrition for microorganisms. Bacteria, fungi, and algae are bioremediation agents in polluted soil. This study aims to obtain a consortium of bacteria from shallot soils capable of reducing Chlorpyrifos.

2. MATERIALS AND METHODS

2.1. Soil sampling

Soil samples were taken by purposive random sampling on shallot fields. Soil sampling in Wanasari and Songgom District, Brebes Regency, Central Java Province, Indonesia.

2.2. Identification of bacterial consortium

Sample inoculum was streak plated onto SEA medium. A total plate count method monitored microbial growth during the isolation process. Furthermore, a bacterial resistance test was carried out against chlorpyrifos pesticides by adding 100 ppm of chlorpyrifos pesticides into nutrient agar media. Bacteria that show tolerance to increased concentrations of Chlorpyrifos will be selected as part of the consortium of microorganisms.

2.3. Molecular identification

Analysis of bacterial diversity using next-generation sequencing (NGS). PCR amplification of the target region using barcode-specific primers. PCR products of appropriate size were selected by 2% agarose gel electrophoresis. The PCR product corresponding to each sample was pooled, end ligated, A-tailed and ligated with an Illumina adapter. Libraries were sequenced on Illumina's first-pair platform to generate 250 bp paired-end reads. Paired reads are based on a unique barcode and split to share the barcode and base sequence. Pair readings were performed using FLASH (V1.2.7). It is an accurate analyzer for paired-end reads when multiple reads overlap with reads from both ends of the same DNA fragment, referring to the splice sequence as a raw identifier. In order to obtain high quality and pure Qiime (V1.7.0) compliant labels, the raw label quality is examined under special screening conditions.

Identifications were compared to the reference SILVA database (Quast et al., 2013) using the UCHIME algorithm to identify chimeric sequences. The chimeric sequence is then removed, yielding an active marker. Sequence analysis was performed using Uparse software using all valid tags. Sequences with 97% similarity were assigned the same Operational Taxonomic Unit (OUT). A representative screen is performed in each OTU for further study. Use of the Qiime-in-Mothuri method for each co-sequence against the SILVA SSUrRNA database. Species labeling in each taxonomic order. Muscle can quickly compare multiple arrays to derive phylogenetic relationships of all representative OTU sequences. Operational Taxonomic Unit (OTU) normalization was performed with the standard sequence number corresponding to the subspecies sample. Alpha diversity analysis was also performed. Alpha diversity is used to determine the biodiversity complexity of a sample using the Shannon index. The model diversity index was calculated by QIIME (version 1.7.0).

Table 1. Colony morphology of the KBB and KH bacterial consortium

Consortium code	Isolate	Colony form	Elevation	Edge	Colour	Surface
KBB	1	Round with scalloped margin	Umbonate	Lobate	Creamy white	Glossy
	2	Irregular and spreading	Umbonate	Undulate	Creamy white	Glossy
	3	Round with scalloped margin	Umbonate	Lobate	Creamy white	Glossy
	4	Irregular and spreading	Umbonate	Undulate	Creamy white	Glossy
	5	Round	Umbonate	Entire	Orangish	Glossy
	6	Round	Umbonate	Entire	Orangish	Glossy
	7	Irregular and spreading	Umbonate	Lobate	Creamy white	Glossy
	8	Irregular and spreading	Umbonate	Lobate	Creamy white	Glossy
	9	Irregular and spreading	Umbonate	Undulate	Creamy white	Glossy
	10	Round	Umbonate	Entire	Orangish	Glossy
	11	Round with scalloped margin	Umbonate	Undulate	Creamy white	Glossy
	12	Round	Convex	Entire	Orangish	Glossy
	13	Irregular and spreading	Umbonate	Irregular	Creamy white	Glossy
	14	Round with scalloped margin	Umbonate	Undulate	Creamy white	Glossy
	15	Round	Convex	Entire	Creamy white	Glossy
	16	Irregular and spreading	Umbonate	Lobate	Creamy white	Glossy
KH	1	Round with scalloped margin	Umbonate	Entire	Creamy white	Glossy
	2	Round	Umbonate	Entire	Creamy white	Glossy
	3	Round	Umbonate	Entire	Creamy white	Glossy
	4	Irregular and spreading	Umbonate	Undulate	Creamy white	Glossy
	5	Round with raised margin	Crater	Entire	Creamy white	Glossy
	6	Irregular and spreading	Umbonate	Irregular	Creamy white	Glossy
	7	Irregular and spreading	Raised	Irregular	Creamy white	Glossy
	8	Irregular and spreading	Umbonate	Irregular	Creamy white	Glossy
	9	Round with scalloped margin	Umbonate	Lobate	Creamy white	Glossy
	10	Round with scalloped margin	Umbonate	Lobate	Creamy white	Glossy
	11	Round with scalloped margin	Umbonate	Lobate	Creamy white	Glossy
	12	Round with scalloped margin	Umbonate	Lobate	Creamy white	Glossy
	13	Irregular and spreading	Turn into drop	Undulate	Creamy white	Glossy
	14	Irregular with raised margin	Umbonate	Lobate	Creamy white	Glossy
	15	Irregular and spreading	Crater	Undulate	Broken white	Glossy
	16	Irregular with raised margin	Crater	Undulate	Broken white	Glossy

