The Biotransformation and Biodecolorization of Methylene Blue by Xenobiotic Bacterium *Ralstonia pickettii*

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Received: May 10, 2021
Accepted: August 26, 2021

DOI: 10.22146/ijc.65806

Abstract: *The biotransformation and biodecolorization of methylene blue (MB) dye using the bacterium* Ralstonia pickettii *was investigated. This experiment was conducted in a nutrient broth (NB) medium after adding MB at 100 mg L^{-1} concentration. Approximately 98.11% of MB was decolorized after 18 h of incubation. In addition, the metabolic products detected by LC-TOF/MS were Azure A (AA), thionine, leuco-MB, and glucose-MB, which indicated the MB degradation through a reductase that attacked the heterocyclic central chromophore group present in the structure. Moreover, azure A and thionine fragments resulted from the* N*-demethylase enzyme that attacked the auxochrome group. Thus, this research was assumed to be the first scientific report suggesting the potential to use* R. pickettii *in the biodecolorization and biotransformation of dye waste, particularly MB.*

Keywords: *decolorization; biotransformation; xenobiotic bacteria;* Ralstonia pickettii*; methylene blue*

■ INTRODUCTION

Water pollution has become a major problem that can negatively impact humans and the environment [1]. Synthetic dyes are typical water pollutants widely used in the cosmetic, pharmaceuticals, textile, printing, and food industries [2]. In addition, approximately 280,000 tons of dye wastes are discharged into the environment, especially water bodies, without any treatment [3-4]. These direct exposures negatively affect the ecosystem following the interference with sunlight penetration, lowered dissolved oxygen concentration, and disrupted photosynthetic processes for some aquatic organisms [5]. Moreover, other hazardous effects are observed with biomagnification, carcinogenicity, and mutagenicity towards the environment and human health [6-7]. Methylene blue (MB) is one of the dyes used in leather, cotton, cosmetic, plastic, and silk industries [8], especially in the coloring process, where only 5% is absorbed, while 95% is released as waste to the aquatic environment [9]. The disposal of the MB directly contaminates humans and causes diseases, including diarrhea, eye damage, vomiting, gastric disorders, methemoglobinemia, headaches, and dizziness, especially in pregnant women and babies [10-11]. Furthermore, the death of some aquatic organisms around the disposal area has also been reported [12-13]. This high contamination incidence indicates the need to manage waste before discharge to minimize the negative impacts properly.

Moreover, microbiological treatment approaches using bacteria or fungi are less costly and more effective in handling color substance waste than physical, chemical, photocatalytic, and electrochemical methods [10]. The biodecolorization or biodegradation of dyes ensues through the reductive and oxidative activities produced by various extracellular enzymes, observed at the chromophore and auxochrome sites of the molecules [11]. Specifically, decolorization is initiated by reducing MB at the molecular level with NADPH-dependent dehydrogenase quinone into a form of leuco-MB [14-15]. The attacks on chromophore groups instigate the opening of aromatic rings within the heterocyclic center, which first produces intermediate compounds estimated to cleave the structures further [16]. Meanwhile, other degradation mechanisms include oxidative

demethylation by demethylase enzymes, comprising aminopyrine *N*-demethylase, assumed to interact with the auxochrome group to produce fragmented metabolic products of azure B (AB), azure A (AA), azure C (AC), and thionine [17]. The microbiological approach towards degradation requires various processes influenced by numerous factors, including the reaction substrate type and the wealth of microbial genetic strains [13]. Therefore, different microbes act through various mechanisms produce diverse results after waste treatment [18]. However, some species demonstrate the degradation ability, including *Daedalea dickinsii* [16], *Bacillus subtilis* strain MTCC 441 [19], *Ralstonia eutropha* [20], *Alcaligenes* species [5], *Alcaligenes faecalis* [21], *Aspergillus niger* LAG [22], and *Rhodococcus* strain UCC 0003 [23]. These microbes potentially remove more than 85% MB and are confirmed to affect degradation cases with other xenobiotic wastes.

Ralstonia pickettii is a xenobiotic bacterial with the capacity to biodegrade some organic pollutants. This phenomenon is confirmed by numerous reports obtained with various related wastes, resulting from the metabolic complexity alongside the encoding genes for biodegradation enzymes [24]. Furthermore, the toluene-3-monooxygenase, phenol/cresol hydroxylase [25], and meta-cleavage pathway enzymes are secreted to convert catechol and methyl catechol tricarboxylic acid cycle intermediates [26]. These intrinsic constituents promotes the ability to decompose a variety of wastes, including DDT [18], crude oil [27], phenol [28], chlorobenzene [29], toluene and meta-cresol [30], BTEX (benzene, ethylbenzene, toluene, xylenes) [31] and 2,4,6-trichlorophenol [30]. The application of *R. pickettii* as a bioremediation agent was more advantageous than other bacteria because of the non-detection as a phytopathogen or pathogen of animals. In addition, reports have shown significant resilience in low-nutrient environments [30], and it is also considered a good choice for the biodecolorization and biodegradation of MB as well as for broader purposes such as degradation of xenobiotic pollutants (toluene and trichloroethylene) [30], crude oil [27] and pesticide [32]. Therefore, the biotransformation and biodecolorization of MB dye using the bacterium *R. pickettii* were investigated in this study.

