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Toxicity Reduction of Antiseptic Triclocarban by a Newly Isolated Strain Sphingobacterium sp. MC43

Merry Krisdawati Sipahutar[1*]

[1]Occupational Safety and Health Study Program, Faculty of Vocation, Balikpapan University, Balikpapan, East Kalimantan, 76114, Indonesia; e-mail: merry.k@uniba-bpn.ac.id

ABSTRAK

Triclocarban, agen antimikroba yang digunakan dalam produk farmasi dan perawatan pribadi, terdegradasi secara tidak sempurna selama pengolahan air limbah. Namun, sangat sedikit informasi tentang biodegradasi triclocarban yang telah dipublikasikan. Penelitian ini adalah yang pertama menggambarkan biodegradasi triclocarban dan pengurangan toksisitas menggunakan *Sphingobacterium* sp. MC43, strain bakteri pemacu pertumbuhan tanaman yang baru ditemukan. *Sphingobacterium* sp. MC43 ini adalah strain baru yang diisolasi dari tanah pertanian dengan riwayat penggunaan pestisida. Karakterisasi in vitro menunjukkan bahwa *Sphingobacterium* sp. MC43 dapat menggunakan triclocarban sebagai satu-satunya sumber karbon dan pada konsentrasi yang tinggi (30 µM), sedangkan uji fitotoksisitas digunakan untuk mengevaluasi detoksifikasi triclocarban. Triclocarban pada konsentrasi 30 µM terdegradasi +/-50% dalam waktu kurang dari 72 jam. *Sphingobacterium* sp. MC43 mampu mendegradasi triclocarban lebih efektif setelah adanya penambahan sumber karbon lain seperti asetat, karboksimetil selulosa, dan asam suksinat serta sumber nitrogen seperti natrium asetat dan urea. Untuk menghindari efek berbahaya triclocarban pada tanaman, *Vigna radiata* digunakan untuk mempelajari bagaimana *Sphingobacterium* sp. MC43 dapat mengurangi toksisitas triclocarban. Paparan triclocarban menyebabkan kerusakan pada tanaman *Vigna radiata*, sedangkan bioaugmentasi *Sphingobacterium* sp. MC43 secara signifikan mengurangi kerusakan tanaman *Vigna radiata*. Ini kemungkinan karena biomassa dan daya degradasi bakteri telah tumbuh. Hasil ini menyiratkan bahwa strain ini memiliki efisiensi bioremediasi triclocarban yang tinggi dan berpotensi mengurangi serapan triclocarban di *Vigna radiata*.

***Kata kunci**:* toksisitas triclocarban, bakteri pemacu pertumbuhan tanaman, isolasi, pereduksi toksisitas, antiseptik

ABSTRACT

Triclocarban, an antimicrobial agent used in pharmaceutical and personal care products, is incompletely degraded during wastewater treatment. However, very little information about biodegradation has been published. This study is the first to describe triclocarban biodegradation and toxicity reduction using *Sphingobacterium* sp. MC43, a recently discovered plant-growth-promoting bacterium. *Sphingobacterium* sp. MC43 is a newly isolated strain isolated from agricultural soil with a history of pesticide use. The in vitro characterizations showed that *Sphingobacterium* sp. MC43 could use triclocarban as the only carbon source and at high concentration (30 µM), whereas phytotoxicity assays were used to evaluate the detoxification of triclocarban. Triclocarban at 30 µM concentration was 50% degraded in less than 72 hours. *Sphingobacterium* sp. MC43 was able to degrade triclocarban more effectively in recognition to additional carbon sources like acetate, carboxymethyl cellulose, and succinic acid as well as nitrogen sources like sodium acetate and urea. Concerned about triclocarban's harmful effects on plants, *Vigna radiata* was used to study how *Sphingobacterium* sp. MC43 can reduce toxicity. Triclocarban exposure caused damage to *Vigna radiata* plant, whereas *Sphingobacterium* sp. MC43 bioaugmentation significantly reduced this damage. This is likely because the bacteria's biomass and degrading power have grown. These results imply that this strain has a high triclocarban bioremediation efficiency and potentially reducing triclocarban uptake in *Vigna radiata*.