Notes: KBB (Bacterial Consortium of Shallot Field), **KH (Control)**

2.4. Bacterial diversity index

The species diversity index determines the diversity of bacterial species in the consortium. The Shannon of General Diversity formula calculates the bacterial diversity index:

$$(H') = -(ni\ N) \log (ni\ N) \quad [1]$$

Where, ni is the significance index of type I, N is the total value of the significant index. The Shannon-Wiener species richness index is defined as follows (Paiki et al., 2018):

1. If H> 3 suggests that the level of bacterial diversity is high
2. If 1≤ H ≤ 3 suggests that the level of bacterial diversity is moderate
3. If H < 1 suggests that the level of bacterial diversity is low.

2.5. Chlorpyrifos biodegradation by the bacterial consortium

Chlorpyrifos pesticide degradation test on KBB (Bacterial Consortium of Shallot Field) samples was carried out as growing cells and supernatant.

2.5.1. Growing cells

This process begins with making a microbial starter by inoculating a consortium of bacteria into 10 ml of nutrient broth (NB) media, then incubating and shaking until the optical

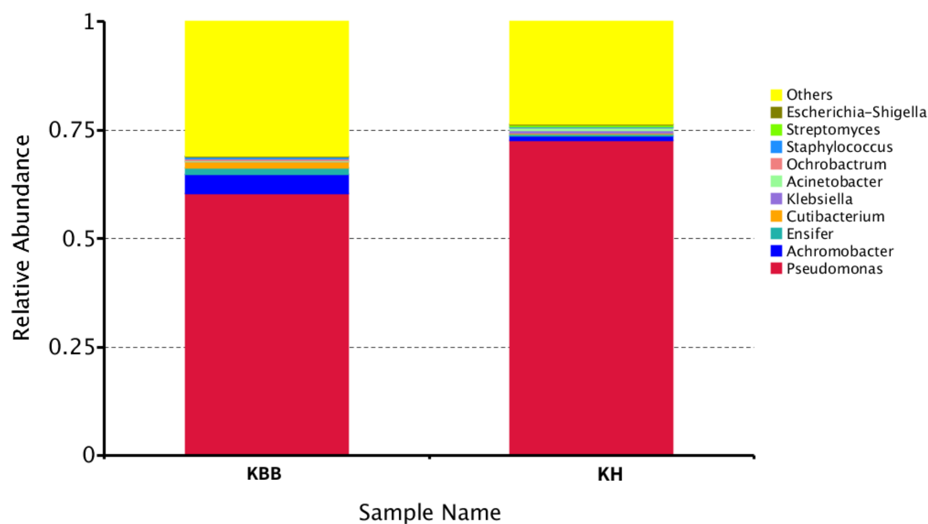

Figure 1. Relative abundance of bacterial consortium

density (OD) value reaches 1 by measuring using a spectrophotometer. Next, 0.5 ml of starter was taken (first starter), inoculated into 20 ml of NB media, then incubated and shaken until the OD value reached 1 (second starter). Then proceed by taking each 2.5 ml of the second starter and putting it into 250 ml of NB medium, then incubate and shake until it reaches the logarithmic peak phase (16 hours). Furthermore, pesticides with a concentration of 100 ppm were added. Then set for ten days and observed every two days. Sampling is carried out every two days to determine the level of pesticide residues. Then the bacterial growth was measured using a spectrophotometer at a resonance line of 600 nm.