■ EXPERIMENTAL SECTION

Materials

The bacterium *R. pickettii* NBRC 102503 (NITE Biological Resources Center, Chiba, Japan) was collected from the Microbial Chemistry laboratory. This specimen was maintained as a culture on nutrient agar (NA; Merck, Darmstadt, Germany), while methanol (Merck, 99%), MB (Merck, C.I. 52015), and D-glucose (Merck-Germany) were purchased from Sumber Ilmiah Persada (SAP, Indonesia).

Procedure

Biodecolorization of methylene blue by R. pickettii

A single *R. pickettii* culture was used to biodecolorize MB, which involved inoculating 10% pre-incubated bacteria into an Erlenmeyer flask containing 50 mL nutrient broth and then incubation at 30 °C for 30 h [33]. Non-inoculated flasks were prepared as controls. Subsequently, the MB was added to attain a final concentration of 100 mg L^{-1} before the cultures were placed in a shaker incubator (VELP scientifica) for 18 h at 30 °C with a shaking speed of 120 rpm. The entire process was monitored for 0, 3, 6, 9, 12, 15, and 18 h, with biomass separation, was performed at each time frame to obtain the supernatants, using 2000 G centrifugation for 10 min [16]. Furthermore, absorbance measurements were carried out with a UV-VIS spectrophotometer at a 400–800 nm wavelength. In addition, the control group contained a 100 mg L^{-1} concentration mixture of NB and MB, while the percentage of sample decolorized was evaluated using the following Eq. (1).

$$\% \text{ Decolorization} = \frac{Ao - At}{Ao} \times 100\% \quad (1)$$

whereas Ao was the absorbance of the control solution while At was the absorbance of the treated sample.

Analysis of metabolic products

The metabolic products were analyzed by evaluating the filtrates through LC-TOF/MS (AB SCIEX). TM RSLC Acclaim 120 C18 column was also used with a 2.1 × 100 mm dimension and set to 33 °C temperature

[16]. In addition, ionizing electrospray (ESI) served as a source of ionization within a mass range of 50–500, where the sample elution flow rate was determined and graded at 0.3 mL min^{-1} for 4 min and 0.4 mL min^{-1} for 6 min. The methanol:water mixture (99:1 v/v) served as the mobile phase within the first 3 min and followed by 61:39 v/v on the remaining 7 min.

■ RESULTS AND DISCUSSION

Biodecolorization Study

This study expresses the *R. pickettii* ability to degrade MB in a NB medium. In addition, each culture was inoculated with 1 mL of pre-incubated bacterium (1 mL bacterium was equivalent to 1.44×10^{13} CFU), and incubated at 30 °C for 18 h, with a biodecolorization monitor time of 0, 3, 6, 9, 12, 15, and 18. Fig. 1 showed the absorbance profile versus wavelength in which 670 nm was determined to be the optimum wavelength. The absorbance pattern profiles at H0 to H9 showed a small decrease in intensity, although there was a significant difference with the absorbance control MB. These conditions indicated the inability *R. pickettii* during incubation time at 0–9 h to show structural degradation activities or transformation performance. However, H12-H18 (incubation 12 and 18 h, respectively) demonstrated a significant change in the absorbance pattern. In addition, *R. pickettii* was capable of thoroughly countering the MB at H18. The elevated cell biomass also contributes to further dye adsorption and reduced absorbance [16].

The optimum decolorization of MB (100 mg L^{-1}) by *R. pickettii* was observed at 18 h (up to 98.11%). This process ensued from the inception of incubation, where 23.33% was reported. In addition, the t-test indicated significant differences in decolorization between 0 to 12 h, which was stationary from 12 to 18 h (Table 1). Thus, the effects of *R. pickettii* were related to the extracellular enzyme content during the incubation period [23]. Moreover, every microorganism demonstrates varying decolorization strength, possibly attributed to the discrepancies in microbe strains or species, incubation time, dye concentration, and other performance-related supporting factors [21,34].