Keywords: triclocarban toxicity, plant growth promoting bacteria, isolation, toxicity reduction, antiseptic

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1. INTRODUCTION

Over the last decade, a persistent antiseptic triclocarban (3,4,4'-trichlorocarbanilide) is extensively used in various pharmaceutical and personal care products (PPCPs), such as deodorants, cosmetics, soaps, shampoos, mouthwash, toothpastes, lotions and skin care creams, and is widely applied in detergents as sanitation, at concentrations ranging from 0.1 to 3% (w/w), for its

capability to inhibit bacteria, fungi and yeast behaviors (Chen et al., 2021; Cheng et al., 2022). The intensive use and recalcitrant property of triclocarban lead to its accumulation in farmland and water resources by treated and untreated biosolid and sewage due to the imperfect handling of waste water treatment facilities (Li, Le-Minh, et al., 2022). The widespread uses of triclocarban have led to continuous emissions of this chemical into the environment. Consequently, this chemical is ubiquitously present at high concentrations in the aquatic systems based on widely reported measured environmental concentration data in different environmental systems worldwide, especially in developed countries (Vimalkumar et al., 2018).

In recent years, worries about the possible effects of the aforementioned antimicrobial agents on human and animal health have been voiced (Barriere, 2015). These substances are regarded as a class of newly emerging endocrine disruptors that interfere with human reproduction and cause immunological dysfunction (Tijani et al., 2016). Studies have demonstrated their toxicity to aquatic organisms like fish, invertebrates, and algae. Human exposure to parabens and the genesis of breast cancer may be related in some way (Tarnow et al., 2013). In developing countries, however, lack of measured environmental concentration data is a major issue, and therefore, inhibits effective risk assessment of this chemical. When the tertiles of triclocarban in urine were treated as a continuous variable, a positive exposure–response relationship was found with general overweight/obesity (Hu et al., 2022).

Due to its toxicity not only to environmental microbes, but also to human, the treatment of triclocarban is required. Microbial degradation is considered the main mechanism of triclocarban transformation in soil and water. Several studies focused on the toxicity reduction of triclocarban by using microorganisms such as soil bacterial communities (Thelusmond et al., 2019); *Rhodococcus rhodochrous* BX2 (Li, Sun, et al., 2022); *Actinobacteria* and *Proteobacteria* (Thelusmond et al., 2018); *Pseudomonas fluorescens* MC46 (Sipahutar et al., 2018); and *Shpingobacterium* strain (Liang et al., 2020; Sipahutar & Vangnai, 2017; Yun et al., 2017). However, study for the ability of *Sphingobacterium* strain to reduce the toxicity of triclocarban is scarce.

Therefore, the main objective of this study was to isolate a bacterium, *Sphingobacterium strain* sp. MC43 from agriculture soil with history of pesticide use. Strain sp. MC43 was evaluated on behalf of its ability to degrade triclocarban under aerobic and anaerobic conditions. Degradation of triclocarban were detected by HPLC and analyzed by LC-MS. Biodegradation pathway of triclocarban was proposed based on the degradation pattern. Toxicity assessments of triclocarban by strain sp. MC43 were performed thru phytotoxicity study onto plant seeds *Vigna radiata*. To the best of our knowledge, the current study is the first report on the degradation and detoxification of triclocarban by *Sphingobacterium strain* sp.

2. METHOD

2.1. Chemicals, culture medium and bacterial cultivation conditions

Triclocarban (99% purity; Sigma-Aldrich, USA) was among the analytical grade compounds. Acetone was used to dissolve triclocarban stock solution before being diluted to the specified concentration. The mineral-salt medium (MSM) used for cultivation had a pH of 7.0±0.1 and contained the following amounts of salt per liter of deionized water: NaH2PO42H2O 0.66 g/L, Na2HPO4 5.8 g/L, KH2PO4 3 g/L, NaCl 0.5 g/L, MgSO4 0.25 g/L, and NH4Cl 2 g/L. As much as 15.0 g/L of agar was transfered to the solid medium. Unless otherwise stated, bacterial cultivation was carried out in a 250 mL Erlenmeyer flask at 30 °C with a rotary shaker spinning at 150 rpm using an inoculum of 4% (v/v) in a 100 mL MSM medium supplemented with triclocarban.