2.5.2. Supernatant

The process begins with making a microbial starter by inoculating a consortium of bacteria into 10 ml of NB media. Next, incubating and shaking until the OD value reaches 1 by measuring using a spectrophotometer. Next, 0.5 ml of starter was taken (first starter), inoculated into 20 ml of NB media, then incubated and shaken until the optical density value reached 1 (second starter). Then proceed by taking each 2.5 ml of the second starter and putting it into 250 ml of NB medium, then incubating and shaking until it reaches the logarithmic peak phase (16th hour). Then, cell biomass was separated from the supernatant by centrifugation at 10,000 rpm for 5 min at 4 °C. The resulting supernatant was placed in a sterile Erlenmeyer flask and pesticides were added at a concentration of 100 ppm. As a control, 100 ppm of pesticide was administered with bacterial inoculation. Treatment and management were incubated for ten days. Sampling is carried out every two days to determine the level of pesticide residues. Then the bacterial growth was measured using a spectrophotometer at a resonance line of 600 nm. Changes in pesticide levels using GC-MS.

3. RESULTS

3.1. Isolation and morphological identification of bacterial consortium

Based on data on isolation and testing of bacterial resistance to chlorpyrifos on shallots and forest plantations, sixteen bacteria are likely to be resistant to chlorpyrifos pesticides in each location. The results of morphological observations of two bacterial isolates consortium coded KBB (Bacterial Consortium of Shallot Field) and KH (Control) are shown in Table 1. The spreading is round with shell edges and irregular with raised edges. Colony edges are flat, curved, irregular, and wavy. All isolates had different elevations, such as convex, button-like, and crater-like. The colour or pigmentation varies; some are yellowish-white, orange-white, and bone white.

3.2. Molecular identification and diversity index of the bacterial consortium

Most *Operational Taxonomy Units* (OTUs) can identify bacterial taxonomy down to the species level, regardless of the length of DNA used (250 bp). However, some bacteria can only be determined at the genus level, namely *Streptomyces, Ochrobacterium, and Klebsiella*. Based on identifying bacterial diversity in the consortium, the homology ranged from 72.725-100% with ten genera and eight species of bacteria. The bacterial genera found in the KBB consortium were *Pseudomonas, Achromobacter, Cutibacterium, Ensifer, Ochrobactrum, Staphylococcus, Escherichia shigella, Acinetobacter*, and *Klebsiella.* The bacterial species found in KBB were *Cutibacterium acnes, Staphylococcus lentus, Ensifer adhaerens, Achromobacter insolitus, E. Coli, Acinetobacter baumanii, Acinetobacter radioresistance* , and *Pseudomonas nitroreducens* . The chart of bacterial diversity in KBB can be seen in Figure 2 and Table 3.

The bacterial genera contained in the KH consortium are *Pseudomonas, Achromobacter, Klebsiella, Acinetobacter, Ensifer, Streptomyces, Staphylococcus, Cutibacterium, Streptomyces*, and *Escherichia shigella*.