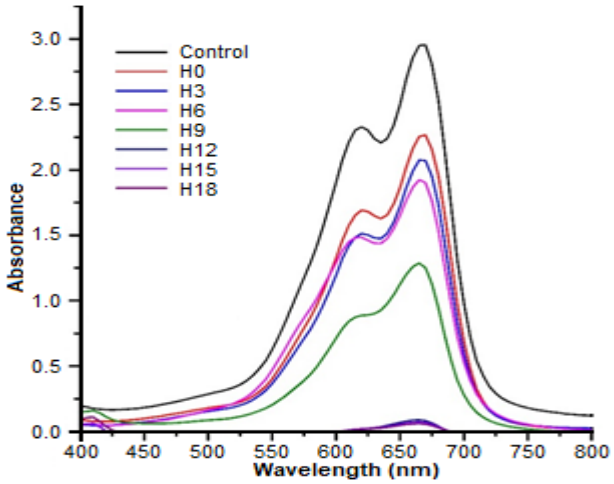

Fig 1. Methylene blue (MB) decolorization absorbance profiles of *R. pickettii*. Data are means (n = 3), where H0 = 0 h, H3 = 3 h, H18 = 18 h incubation

Table 1. Biodecolorization of MB by *R. pickettii* in nutrient broth during 18-day incubation period

Incubation time (h)	Absorbance	% Decolorization
Control	2.95 ± 0.01	-
0	2.27 ± 0.07 [a]	23.33 ± 0.68 [a]
3	2.07 ± 0.11 [b]	29.94 ± 0.42 [b]
6	1.90 ± 0.02 [c]	35.64 ± 0.22 [c]
9	1.25 ± 0.21 [d]	57.71 ± 0.27 [d]
12	0.08 ± 0.04 [e]	97.21 ± 0.43 [e]
15	0.07 ± 0.02 [e]	97.70 ± 0.01 [e]
18	0.06 ± 0.03 [e]	98.11 ± 0.22 [e]

The decolorizing ability of *R. pickettii* was seamless in contrast with other bacteria species, although some bacteria showed satisfactory results, such as *Alcaligenes* species, with maximum decolorization of 88.06% (100 mg L^{-1}, 40 d) [5]; *Camamonas aquatica* at 67% (50 mg L^{-1}, 96 h) [35]; *B. subtilis* strain MTCC 441 at 91.68% (20 mg L^{-1}, 6 h) [19]; *Bacillus thuringiensis* 016 with 95% (25 mg L^{-1}, 2 h) [36]; and *Bacillus* sp. strain MZS10 at 93.55% (0.04 g L^{-1}, 14 h) [14]; as well as *Bacillus licheniformis* strain IFO 12200, *Bacillus circulans* strain IFO 13626, and *Bacillus pomilus* strain IFO 12092, with a maximum of 97.5%; 88.0%; 65.0%, respectively (1.1×10^{-5} mol L^{-1}, 24 h) [37]. In addition, some fungi also confer these effects on MB, although longer incubation is time required. These fungi were *Phanerochaete chrysosporium*, with maximum decolorization of 84.8%

(0.4 g L^{-1}, 22 d) [36]; *D. dickinsii* at 54% (50 mg L^{-1}, 14 d) [16], while *Phaseolus coccineus*, *Coriolus versicolor*, *Fomes fomentarius*, *Trametes suaveolens*, and *Stereum ostrea* decolorized approximately 40% (100 mg L^{-1}, 20 d) [38].

The early interaction with MB ensues on the cell wall surface, which is achieved through adsorption and ionic interactions between the negative charge of the carboxylate (–COOH) or hydroxyl (–OH) group and the positive MB charge [19]. The reactions possibly occur via the reduction of MB to leuco-MB. In addition, extracellular enzymes, including NADH/NADPH-dependent reductase, are widely reported to be responsible for the procedure, as shown in Fig. 2, where colorless Leuco-MB was generated [39]. This reaction also requires oxidative dehydrogenation to yield products characterized by the potential to produce hydrogen peroxide with functionalities through other media or to be decomposed into O_2 and H_2O molecules [40]. This reaction demonstrates redox properties with MB, hence the tendency to use staining and cell density detection. In addition, color loss indicates a substantial cell population implicated in surface adsorption and reduction of MB [41].

The dye decolorization process ensued through a different mechanism for various microorganisms[23,32], where the carbon and nitrogen sources influence the cell's ability to act effectively [14]. The procedure possibly occurs through enzyme and non-enzyme (Fenton) mechanisms, and the latter is adopted with brown-rot fungi [16,42-43]. In addition, some other enzyme groups exploited for dye decolorization include azoreductase [44], lignin peroxidases [38], laccase [45], manganese peroxidase [17], peroxidase and polyphenol oxidase [39], and microperoxidase-11 [46]. The MB in this current investigation was exposed to biotransformation and biodegradation. In addition, the resulting metabolic products were generated through reduction and demethylation reactions on the structure. These mechanisms ensure the support provided by the extracellular enzymatic activities of *R. pickettii* in a liquid NB medium [38].