2.2 Pure strain isolation and identification

Using soil samples that had previously been treated with pesticide, the enrichment culture technique was used to isolate bacteria that could break down triclocarban. By mixing 5 g of the collected soil sample with 100 mL of the mineral salts medium, the triclocarban biodegrader population was first enriched (MSM). MSM was given a 30 M addition of triclocarban as the only source of carbon and energy. The flasks were placed on an orbital rotary shaker at 150 rpm and incubated at 30°C for 1 day. On MSM agar plates with 30 M of triclocarban as the carbon and energy source, a 0.2 mL aliquot was applied. Colonies on the plates that were still alive after two days at 30 °C were chosen to determine whether they could break down triclocarban (Monod, 1949). By sequencing the partial 16*S* rDNA, a pure bacterial strain with the best triclocarban biodegradation and cell proliferation capacity was further found (STONE et al., 1995). Polymerase chain reaction (PCR) amplification was carried out using a standard set of primers for bacteria: forward primer (27f: 5'-AGA GTT TGA TCC TGG CTC AG-3') and reverse primer (1492r: 5'-GGT TAC CTT GTT ACG ACTT-3'). The PCR procedure included a pre-denaturation stage at 94 °C for three minutes, 30 cycles of 95 °C for thirty seconds, 57 °C for thirty seconds, 72 °C for fifteen seconds, and a final ten-minute cycle of 72 °C for chain extension. Sequencing and amplicon purification were done. The sequences were then made available for the NCBI's BLAST search to find closely related sequences. Mega software and the neighbor-hood joining method were used to create a phylogenetic tree (Version 6). A bootstrap value of 1000 was used to obtain confidence estimates for the phylogenetic tree topologies.

2.3 Growth and triclocarban degradation experiments

For all experiments, cell culture incubated into 5 mL LB liquid medium under continuous shaking at 150 rpm and harvested at the end of the exponential phase. This cell was used as starter. The starter was inoculated at a 8% (v:v) level into 100 mL MSM (pH 7) containing 30 µM triclocarban before being incubated at 30 °C and 150 rpm in a rotary shaker. The experiment was conducted under growth dependent. Stock solution of triclocarban (100 mM dissolved by acetone) was added to the flasks (250 mL), and the acetone was allowed to evaporate before addition of MSM media. Uninoculated cultures, as abiotic controls, were also prepared in the same way as above. The degradation experiments were conducted under aerobic condition. All solution flasks were vigorously shaken and then incubated in room temperature. At predetermined time intervals, the culture samples were withdrawn to measure both residual triclocarban and OD600nm. The degradation of triclocarban was determined by taking 1 mL volume from each vial and centrifuging the solutions at 10,000 rpm for 10 min to remove the cells. The residues of triclocarban in the supernatant was analyzed by HPLC (LC-20AT, Shimadzu) as per the following conditions: column temperature: 40 °C, wavelength: 254 nm, flow rate: 1 mL/min, mobile phase: acetonitrile (ACN) : UP water (70 : 30), and injection volume: 20µL. Quantitative data was obtained by comparing the peak area of unknown peaks with that of the standard compounds.

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2.4 Effect of supplemental of co-substrates on triclocarban degradation by *Sphingobactrum sp MC43*

Additional co-substrates (carbon and nitrogen sources) was also investigated to study their influence on triclocarban degradation by *Sphingobacterium* MC43 in MSM liquid culture under growth dependent experiment. The experiment was modified based on the protocol of Wang et al., 2016 (Wang et al., 2016): 1 g/L carbon sources (1. acetate, 2. carboxymethyl cellulose or CMC, 3. succinic acid, and 4. glucose) and 1 g/L nitrogen sources (1. ammonium sulfate, 2. ammonium nitrate, 3. sodium nitrate, and 4. urea). 8 mL of *Sphingobacterium* sp. MC43 cell suspension was added to each flask containing 100 mL of MSM medium, 30 µM concentration of triclocarban, and each co-substrate. Flasks without the addition of additional substrates served as control. At predetermined time intervals, the culture samples were withdrawn to measure both residual triclocarban and OD600nm.

2.5 Plant characteristics in growth chamber experiments

This study also examined plant properties through seed germination and seedling development. In accordance with Toyama et al. (2011), mung bean (*Vigna radiata*) seeds were sterilized, pre-germinated for three days, and then aseptically transferred into a 500 ml bottle jar containing 300 ml of half-strength semisolid Hoagland's solution (0.3%) agar containing 30 µM triclocarban supplemented with 1 ml of bacterial suspension of *Sphingobacterium* sp. MC43:triclocarban (Hoagland's solution plus 30 µM triclocarban and Sphingobacterium sp. MC43) served as a positive control, triclocarban (Hoagland's solution plus 30 µM triclocarban served as a negative control, and Blank (Hoagland's solution without both triclocarban and bioaugmented *Sphingobacterium* sp. MC43) served as a negative control). For each treatment, three replicates were employed. The bottles and jars were set up in a growth chamber with a cycle of 14 hours of light and 10 hours of darkness, a constant temperature of 25 °C, and an 80% humidity level (Yang et al., 2011). After 7 days of incubation, the plant's wet weight, length, and root length were measured. The biomass of each treatment group was used to calculate the wet weights of the plants, whereas the plant and root lengths were measured in centimeters.