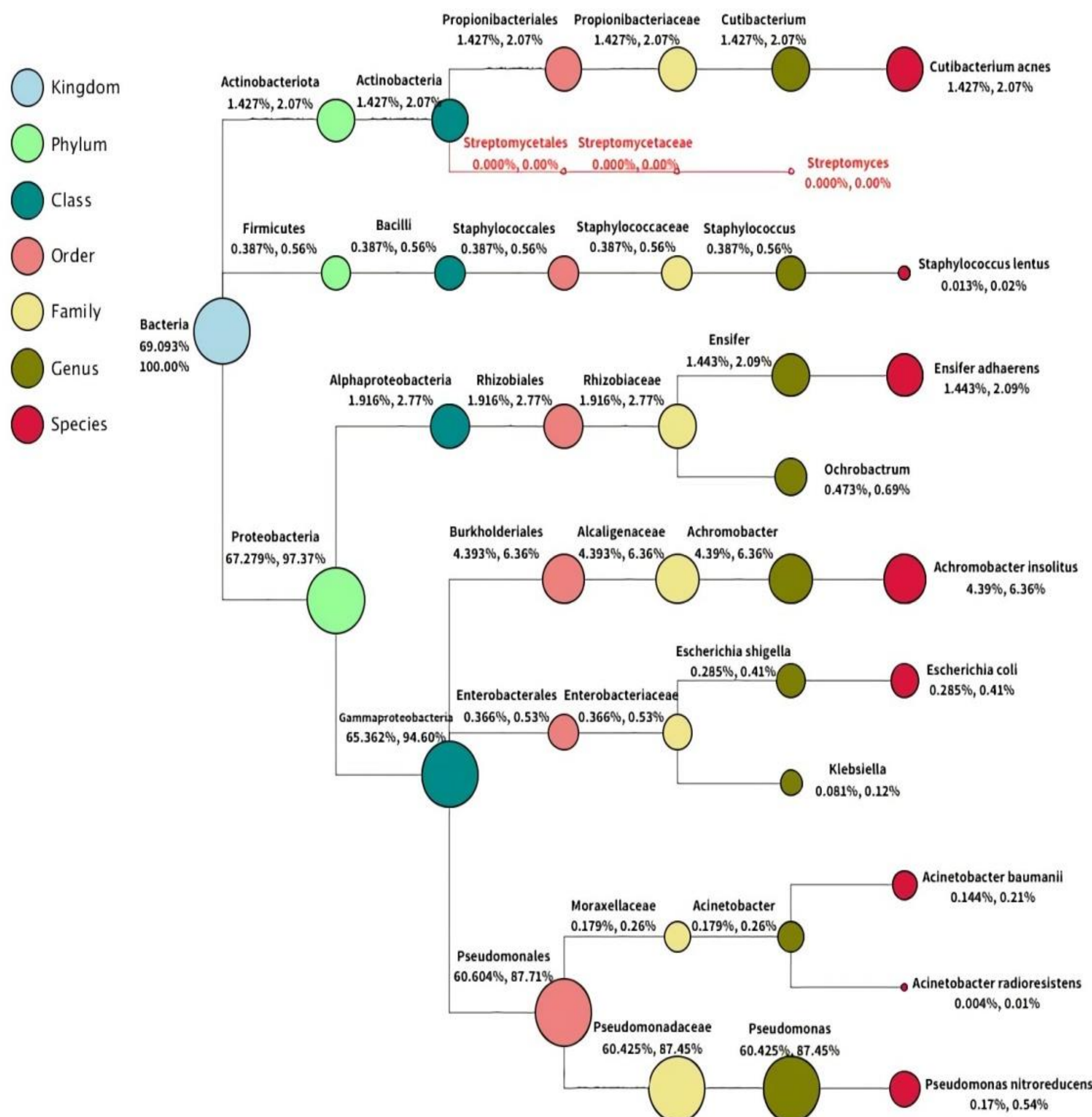

Figure 2. Taxonomy tree of KBB (Bacterial Consortium of Shallot Field)

The bacterial species found in KH were C *utibacterium acnes, Staphylococcus lentus, Ensifer adhaerens, Achromobacter insolitus, E. Coli, Acinetobacter baumanii,* and *Pseudomonas nitroreducens*. The chart of bacterial diversity in KH can be seen in Figure 3 and Table 3. The KBB diversity index reaches 2,040, while in KH, it reaches 1,467. The diversity index of the KBB and KH bacterial consortium can be seen in Table 2.

3.3. Chlorpyrifos pesticide degradation test

The degradation test of chlorpyrifos pesticide by a consortium of bacteria was carried out by measuring the growth rate of the consortium. The consortium rate was observed so that the time of adding chlorpyrifos pesticide to the sample inoculum was correct. The addition of pesticides was carried out in the logarithmic phase with the aim that the bacterial consortium could use the chlorpyrifos pesticide as a source of nutrition.

Based on the measurement results of the bacterial growth rate, the KBB consortium experienced an adaptation phase (lag phase) at 0 to 4 hours, a logarithmic phase at 6 to 16 hours, a stationary phase at 18 to 40 hours, and began to experience the death phase at the 42 hours. Nutrient exhaustion and product accumulation inhibitors such as acids are some of the factors that affect cell death. Apart from knowing the time and age of the microbial culture to be harvested, a growth curve can be used to determine the length of microbial generation time. Knowing the time of each microbe's generation can predict each microbe's population in a certain period and their activity in metabolic processes. The curve growth of the bacterial consortium can be seen in Figure 4. KBB in the form of *growing cells* has a 94,43% decrease in ppm levels of the pesticide chlorpyrifos. The ability of the KBB *supernatant* to degrade chlorpyrifos was 98.88%. The reduction in chlorpyrifos pesticide levels by KBB can be seen in Figure 5.

