REVIEW ARTICLE

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Microbial Biotransformation Of Anthraquinone And Biological Activity: A Review

Qamar Uddin Ahmed[1][*], Nursyafiqqah Najwa Binti Amran[1]

ABSTRACT

Anthraquinones are known to have multiple biological activities such as antioxidant, cytotoxicity, antibacterial, antifungal, and antimalarial activities. Despite that, microbial biotransformation of anthraquinones has been extensively carried out to modify or synthesise new anthraquinone derivatives which were technically hard to be chemically synthesised. It is an easy way to discover and synthesise new medicinal compounds having potent pharmacological properties. The synthesis of desired compounds requires less time and less energy. It also promises low cost of production and less environmental impact. Some of the reactions involved were dehalogenation, hydrolysis, oxidation, and glycosylation. The new compounds might possess new biological activities or might exert a better pharmacokinetic profile than the parent compound. However, until recently, there was no *in-vivo* study had been done to evaluate the pharmacodynamics profile of microbial biotransformed anthraquinones. If the *in-vitro* study is successful, we can expect a high probability of a positive result in an *in-vivo* study. Nonetheless, microbial biotransformation is an alternative way to discover new future drugs alongside the synthetic pathway. This review summarises articles related to microbial biotransformation of anthraquinones for the past 20 years.

HOW TO CITE THIS ARTICLE:

*Corresponding author:

Email address: quahmed@iium.edu.my

Authors' Affiliation:

[1] *Department of Pharmaceutical Chemistry, Kulliyyah of Pharmacy, International Islamic University Malaysia, Jalan Sultan Ahmad Shah, 25200 Kuantan, Pahang, Malaysia.*

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Introduction

Microbial biotransformation is a process of utilising microorganisms either bacteria or fungi to transform organic compounds to their consequent products. Microbial biotransformation can be classified into enzymatic and non-enzymatic in nature. The enzymes which carry these microbial biotransformation are present either within the lipophilic membranes of smooth endoplasmic reticulum or in the mitochondria (1,2). Nevertheless, they strive for the same aim which is developing innovative options of metabolites with low waste production and less environmental impact (3,4). Technically, microbial biotransformation is associated with low cost of production (5). With that principle in mind, microbial biotransformation is called as "Green Chemistry" which is one of the modernisation aspects alongside with nanotechnology, microdosing etc. (3,4,6).

Microbial biotransformation is an alternative way to synthesise new medicinal compounds having potent pharmacological properties. The microbes can selectively introduce specific functional groups at certain positions which are relatively difficult to be synthetically synthesised. These resultant compounds may possess more potent or new biological activities, thus exhibit better pharmacological efficacy. The microbes may also remove certain functional groups or degrade the compounds completely to detoxify the compounds. The synthesis of a desired compound requires less time, less cost and less energy, unlike synthetic pathways which involve a long process to add a particular functional group at a particular position. Consequently, synthetic pathways need more time and cost a lot of money.

The study of microbial biotransformation in pharmaceutical research and development has been done vastly to certain naturally occurring secondary metabolites such as steroids (7) and flavonoids (8) to obtain analogs with better pharmacokinetic parameters. The study has emerged even though the discovery and evaluation of biological properties towards a myriad of secondary metabolites are yet to be done (3,9–11). However, there is not much literature recorded on anthraquinone.

Secondary metabolites obtained from the medicinal plants may be insignificant to them for maintaining their life process, but they do have roles in environment adaptation and defence mechanisms (12,13). Unlike primary metabolites such as amino acids, carbohydrates and lipids, the production of secondary metabolites are profoundly influenced by the plant's stage of development and physiological changes. That explains the low production of secondary metabolites in almost every plant (12,14). Despite their low productivity, secondary metabolites have shown their significance and benefit in pharmaceutical areas (15). In fact, some of them have been successfully isolated and marketed as drugs, such as cocaine, morphine and quinine etc. (12).

Anthraquinone (**1**) is a naturally occurring aromatic compound derived from 9,10- dixoanthracene (16). The chemical structure is shown in Figure 1. It is the biggest naturally pigmented group (5,17–19) which can be found in certain plants, insects, fungi and lichens (16), such as *Rubiaceae* sp. (20), *Fusarium* sp. (21) and *Spermacoce latifolia* (22). Its shade varies from yellow to gray-green.
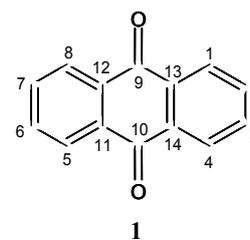

Figure 1: Chemical structure of anthraquinone (**1**).