2.6 Phytotoxicity

The toxicity level of the degradation metabolites was compared to that of the parental compound, triclocarban, using phytotoxicity to assess biodegradation efficiency. The degradation metabolites collected at 72 h during the time-course biodegradation test with 30 M triclocarban concentration were extracted with acetone, dried, dissolved to the initial volume in distilled water, and tested for toxicity. A seed germination bioassay was used to assess the phytotoxicity of treated samples (Haq & Kalamdhad, 2021). Five *Vigna radiata* seeds were surface-sterilized and placed on filter paper in each petridish soaked with 2-ml of the test solution (triclocarban or its degraded metabolites by Sphingobacterium sp. MC43) or deionized water as a control (Toyama et al., 2011). The plates were incubated in a growth chamber for 72 hours in a dark environment at a constant temperature of 25 °C, 80% humidity, and a cycle of 14 h of light and 10 h of darkness (Yang et al., 2011). At the conclusion of the exposure period, the level of toxicity was evaluated in terms of the suppression of seed germination and the length of their sprouts in

comparison to those of the control, where distilled water indicated no toxic effect on the seeds and the longest sprout possible.

2.7 Statistical analysis

All the tests were conducted in three replications and data were statistically analyzed and significant difference among treatments means were calculated by one way analysis of variance (ANOVA) with Tukey's multiple comparison test at 5% probability level using Graphpad Prism, version 5.0.03 (CA, USA).

3. RESULT AND DISCUSSION

3.1 Isolation and identification of triclocarban-degrading strain *Sphingobacterium* sp. MC43

A total of 4 strains of triclocarban-degrading bacteria were obtained by enrichment processes and after incubation on agar plates supplemented with triclocarban from soil samples that were historically contaminated with pesticide. Using MSM medium containing triclocarban as the only carbon and energy source, these bacterial strains were evaluated for growth (OD600 nm). The MC43 strain demonstrated triclocarban tolerance and growth efficiency. As a result, MC43 was picked for more research. Being the only carbon and energy source, triclocarban was effectively broken down by MC43. It is a short rod-shaped, gram-negative, non-spore, motile bacteria. The length of the MC43 16S rDNA amplified was 1467 bp nucleotides. The 16S rRNA sequence analysis made it abundantly evident that MC43 belonged to the *Sphingobacterium* genus. As a result, this strain was determined to be a *Sphingobacterium* species strain (named *Sphingobacterium* sp. MC43). A phylogenetic tree of *Sphingobacterium* sp. MC43 was created using the neighbor-joining method after alignment with other 16S rRNA sequences in GenBank. Figure 1 shows that MC43 is closely related to *Sphingobacterium* sp. N7 (GenBank accession number KF987808), a bacterium known for degrading hydrocarbons.

The ability of *Sphingobacterium* sp. to degrade harmful substances has been documented by numerous investigators. *Sphingobacterium spiritovorum* from sandy soil was found to be capable of degrading benzo(a)pyrene (Alias et al., 2022), *Sphingobacterium mizutaii* LLE5 isolated from activated sludge can degrade sulfamethoxazole (Song et al., 2021), *Sphingobacterium mizutaii* S121 isolated from contaminated soil is promoted as tetracycline degrading bacterium (Tan et al., 2022), *Sphingobacterium multivorum* B-3 from sewage, activated sludge, and soil has capability to degrade hexaconazole (An et al., 2020), a psychrotolerant *Sphingobacterium* sp. C1B isolated from the apple orchard is able to degrade organophosphorus pesticide chlorpyrifos (Verma et al., 2020), *Sphingobacterium* sp. KM-02 isolated from polycyclic aromatic hydrocarbon-contaminated soil has capability to remove fluorene (Nam et al., 2015), and *Sphingobacterium* sp. strain RTSB isolated from a petroleum-contaminated soil competent of utilizing acenaphthene (Mallick, 2019). The ability of *Sphingobacterium* sp. to digest triclocarban in pure culture, however, has never been reported before in a study.

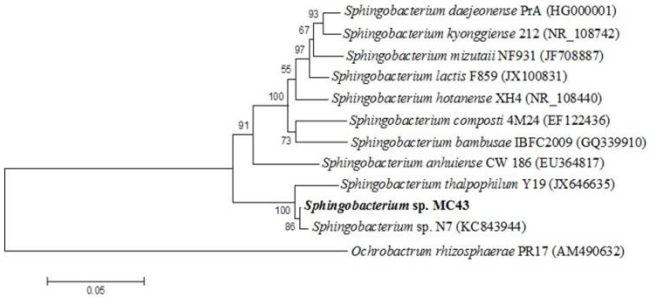

Figure 1. The phylogenetic tree for **MC43 (1467 bp)** and related strains based on **16S rRNA gene sequences**, showing a high degree of similarity (99%) to *Sphingobacterium* sp. N7.