Identification of Metabolic Products

The MB metabolic products generated after biodegradation treatment with *R. pickettii* were identified using LC-TOF/MS, as shown in Fig. 3. Furthermore, the LC-TOF/MS chromatogram demonstrates 4 metabolic product peaks, where one with a retention time of 5.57 min indicated MB molecule at m/z = 284 [14,16]. Remarkably, the intensity was lower than the control, further confirming the sample transformation within the incubation period, following the incidence of extracellular metabolic products activities. Fig. 3 showed the 4 peaks, characterized by retention times of 2.67, 5.01, 6.49, and 7.79 min, with each possessing an m/z value of 256 (azure A) [14,17], 224 (thionine) [47], 285 (leuco-MB) [15], and 447 (glucose-MB) [14], respectively.

The yields from each microorganism comprised different fragments of compounds, which applied bacteria or fungi outputs [5,16]. In addition, photocatalytic decomposition processes yield a variety of smaller and large molecular fragments [47]. The mechanisms mostly adopted currently involve the working principle of microbes, photocatalytic, and a combination of both mechanisms. Fig. 3 showed the LC-TOF/MS chromatogram and further demonstrated *R. pickettii* transforming MB into some metabolic products, including azure A, thionine, Leuco-MB, and Glu-MB. Moreover, certain bacteria or fungi were identified to yield similar output, although some of them were also different. The *Alcaligenes* species yielded 4-amino-1-benzylpiperidinium dichloride ($C_{12}H_{18}ClN_2$) and

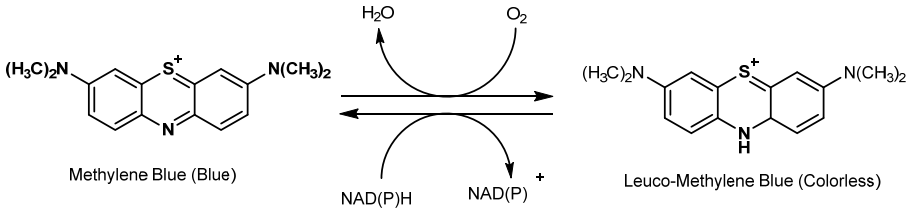

Fig 2. Redox cycling of Methylene blue (MB)