It has various notable biological benefits and has been studied since years (16). Generally, anthraquinones display remarkable antioxidant (23), cytotoxicity (17,24), antibacterial, antifungal (25), antituberculosis and antimalarial activities (17). The cytotoxicity activity involves the suppression of cancer cell proliferation at DNA level, specifically by inhibiting topoisomerase II activity (24). Anthraquinone viz. xantholipin (**2**) is the precursor for microbial polycyclic xanthone antibiotics (26) . Its chemical structure is shown in Figure 2. Thanks to its xanthone ring nucleus, xantholipin strongly covers Gram-positive bacteria and possesses inhibitory effect on heat-shock protein (HSP47). Thus, it becomes the lead drug for fibrotic diseases (27–30). Aside from that, xantholipin also has potent antitumor, antifungal, and anticoccidial activities (27,31–35).
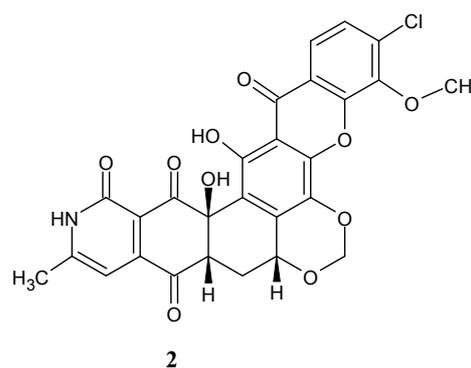

Figure 2: Chemical structure of xantholipin (**2**).

Anthraquinones of *Rhamnus* species possess antimicrobial activities against several pathogenic microbial species such as *Staphylococcus aureus, Pseudomonas aeruginosa, Escherichia coli, Microsporum gypseum, Aspergillus niger* and *Candida albicans* (23). In industrial perspective, the significance of anthraquinone can be seen in the production of synthetic dyes (5,25) and hydrogen peroxide (5,36). Previously, the synthesis of anthraquinone derivatives favoured chemical pathways which required extra budget and included a high number of side-products (5,37). In addition, chemical glycosylation not only offers limited regioselectivity, but also low product stereospecificity (38,39). However, with the arising of microbial biotransformation strategy, all those limitations can be defeated (5,38). This review mainly summarises among the frequent microbial biotransformations done to anthraquinone.

Methodology

Search engines included PubMed, Google Scholar, IIUM Discovery, ScienceDirect and Scopus databases for articles published from 2000 to 2020 using keywords *"Microbial Biotransformation of Anthraquinone"*. Overall, all articles directly and indirectly related to the keywords were considered and included in this review. Articles which were beyond year 2000 were retrieved from the list of references of research and review papers used in this review. Those papers were weighted for their significance and importance in this review and were considered deliberately to be included.

Results

MICROBIAL TRANSFORMATION:

GLUCOSYLATION REACTION

Glucosylation by Absidia coerulea and Mucor spinosus

Glycosylation is a process of adding sugar molecules to the parent compound. It is an effective strategy to modify the pharmacokinetic profile of the metabolites (40) by increasing their hydrophilicity as well as enhancing their bioavailability and stability (38,41,42). Some fungi such as *Alternaria alternata, Mucor spinosus, Mucor lamprosporus, Mucor polymorphosporus, Mucor rouxianus, Aspergilus flavus, Aspergillus niger, Absidia coerulea, Cephalosporium coremioides, Cunninghamella elegans, Cunninghamella blakesleana, Curvularia lunata, Fusarium aveneceum, Gliocladium roseum, Penicillium melinii* and *Syncephalastrum racemosum* were screened for their abilities in transforming the selected anthraquinone molecules into their respective glycosylated metabolites (43). Combine all those, only *Mucor spinosus* and *Absidia coerulea* were successful, with the latter being the most potent fungus in adding sugar molecule to chrysophanol (**3**), physcion (**4**), emodin (**5**) and aloe-

emodin (**6**) (43). The metabolites were chrysophanol 8-*O-ß*-d-glucoside (**7**), physcion-1-*O-ß*-d- glucopyranoside (**8**) and physcion 8-*O-ß*-d-glucoside (**9**), emodin 6-*O-ß*-d-glucoside (**10**) and aloe-emodin 1-*O-ß*-d-glucoside (**11**), respectively (43–45). The yields ranged from 6-14% (43).