3.2 Bacterial growth and triclocarban biodegradation by *Sphingobacterium* sp. MC43 under aerobic condition

Triclocarban degradation by *Sphingobacterium* sp. MC43 was studied using the batch experiments at aerobic condition. *Sphingobacterium* sp. MC43 inoculated into media containing triclocarban as the sole carbon and energy source grew exponentially without any lag phase under aerobic condition. No bacterial growth was found in abiotic control flasks containing MSM medium and triclocarban. Triclocarban started to decrease in aerobic liquid cultures without any lag phase. This result reflected a rapid bacterial adaptation *of Sphingobacterium* sp. MC43 to triclocarban. Analysis of residual triclocarban in any of the sterilized control flasks showed less than 5 % degradation due to abiotic processes, in Figure 2.

Many microorganisms have limited capability in degrading triclocarban (Miller et al., 2010; Cha & Cupples, 2010). *Sphingobacterium* sp. MC43 was able to degrade approximately 50% triclocarban at an initial concentration of 30 µM under aerobic conditions after 72 hours. However, it is the first study reporting *Sphingobacterium* sp., as pure culture, that is able to degrade triclocarban.

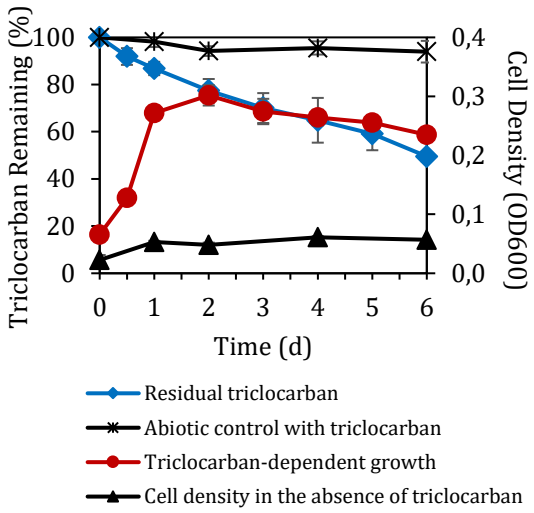

Figure 2. Triclocarban degradation and growth profile of *Sphingobacterium* sp. MC43 at 30 µM triclocarban concentration. Error bars indicate the standard deviation of 3 replicates.

3.3. Effects of different carbon and nitrogen sources on bacterial growth and triclocarban degradation by *Sphingobacterium* sp. MC43

In Figures 3 and 4, *Sphingobacterium* sp. MC43 was used to examine the effects of various carbon and nitrogen sources on bacterial growth and the breakdown of triclocarban. While other types of carbon and nitrogen sources used a very moderate impact on the number of cells of strain *Sphingobacterium* sp. MC43, the presence of 1 g/L glucose and succinic acid considerably increased the cell proliferation of *Sphingobacterium* sp. MC43. *Sphingobacterium* sp. MC43 considerably boosted triclocarban degradation (100% in 72 h) over the control, MSM alone (40%), when carbon sources such acetate, carboxymethyl cellulose, and succinic acid were added. However, adding glucose marginally reduced triclocarban degradation (20%). The breakdown of triclocarban by *Sphingobacterium* sp. MC43 was also improved by the addition of nitrogen sources, such as sodium acetate and urea, by 80% and 90% in 72 hours, respectively. However, the addition of ammonium sulfate and sodium nitrate had less of an effect (between 50 and 55%).

Carbon, such as acetate, carboxymethyl cellulose, and succinic acid, and nitrogen, such as ammonium sulfate, ammonium nitrate, sodium nitrate, and urea, had no effect on the specific degradation rate of triclocarban by *Sphingobacterium* sp. MC43 when compared to MSM alone, whereas adding ammonium sulfate and sodium nitrate nitrogen had a slightly significant effect. Glucose, on the other hand, clearly inhibited triclocarban degradation, despite the fact that its addition to the medium could significantly increase microbial biomass. The addition of readily accessible carbon sources may boost microbial biomass and improve the ability of bacteria to degrade materials. That is consistent with this study's findings that the addition of glucose promoted the breakdown of polychlorinated biphenyls (PCBs) by *Pseudomonas stutzeri*, but the addition of these substrates reduced PCBs biodegradation in other studies (Murínová et al., 2014).