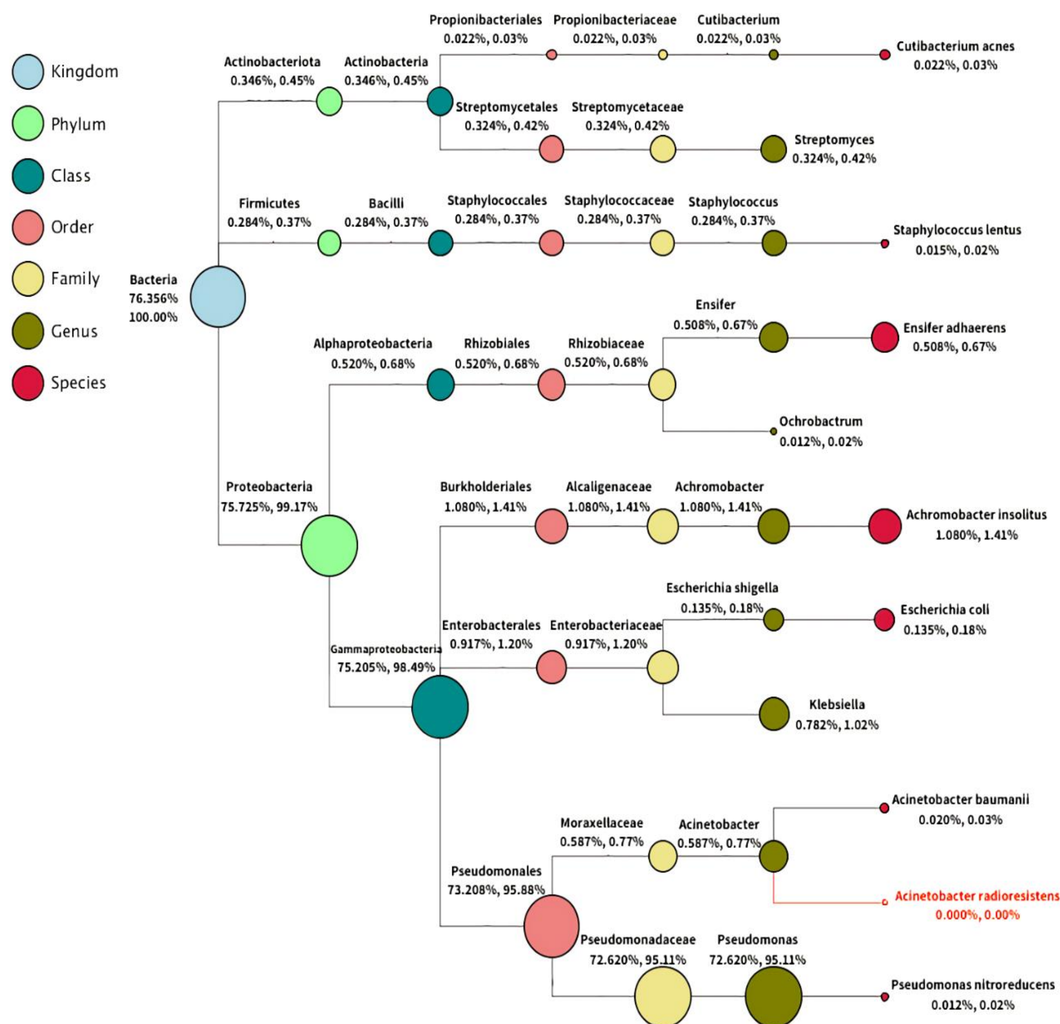

Figure 3. Taxonomy tree of KH (Control)

Table 2. Bacterial diversity index

Sample Name	Diversity Index
KBB	2,040
KH	1,467

Table 3. Percentage of bacterial species in the KBB consortium

Species	Rate of Bacterial Species (%)	
	KBB	KH
Cutibacterium acnes	2,07	0,03
Staphylococcus lentus	0,02	0,02
Ensifer adhaerens	2,09	0,67
Achromobacter insolitus	6,36	1,41
Escherichia Coli	0,41	0,18
Acinetobacter baumanii	0,21	0,03
Acinetobacter radioresistens	0,05	-
Pseudomonas nitroreducens	0,54	0,02