Chrysophanol (**3**) with a chemical name of 1,8-dihydroxy-3-methylanthraquinone had shown good antimicrobial and anti-inflammatory activities (46,47). In the presence of *Absidia coerulea* or *Mucor spinosus*, chrysophanol was glucosylated into chrysophanol 8-*O- ß*-d-glucoside (**7**). The optimum condition for *Absidia coerulea* was 150 rpm and 27 °C, whilst for *Mucor spinosus* was 150 rpm and 28 °C. The reaction was completed in 48 h for *Absidia coerulea* and 5 days for *Mucor spinosus*, respectively. The reaction is illustrated in Figure 3. The yields for *Absidia coerulea* and *Mucor spinosus* were 10% and 9%, respectively (43, 44). Both parent compound and metabolite were tested for *in vitro* platelet aggregation induced by collagen and thrombin. Based on the IC_{50} values, the resultant compound was regarded as more potent antiplatelet and anticoagulant agent than the parent compound (48). The IC_{50} values are shown in *Table 1*.

The chemical name of physcion (**4**) is 1,8-dihydroxy-3-methoxy-6-methyl-9,10- anthraquinone. Within 5 days, *Mucor spinosus* could biotransform physcion into physcion-1-*O-ß*-d-glucopyranoside (**8**), while *Absidia coerulea* could biotransform physcion into physcion 8-*O-ß*-d-glucoside (**9**) (44). The reaction is illustrated in Figure 4. The yield for *Absidia coerulea* was 6% (43). Physcion was known to have antibacterial and antifungal activity (49,50). However, no biological   activity for the above two metabolites of physcion viz., **8** and **9** has so far been evaluated.

Another example is emodin (**5**). It has chemical name of 1,6,8-trihydroxy-3- methylanthraquinone. Within 48 h, emodin was biotransformed into emodin 6-*O-ß*-d-glucoside (**10**) in the presence of *Absidia coerulea*. The optimum condition was 150 rpm and 27 °C (44). The reaction is shown in Figure 5. The yield was 14% (43). Emodin was known to have potent anticancer activity (44,51). It could impede human cytomegalovirus development (52,53). Besides, it had potential for the treatment of metabolic disorder as well as type 2 diabetes mellitus due to its potent 11β-HSD1 selective inhibition (54). Similar as physcion's metabolites, there is no biological activity of emodin 6-*O-ß*-d-glucoside has ever been evaluated until now.

Aloe-emodin (**6**) is 1,8-dihydroxy-3-(hydroxymethyl)anthraquinone. It had strong stimulant-laxative action (43,44). In the presence *Absidia coerulea*, aloe-emodin was biotransformed to aloe-emodin 1-*O-ß*-d-
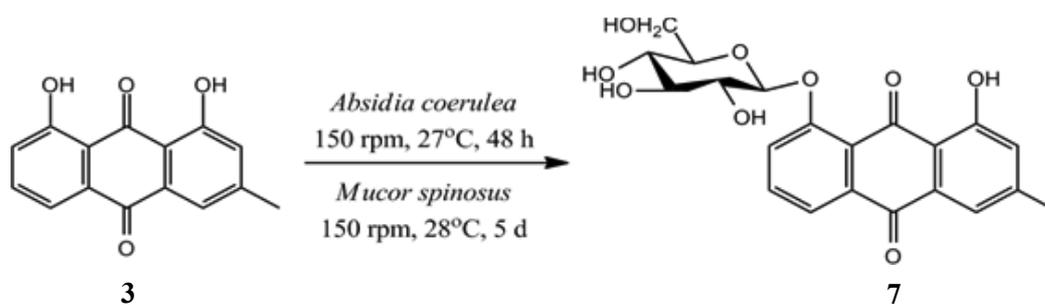
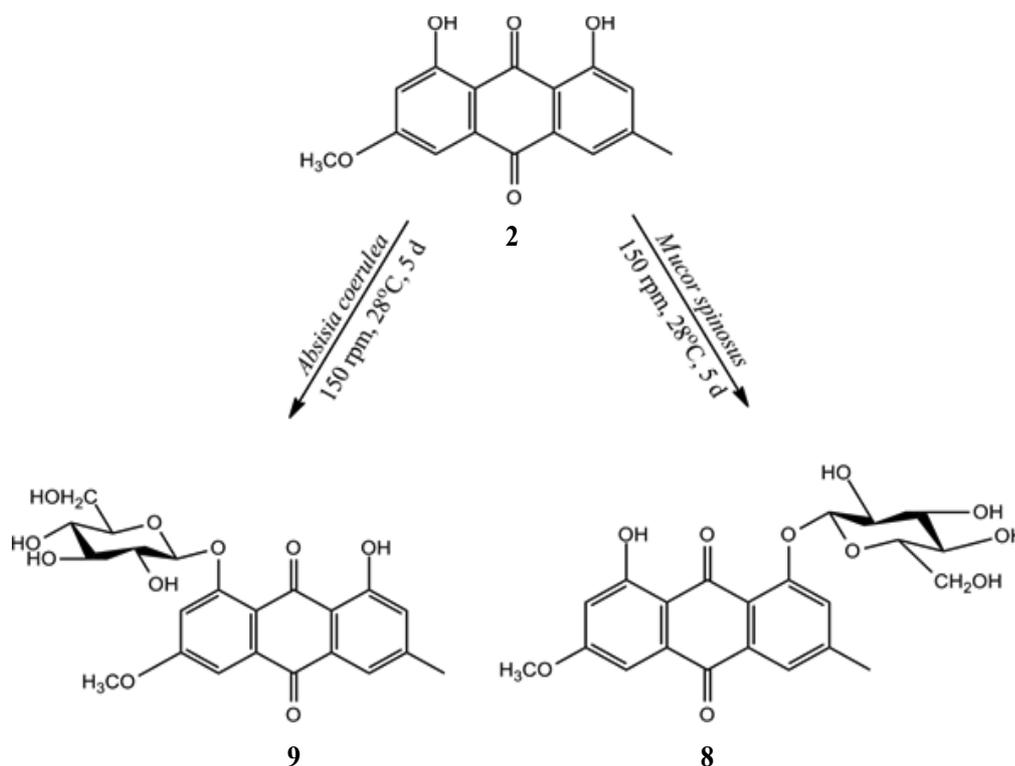Figure 3: Glucosylation of chrysophanol **(3)** into chrysophanol 8-O-ß-d-glucoside **(7)**.