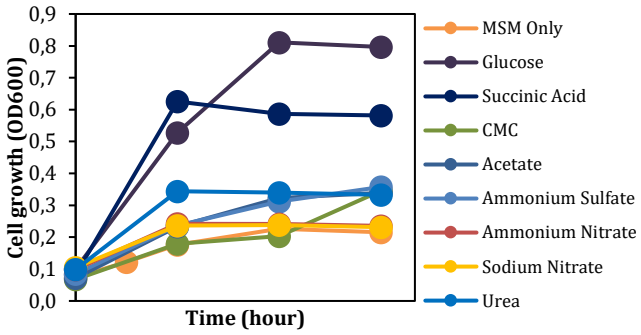

Figure 3. Effect of additional carbon and nitrogen sources on bacterial growth *Sphingobacterium* sp. MC43.

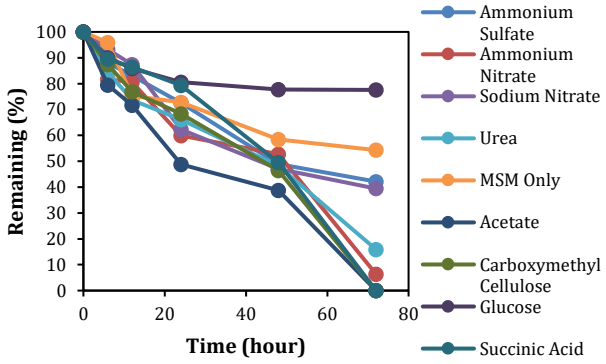

Figure 4. Effect of additional carbon and nitrogen sources on biodegradation of triclocarban by *Sphingobacterium* sp. MC43.

3.4 Phytotoxicity assessment of triclocarban and the degraded metabolites

Vigna radiata seedlings were exposed to Hoagland's solution with 30 µM triclocarban containing *Sphingobacterium* sp. MC43 to determine whether it may accelerate plant growth in polluted conditions. The effects of biologically treated and untreated effluent at concentrations of 30 µM triclocarban on seed germination were assessed. The findings demonstrated that when exposed to untreated effluent at a concentration of 30 µM triclocarban (negative control) in hydroponic settings, the plant wet weight, plant length, and root length dramatically decreased 52%, 33%, and 45%, respectively, in contrast to the positive control. Whereas, in the contaminated effluent, *Sphingobacterium* sp. MC43 strain increased Vigna radiata wet weight (75%), plant length (25%), and root length (30%). The findings show that the MC43 strain of *Sphingobacterium* sp. significantly increased plant growth and triclocarban degradation in the

contaminated wastewater (Figure 5a,b,c).

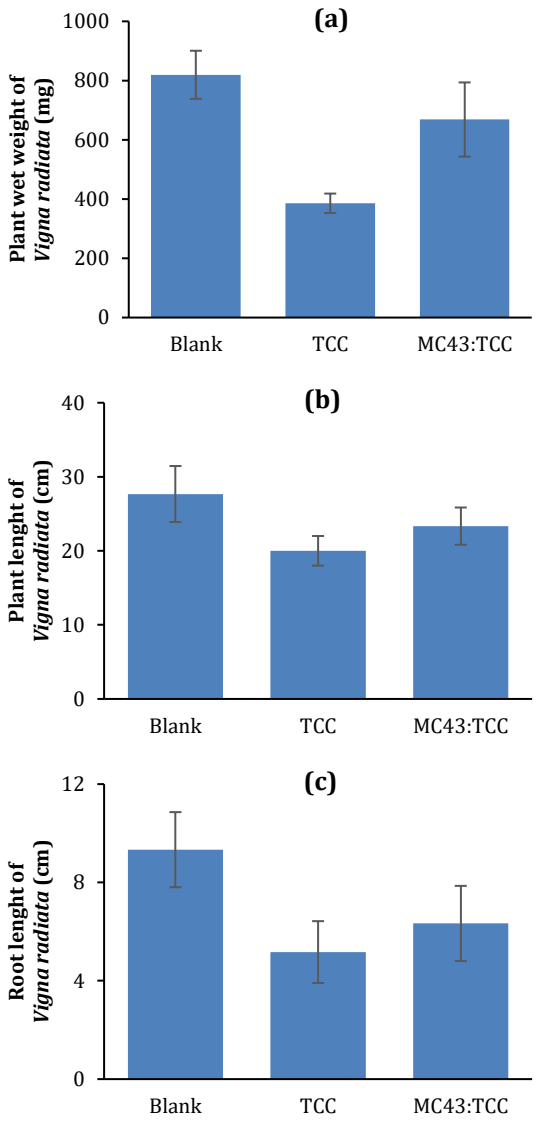

Figure 5. Plant characteristics: plant wet weight (a), plant length (b) and root length (c) of *Vigna radiata* of Blank (Hoagland's solution without both TCC and bioaugmented *Sphingobacterium* sp. MC43) served as a positive control, TCC (Hoagland's solution plus 30 µM TCC) served as a negative control, and MC43:TCC (Hoagland's solution plus 30 µM TCC and *Sphingobacterium* sp. MC43), measured one week after incubation. The data are mean ± standard deviation from triplicate treatments, each of which with at least 5 seeds, and assessed using Tukey's multiple comparison test significant differences at P ≤ 0.05.