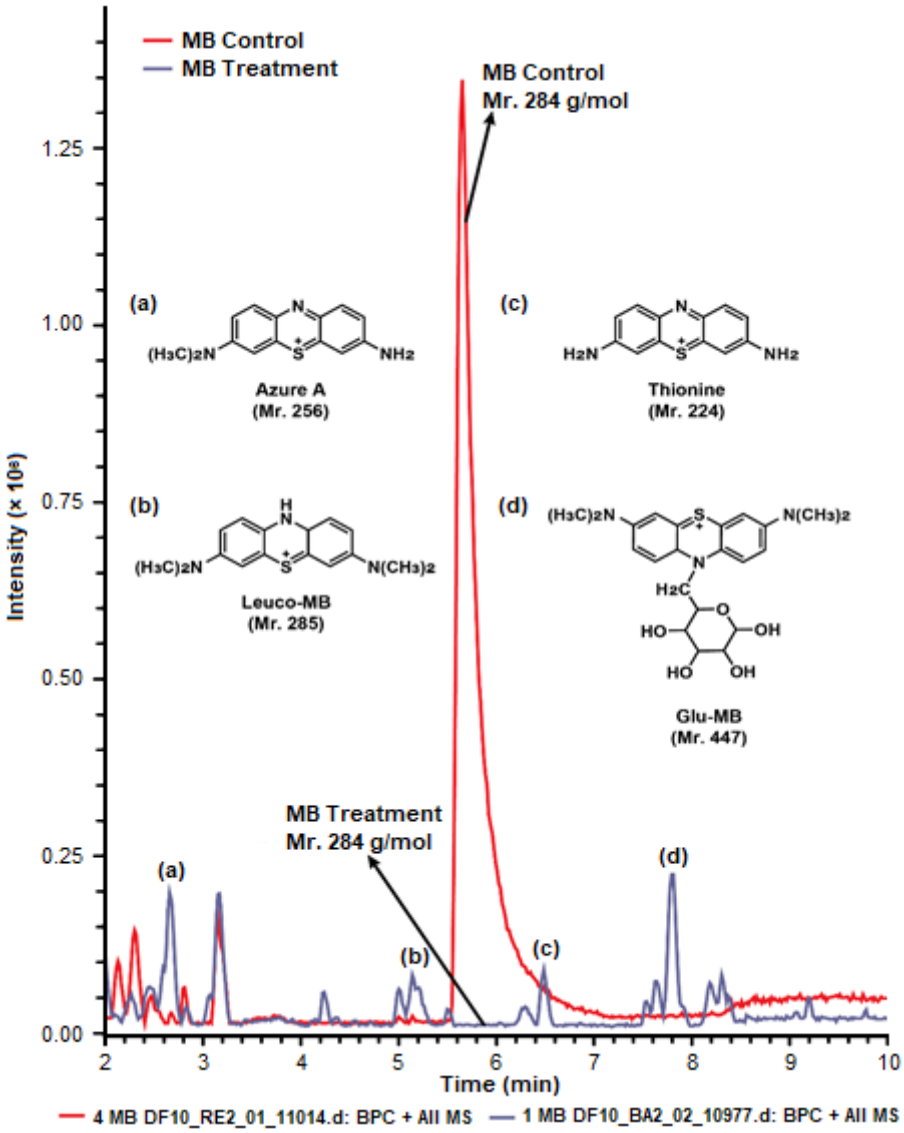

Fig 3. LC-MS chromatogram profile of MB transformation by *R. pickettii*

4,4'-diaminodiphenyl sulfide ($C_{12}H_{12}N_2S$) [5], while azure B (m/z = 270); 3,7-bis (dimethylamino)-4aH-phenothiazin-5-one (m/z = 300), and 4-(dimethylamino)-2-[*m*-(dimethylamino) phenyl sulfinyl] benzenamine were observed with *D. dickinsii* (m/z = 303) [16]. Fig. 4 showed the summary for the proposed MB transformation pathway using 3 microbes.

The molecular fragments identified as products after treatment with photocatalytic and natural oxidizing agents were more diverse, despite the similarity between some metabolic products of bacteria or fungi, including azure A, azure B, and thionine [47]. The MB degradation process using natural MnOx yielded 7 metabolic products, comprising azure B (m/z = 270), azure A (m/z = 256), 2-methyl-2,3,4,5-tetrahydro-1,4-thiazin-1-ium (m/z = 115), 7-amino-2,3-dimethylbenzo[b][1,4]thiazin-1-ium (m/z = 186), benzene-1,4-diamine (m/z = 105), benzo[b][1,4]thiazin-1-ium (m/z = 156), and aniline (m/z = 98.5) [17]. Meanwhile, photocatalysis with materials from sugarcane bagasse cellulose (SBC)-TiO_2 generated fragments of $C_{16}H_{21}N_3SO$, $C_{16}H_{19}N_3S$ (azure B), $C_8H_{12}N_2SO_3$, C_6H_7NO, and C_6H_7N [11]. The use of ZnO:Eu nanoparticles under the sun rays produced a similar outcome as observed with *R. picketii,* while leuco-MB and azure B were identified with *D. dickinsii* [48]. In addition, the microbes and material sources

considerably influenced the variations between specific metabolic products produced. The degradation mechanisms adopted by each bacteria or fungi are associated with the intrinsic enzyme complexity enzymes [4,16,33], while photocatalysis is influenced by the energy source and the material composition [47].

The microbial approach is entirely controlled by extracellular enzyme activities [49]. Furthermore, the effects on synthetic dyes are generally achieved through varied mechanisms to attain a structural transformation. These bacteria and fungi secrete extracellular enzymes after induction in the media. Moreover, *R. pickettii* was presumed to affect modifications to MB by involving the reductase enzymes, including quinone dehydrogenase (QD) dependent NADPH and demethylase. Mainly, QD is an enzyme involved in xenobiotic metabolism processes and confers protection from quinone free radical derivatives [50]. The reductase enzyme, including azo reductase, influences azo dye degradation, with a specific effect on the bond –N=N– [39]. These enzymatic involvements are based on the metabolic products identified in the LC-MS analysis (see Fig. 3).

Proposed Methylene Blue (MB) Transformation Pathway

The identification for metabolic produced during MB degradation, using LC-TOP/MS, and determined based on the approximate pathway after transformation by *R. pickettii* showed in Fig. 4. The proposed procedure recognized the essential roles played by QD in the decolorization process by the hydrogenation of MB to form colorless Leuco-MB. The proposed procedure recognized the essential roles played by QD in the decolorization process by the hydrogenation of MB to form colorless Leuco-MB. This output is more stable, resulting from the Glucose-MB molecules formed by dehydration between the hydroxyl group (–OH) from glucose and the -NH present in MB [14]. Furthermore, QD was produced and identified in some bacteria, including the *Bacillus* sp. strain MZS10 and *Bacillus* sp. strain LD003, following the degradation of dye azure B [50].