4. DISCUSSION

Based on Table 1, the results of isolation and testing of bacterial resistance to chlorpyrifos in bulb and forest plantation soils, there were 16 bacteria potentially resistant to the insecticide chlorpyrifos at each site. Based on Figure 1, most of the bacterial genera found in bacterial consortia KBB and KH are *Pseudomonas sp*., accounting for over 50% of the total bacterial consortium. According to, the *Cutibacterium acnes strains were* more efficient and showed low and moderate levels of metal degradation. Based on research Chaieb et al. (2016), *Staphylococcus lentus* can decolorize dyes with four sulfonate groups (Evans blue) longer than dyes with one (Eriochrome Black T) or two (Congo red) sulfonate groups. Cui et al. (2014) stated that the dye test conducted by *Klebsiella sp.* was influenced by the number of sulfonate groups in the dye. Hegde et al. (2017) showed that *Acinetobacter sp*. can be used to degrade pesticides such as chlorpyrifos and methylparathion. Based on research González-Benítez et al. (2021), an independent culture technique (High-throughput Sequencing Illumina) consisting of *Pseudomonas* and *Cutibacterium* bacteria was identified as arsenic-tolerant bacteria and played a role as plant growth support bacteria.

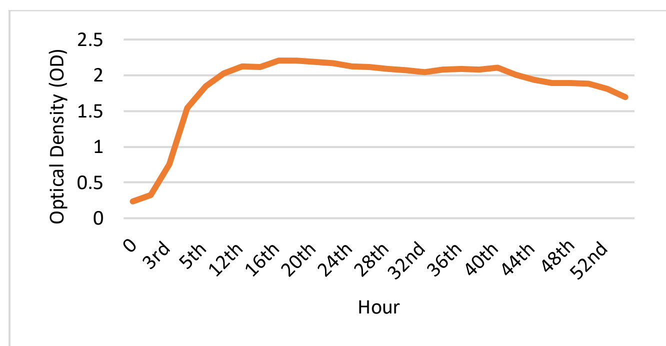

Figure 4. Bacterial consortium growth rate curve of KBB (Bacterial Consortium of Shallot Field)

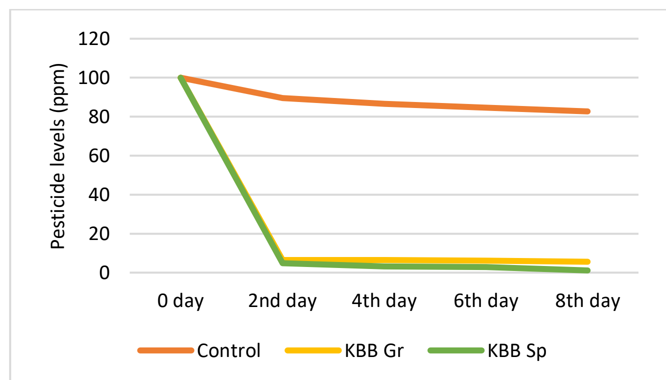

Figure 5. Chlorpyrifos pesticide degradation curve

Notes: KBB Gr (Bacterial Consortium of Shallot Field in the growing cell); KBB Sp (Bacterial Consortium of Shallot Field) in the supernatant).

In research González-Benítez et al. (2021), the biodegradation of polychlorinated biphenyls and biosorption of contaminated water can be carried out by the bacterium *Ensifer adhaerens*. Bacteria from the genus *Ensifer* are very multipurpose applications, as in the research results, which claim *E. adhaerens can* act as *Plant promoting growth bacteria* (PPGB), biosorption, and potential chromium reduction. Kulkarni and Kaliwal (2018) Stated that biodetoxification of coumaphos insecticides can be carried out using *Escherichia coli*.

Pseudomonas and *Achromobacter* are genera of bacteria that specifically act as decomposers of *polycyclic aromatic hydrocarbons* (PAH) (Sopeña et al., 2014). *Pseudomonas sp. is* capable of degrading the degradation products of chlorpyrifos. Ramadass and Thiagarajan (2017) reported that soil degradation increased with the cultivation of cabbage. Meanwhile, in liquid culture, the metabolites could degrade Chlorpyrifos up to 93.5% and 100% within ten days. Hong et al. (2017) stated that the *Achromobacter insolitus* strain could produce biosurfactants that efficiently emulsified various hydrophobic compounds, indicating a potential for bioremediation.