In tropical nations, the nutritious bean crop known as *Vigna radiata* is common and widely grown. The goal of the current investigation is to determine whether triclocarban injury to *Vigna radiata* plants may be reduced by *Sphingobacterium* sp. MC43. The findings demonstrated that in the triclocarban-contaminated medium, *Sphingobacterium* sp. MC43 enhanced plant wet weight, plant length, and root length. Perhaps because it was able to lessen the toxic effects of triclocarban. Uninoculated trials, on the other hand, revealed an inhibitory effect on *Vigna radiata*'s elongation growth. *Sphingobacterium* sp. MC43 enhanced the biomass, length, and root length of *Vigna radiata*.

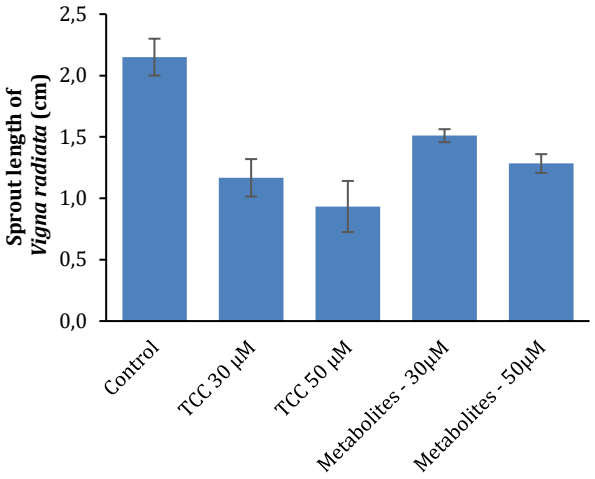

Figure 6. Phytotoxicity assessment of triclocarban at 30 and 50 µM and its degraded metabolites by *Sphingobacterium* sp. MC43 with *Vigna radiata* expressed as sprouting length. The data are mean ± standard deviation from triplicate treatments, each of which with at least 5 seeds, and assessed using Tukey's multiple comparison test significant differences at P ≤ 0.05.

Table 1 ANOVA with Tukey's multiple comparison test conducted on the data of plant wet weight of *Vigna radiata*

Tukey's Multiple Comparison Test	Mean Diff,	q	Significant? P < 0,05?
Blank vs TCC	434,0	8,523	Yes
Blank vs MC22:TCC	151,0	2,965	No
TCC vs MC22:TCC	-283,0	5,558	Yes

Table 2 ANOVA with Tukey's multiple comparison test conducted on the data of plant length of *Vigna radiata*

Tukey's Multiple Comparison Test	Mean Diff,	q	Significant? P < 0,05?
Blank vs TCC	8,675	8,299	Yes
Blank vs MC22:TCC	4,000	3,827	No
TCC vs MC22:TCC	-4,675	4,472	Yes

Table 3 ANOVA with Tukey's multiple comparison test conducted on the data of root length of *Vigna radiata*

Tukey's Multiple Comparison Test	Mean Diff,	q	Significant? P < 0,05?
Blank vs TCC	4,400	7,995	Yes
Blank vs MC22:TCC	1,500	2,726	No
TCC vs MC22:TCC	-2,900	5,270	Yes

Graphpad Prism, version 5.0.03 (CA, USA) was used to perform statistical analysis. ANOVA test followed by the multiple comparison Tukey's test, conducted on all data and separately for each plant characteristic (plant wet weight, plant length, and root plant), showed that plant characteristics of *Vigna radiata* were not significantly different between those treated on Blank (Hoagland's solution without both triclocarban or TCC and MC43) and bioaugmented *Sphingobacterium* sp. MC43 on TCC,

while there were significant differences between those on Blank and TTC alone, as well as between bioaugmented *Sphingobacterium* sp. MC43 and TTC alone (Table 1–3).