The biodecolorization process is generally attributed to enzymatic oxidation or reduction activities. These are some of the various extracellular types involved: H_2O_2

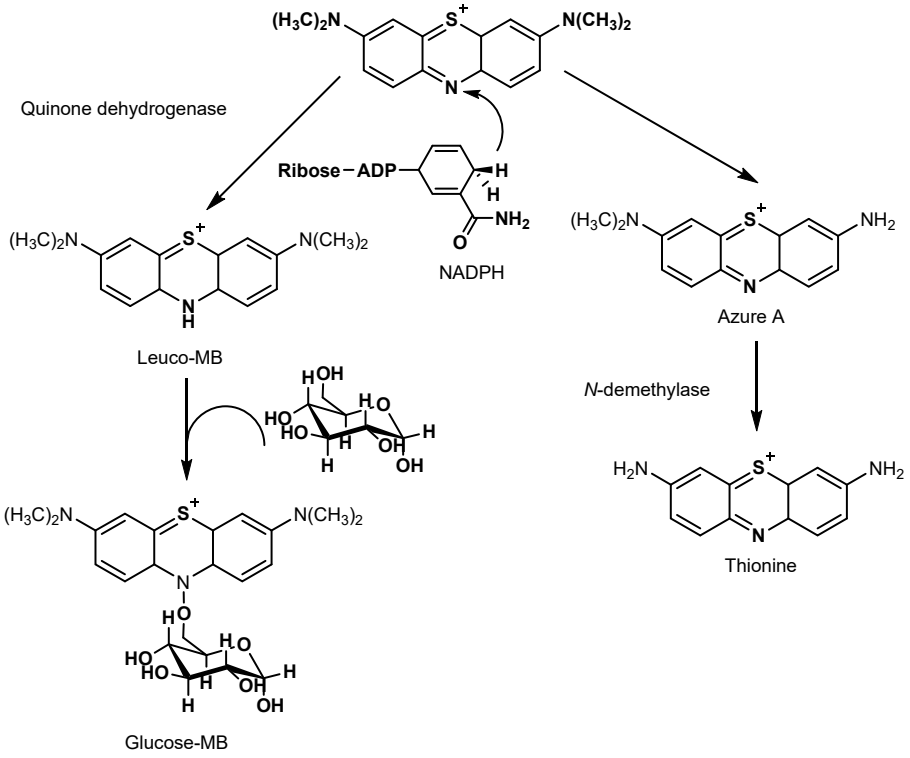

Fig 4. Proposed methylene blue (MB) transformation pathway by *R. pickettii*

independent oxidase, laccase, and azoreductase to degrade the reactive yellow of 84A by *Exiguobacterium* sp. RD3 [51]. Also, *Pseudomonas aeruginosa* BCH is known to secrete tyrosinase, NADH-DCIP reductase, veratryl alcohol oxidase, and laccase are implicated in amaranth azo dye decomposition [52].

Fig. 4 showed oxidative demethylation of MB as another mechanism exploited to form azure A (AA) and thionine molecules. Particularly, demethylation commonly facilitates the conversion into azure B (AB), azure A (AA) or sym-dimethylthionine, Azure C (AC), and thionine [50]. Fig. 5 showed the degradation in potato dextrose broth (PDB), using *D. dickinsii* to produce azure B molecules. Conversely, oxidation with sulfur and reduction at the C=N bond instigated the production of 3,7-bis(dimethylamino)-4aH-phenothiazine-5-one and 4-(dimethylamino)-2-[*m*-(dimethylamino)-phenylsulfinyl] benzenamine [16]. Furthermore, the MB degradation process involving *Alcaligenes* generated a derivative with smaller BM. This effect was attributed to enzymes secreted to perform total demethylation and C=N bond reduction resulting in the 4,4-diaminodiphenyl sulfide fragment [5]. In addition, other metabolic products emanated from demethylation, deamination, and oxidation procedures, with characteristic fragments of 4-amino-1-benzylpiperidinium dichloride [5]. The Lignin peroxidase (LiP) and horseradish peroxidase (HRP) from *P. chrysosporium* instigated the demethylation of MB and azure B. Based on HPLC analysis, the HRP produced more derivatives (AB, AA, AC) than the LiP, while individual activity depended on H_2O_2 concentration [53].

Moreover, there are limited detailed reports on demethylation reaction mechanisms involving microbes, as most were based only on the modification approach towards the groups present on the metabolic product. Therefore, the biodegradation of dyes with *R. pickettii* has not been substantially evaluated, and the predominant enzymes involved are not currently identified. Today, the enzymes identified in *R. pickettii* involved in the degradation of xenobiotic pollutants are oxygenase groups such as catechol-1,2-dioxygenase and hydroxyquinol-1,2-dioxygenase [24,30].