Yadav et al. (2016) reported that within 8 hours, *Pseudomonas nitroreductase* strain AR-3 was able to degrade chlorpyrifos up to 97% and reduce the concentration of pesticide residues up to 3.0 mg L^{-1}. Gilani et al. (2016) stated that bioaugmentation by inoculating *Pseudomonas nitroreductase* strain PS-2 in ryegrass rhizosphere soil for 28 days showed the potential for degradation of up to 100% in all concentrations of chlorpyrifos pesticide compared to uninoculated soil. Hussaini et al. (2013) reveal that *Acinetobacter radioresistance* has demonstrated considerable degradation of chlorpyrifos with 38% degradation and Malathion marginal degradation with 1% degradation, whereas for other pesticides, there was no degradation by this culture.

The diversity index of the two consortiums of KBB and KH bacteria is shown in Table 2. The KBB diversity index reaches 2,040, while in the KH, it reaches 1.467. Analysis result range the data obtained is appropriate with index range criteria for the diversity of species put forward by Shannon Wiener in Paiki et al. (2018) research, which stated that the value <1 indicates low bacterial diversity, 1≤H≤3 indicates the diversity of bacteria in the medium category, and >3 indicates a high value of bacterial diversity. Diversity index types owned by the two consortiums are in the range of values of more than one and less than 3 (1≤H≤ 3), which means a large diversity index types of bacteria owned by the consortium are included in the medium category.

Testing the degradation ability of chlorpyrifos by KBB begins with calculating the growth rate of the bacterial consortium in Figure 4. Measuring the bacterial consortium growth rate using a spectrophotometer by measuring the changes in optical density (OD) indicates the cell density range. This is as Atolia et al. (2020) said that measurement of bacterial growth rate requires measuring changes in optical density (OD) across the entire cell density range. Bacterial cells in the logarithmic phase will divide rapidly at a constant rate. In the logarithmic phase, bacteria overgrow. In addition, the energy requirements of bacteria in the logarithmic phase were higher than in the lag (adaptation), stationary, and death phases, so the addition of chlorpyrifos pesticide to the sample inoculum was carried out when it reached the logarithmic phase. This is done so that bacteria can use the pesticide chlorpyrifos as a nutrient because, as stated, cells can produce many metabolites needed to meet their growth needs in the logarithmic phase.

This study tested the ability of the KBB bacterial consortium to reduce chlorpyrifos which is in Figure 5, using Nutrient Broth (NB) media so that the carbon source of bacteria does not only come from the pesticide chlorpyrifos. (Imamuddin et al., 2017). It also tested the ability to reduce bacteria on NB media, which showed that bacterial growth had higher yields. On NB media containing 100 ppm carbaryl compared to that in *Minimal Medium* (MM). Based on Figure 5, the test results for the reduction of chlorpyrifos bacteria by the KBB consortium, it was found that the ability to reduce chlorpyrifos pesticide during an eight-day incubation period in the form of *growing cells* was 94.43% and in the form of *Supernatant* 98.88%. In contrast, the control showed a slight decrease in ppm levels in the pesticide chlorpyrifos, as in the study (Mallick et al., 1999) who experienced a decrease in chlorpyrifos content from the uninoculated media also during the incubation period. This is probably caused by the chemical hydrolysis process in the press. The genus *Pseudomonas* bacteria was more dominantly found in the consortium, and the results of the degradation test of chlorpyrifos pesticide by the supernatant showed maximum results. This was due to

the activity of enzymes capable of degrading pesticides. As Parte et al. (2017) mentioned, *Pseudomonas possesses* various enzymes that degrade hydrocarbon compounds, such as hydrolases and various oxygenases. These degradation enzymes are found in catabolic genes present on chromosomes, plasmids, or transposons. Yadav et al. (2016) stated that bacteria degrade by hydrolyzing Chlorpyrifos into diethyl-thiophosphate (DETP) and 3,5,6 trichloro-2-pyridinyl (TCP) and then using the degradation product as a source of nutrition.

5. CONCLUSION

The dominant bacterial genus in the consortium is Pseudomonas, and the dominant bacterial species is *Achromobacter insolitas*. The KBB and KH consortium diversity indices show an intermediate category. The proportion of bacterial consortia degrading chlorpyrifos in the form of KBB-grown cells reaches 94.43% and achieves 98.88% in the supernatant state. The KBB bacterial consortium can reduce the chlorpyrifos content in the liquid medium. Therefore, the KBB bacteria consortium may be used as a bioremediation agent in land contaminated with the pesticide chlorpyrifos.

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Declaration of Competing Interest

The authors declare that no competing financial or personal interests may appear and influence the work reported in this paper.

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