Table 4 ANOVA with Tukey's multiple comparison test conducted on the data of sprout length of *Vigna radiata*

Tukey's Multiple Comparison Test	Mean Diff,	q	Significant? P < 0,05?
Control vs TCC 30 µM	1,050	9,038	Yes
Control vs TCC 50 µM	1,350	11,62	Yes
Control vs Metabolites - 30µM	0,2733	2,353	No
Control vs Metabolites - 50µM	0,5400	4,648	No
TCC 30 µM vs Metabolites - 30µM	-0,7767	6,686	Yes
TCC 50 µM vs Metabolites - 50µM	-0,8100	6,973	Yes

The same pattern was also obtained for the phytotoxicity assessment of triclocarban at concentrations of 30 and 50 µM and its degraded metabolites by MC43 with *Vigna radiata*. No significant differences among the control and metabolites of MC43 at both 30 and 50 µM treatments were observed. The Tukey's test demonstrated that there were significant differences between control and TCC for both concentrations, as well as between sprout length on metabolites and TCC at both concentrations.

Toxicology assessment is strictly necessary as a final test step for risk warrant because the most crucial objective of the pollutant biodegradation process towards a safe environment is to eliminate or minimize the overall toxicity caused by the parental toxic pollutant or toxic metabolites formed during the degradation. To confirm that *Sphingobacterium* sp. MC43 strain biodegradation capacity resulted in triclocarban toxicity reduction, current investigation applied the following phytotoxicity assessment procedures. The primary reason for doing the phytotoxicity study was the safety issue raised by the possibility that triclocarban contamination in agricultural areas could have an adverse effect on plant growth. Accordingly, triclocarban 30 and 50 µM as well as their respective degraded metabolites collected at 72-h of the degradation interval were tested for toxicity using *Vigna radiata* legume seeds (Figure 6). The findings were then contrasted with seeds that had grown normally in water after 72 hours of incubation, which served as positive controls. For *Vigna radiata* seeds, the maximum sprouting length was 2.15 ± 0.15 cm. Triclocarban at both concentrations had a notable negative impact on *Vigna radiata* seed and inhibited or slowed root elongation by >45% as evidenced by sprouting that was more than half as short as that of the controls (Figure 6). When the seeds were exposed to triclocarban's degradative metabolites at 30 and 50 µM, the root elongation ability was significantly restored because triclocarban at this original concentration was partially degraded at a 72-h period (Figure 2). This finding confirmed that triclocarban has considerable phytotoxicity even at low concentrations, and that the biological treatment with *Sphingobacterium* sp. MC43 strain could significantly reduce the phytotoxicity allied with triclocarban.

With 25% treated effluent, the sprouting length was somewhat longer than with the untreated seedlings. This suggests that toxicity declined after wastewater was treated with bacterial culture *Sphingobacterium* sp. MC43, which may have been brought on by the suspected toxicants being broken down during biological treatment. This evidence strengthens our theory that biological treatment with the present isolate can reduce effluent toxicity significantly while also improving other effluent parameters. Cadmium, metal, arsenic, and chromium effluents were previously documented cases where toxicities were reduced with biological treatment thus promoted plant growth (Wang et al., 2022; Alves et al., 2022; Alka et al., 2020; Vishnupradeep et al., 2022).

The accumulation of antiseptic compound in plants changes their physiological and metabolic processes. All antiseptic compounds have harmful consequences; however each one acts differently inside the plant. Due to structural and molecular changes brought on by toxic compounds, the plant experiences growth retardation, necrosis, chlorosis, and a drop in germination rate (Fernandes & Ghag, 2022; Valivand et al., 2019).

4. CONCLUSIONS

This research attempts to provide a comprehensive study on isolation and characterization of a triclocarban degrading *Sphingobacterium* strain and evaluation of its plant growth promoting traits. For the first time, a pure culture of strain *Sphingobacterium* sp. MC43, capable of degrading triclocarban and plant growth promoting bacterium, was isolated and identified. This novel isolated strain successfully degraded at 30 µM concentration of triclocarban in less than 72 hours under aerobic conditions. Additional carbon, acetate, carboxymethyl cellulose, and succinic acid, as well as nitrogen sources, sodium acetate and urea, aided triclocarban degradation by *Sphingobacterium* sp. MC43, which could be attributed to increased bacterial biomass and degrading capacity. Furthermore, the results showed that this newly isolated strain, which possesses plant growth promoting traits, was critical not only in reducing but also in eliminating the triclocarban. Consequently, *Sphingobacterium* sp. MC43 could be used as a bio-inoculant to remediate triclocarban and also sustaining agronomic production programs in triclocarban-contaminated areas. However, the author recognizes that this study has limitation in terms of a lack of data on what enzyme makes MC43 so effective at reducing triclocarban toxicity. It is therefore hoped that additional genomic research

will be done to identify the enzymes possess by this bacterium that allow MC43 to remove triclocarban and it's toxicity.

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