Furthermore, there have been extensive studies on demethylation, including the oxidative aminopyrine approach, involving various enzyme types, e.g., microsomal cytochrome P-450, HRP, metmyoglobin, and protohemin [54]. The catalytic demethylation on dye

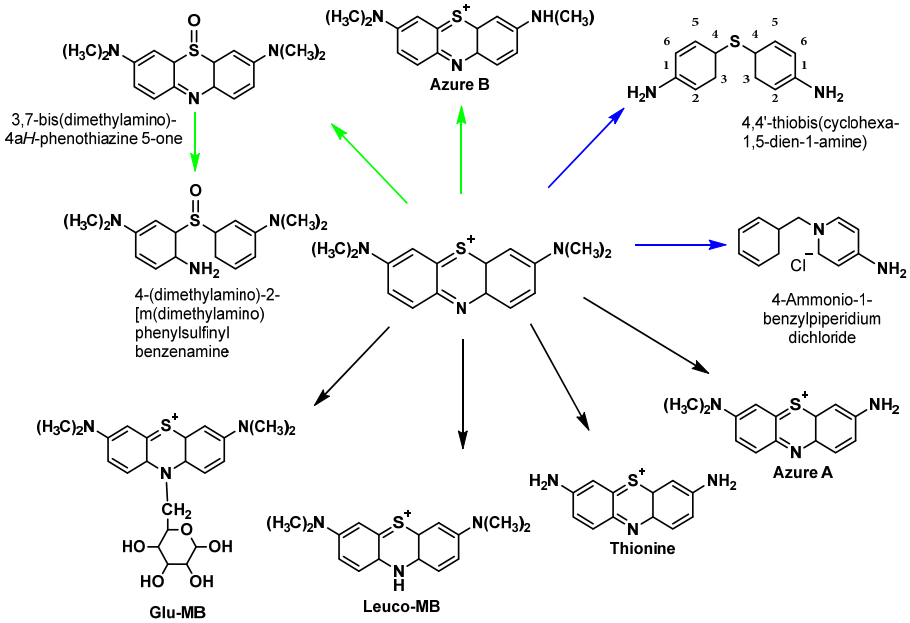

Fig 5. Summary of the proposed methylene blue (MB) transformation pathway by *R. pickettii* (black line; this study), *Alcaligenes* (blue line) [21], *D. dickinsii* (green line) [16]

structure was reported to have occurred during biodegradation through a similar mechanism, although with aminopyrine *N*-demethylase (AND). This pathway was confirmed during the bio-decomposition of Direct Blue-6 by *Pseudomonas desmolyticum* strain NCIM 2112 [55] and methyl red by *Brevibacillus laterosporus* strain MTCC 2298.

In addition, several enzyme types were also involved in the structural biotransformation of methyl red, including lignin peroxidase, laccase, NADH-DCIP reductase, and malachite green reductase [10]. The AND catalytic activity was also detected after the decolorization of Red BLI by *Pseudomonas* sp. SUK1 [56]. Moreover, the demethylation mechanisms exhibited with some dyes involve the activity of various enzymes, encompassing laccase at Brown 3REL [57], lignin peroxidation to convert N,N,N',N',N",N"-hexamethylpararosaniline (crystal violet) into N,N,N',N',N"-penta-, N,N,N',N"-tetramethyl pararosaniline, and N,N',N"-trimethylpararosaniline [58]. The demethylation at crystal violet ensued in the intermediate compounds Michler's Ketone by *Shewanella* sp. strain NTOU1 [59].

Furthermore, MB degradation through microbial mechanisms is similar to the photocatalytic method, based on generated metabolic products. The latter showed decomposition or structural transformation by attacks on the chromophores and auxochrome units. Particularly, *R. pickettii* predominantly decomposed the dye at the auxochrome to yield fragments of demethylation, including azure A and thionine. In addition, similar pathway and metabolic products were reported with *D. dickinsii* [16], and *Bacillus* sp. strain MZS10 [14]. The decomposition through a group of auxochrome with photocatalytic techniques using P2ABSA-modified TiO_2 nanocomposite yielded more organized demethylation products, comprising azure B, A, C, and thionine. However, chemical agents, including manganese oxides, also generate compounds between azure B and A [17]. The chromophore pathway (a conjugation system of N-S on the core aromatic ring) involving attacks by hydroxyl radical, which instigate the opening of a center aromatic ring, is implicated in the production of intermediate compounds. These include 3-((3-(dimethylamino)-cyclo hexa-2,4-dien-1-yl)sulfinyl)-N,N'-dimethyl benzene-1,4-diamine ($C_{16}H_{23}N_3OS$; m/z = 303) [47]. Meanwhile, compounds related to $C_{16}H_{23}N_3OS$ were also recognized during biodegradation using *D. dickinsii* and combined with photocatalytic-biodegradation [11,16].

These biological, chemical, photocatalytic approaches and the combination, initiated with the group of chromophores, facilitate cleavage development at the center aromatic ring of MB and generate monocyclic fragments. Simultaneously, degradation observed on the auxochrome group yields intermediate demethylation products, including azure B, A, and C, and thionine, before advanced decomposition into simpler compounds. Table 2 shows the summary of MB metabolic products after degradation through various means. The intermediate compounds generated, including 3-((3-(dimethylamino)cyclohexa-2,4-dien-1-yl)sulfinyl)-N,N'-dimethyl benzene-1,4-diamine ($C_{16}H_{23}N_3OS$; m/z = 303) were first identified after the sulfhydryl group (–C-S^+=C) was oxidized. An electron rearrangement process characterizes this into C–S(=O)–C, which leads to the opening of a center aromatic ring required before the intermediate compounds are formed. The reaction mechanism involves photocatalysis [47-48], Fenton [60], oxidizing agents [17], and microbes [16], instigated by hydroxyl radical attacks.

Furthermore, advanced degradation is experienced after cleavage at the heterocyclic center to yield a variety of simple fragments, including 4-amino-1-benzylpiperidinium dichloride ($C_{12}H_{18}ClN_2$, m/z = 225.74) and 4,4'-diaminodiphenyl sulfide ($C_{12}H_{12}N_2S$, m/z = 216) [5]. The structure auxochrome of MB are decomposed through oxidative demethylation to produce azure B, A, C, and thionine molecules as intermediates. These specimens are estimated to encounter advanced metabolism for subsequent conversion into simpler compounds. The ability for *R. pickettii* to transform MB on a group of chromophores and auxochrome indicates the potency for microbes to prevent dye wastes. In addition, there are numerous records on the capacity to degrade a variety of xenobiotic wastes due to the genetic complexity used to encode various degradation-related enzymes [24,27-28,30,42].

Table 2. A comparison of different metabolic products for the treatment of MB

Microbial/Material		Metabolic products	m/z	Analytical method	Ref.
D. dickinsii	1	Azure B	270	LC-MS	[16]
	2	3,7-bis(dimethylamino)-4aH-phenothiazin-5-one ($C_{16}H_{19}N_3S$)	300		
	3	4-(dimethylamino)2-[*m*(dimethylamino) phenylsulfinyl] benzenamine ($C_{16}H_21N_3SO$)	303		
Alcaligenes	1	4-amino-1-benzylpiperidinium dichloride ($C_{12}H_{18}ClN_2$)	225.74	GC-MS	[5]
	2	4,4'-diaminodiphenyl sulfide ($C_{12}H_{12}N_2S$)	216.3		
Bacillus sp. *MZS10*	1	Azure B ($C_{15}H_{16}N_3S$)	270	UPLC-MS	[14]
	2	Azure A ($C_{14}H_{14}N_3S$)	256		
R. pickettii	1	Azure A ($C_{14}H_{14}N_3S$)	256	LC-MS	This study
	2	Thionine ($C_{12}H_{10}N_3S$)	228		
	3	Leuco-MB	285		
	4	Glucose-MB ($C_{22}H_{31}N_3SO_5$)	447		
MnOx	1	Azure B	270.1	HPLC-MS	[17]
	2	Azure A	256.1		
	3	7-amino-2,3-dimethylbenzo[b] [1,4]thiazin-1-ium	191.06		
	4	Benzo[b] [1,4] thiazin-1-ium	148.02		
	5	2-methyl-2,3,4,5-tetrahydro-1,4-thiazin-1-ium	116.05		
	6	Benzene-1,4-diamine	108.7		
	7	Aniline	93		
Photocatalysis-Biodegradation	1	3-((3-(dimethylamino) cyclohexa-2,4-dien-1-yl) sulfinyl)-*N*,*N*'-dimethylbenzene-1,4-diamine	303.30	HPLC-MS	[11]
	2	3,7-bis (dimethylamino)-10,10a-dihydro-4aH-phenothiazine 5-oxide	301.14		
	3	2-amino-5-(dimethylamino) benzenesulfonic acid	216.17		
	4	2-aminophenol	109.10		
	5	Aniline	93		
P2ABSA-modified TiO_2 nanocomposite	1	Azure B	270	UHR-TOF-MS	[47]
	2	Azure A	256		
	3	Azure C	242		
	4	Thionine	228		
	5	Phenol	94		
	6	3-((3-(dimethylamino) cyclohexa-2,4-dien-1-yl) sulfinyl)-*N*,*N*'-dimethylbenzene-1,4-diamine	303.30		
	7	2-amino-5-(*N*-methylformamido) benzenesulfonic acid	230		
	8	2-amino-4-hydroxy-5-(methylamino) benzenesulfonic acid	218		
	9	benzenesulfonic acid	158		

■ CONCLUSION

R. pickettii was able to decolorize and degrade the molecular structure of MB. Approximately 98.11% of MB was decolorized after 18 h of incubation. Based on the analysis of metabolic products, *R. pickettii* was revealed to have the ability of MB transformation into Azure A (AA), thionine, leuco-MB, and glucose-MB, which indicated the MB degradation through a reductase that

attacks the heterocyclic central chromophore group present in the structure. Moreover, azure A and thionine fragments resulted from the attacks on the auxochrome group by *N*-demethylase enzyme. This research provides evidence for the potential to use *R. pickettii* in the biodecolorization and biotransformation of dye waste, particularly MB.

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