Figure 4: Glusoylation of physcion (**2**) into physcion-1-O-β-d-glucopyranoside (**8**) and physcion 8-O-ß-d-glucoside (**9**).
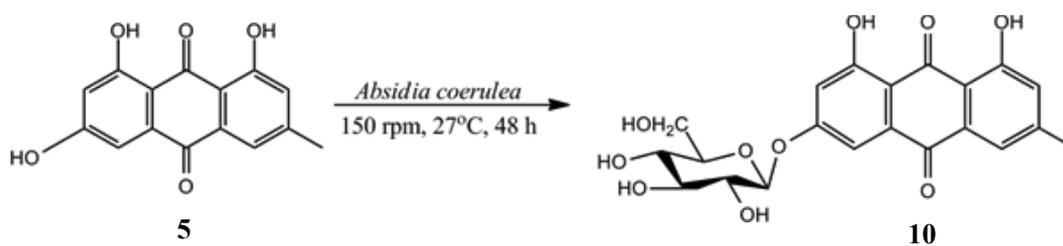

Figure 5: Glucosylation of emodin (**5**) into emodin 6-O-ß-d-glucoside (**10**).

Table 1: IC50 values of chrysophanol and chrysophanol 8-O-ß-d-glucoside (7) on in vitro platelet aggregation induced by collagen and thrombin (48).

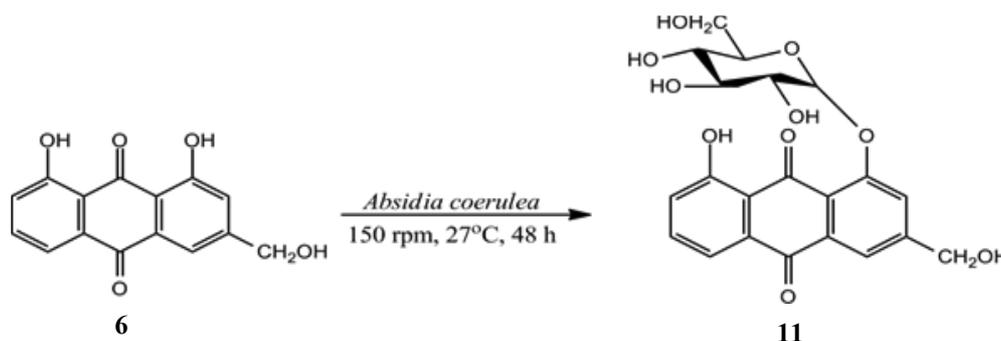| Compounds	Chrysophanol (3)	Chrysophanol-8-*O*-*ß*-d-glucoside (7)
IC$_{50}$ on *in vitro* platelet aggregation induced by collagen (μM)	>100	33.4 ± 0.2
IC$_{50}$ on *in vitro* platelet aggregation induced by thrombin (μM)	>100	25.9 ± 0.2

Figure 6: Glucosylation of aloe-emodin (**6**) into aloe-emodin 1-O-ß-d-glucoside (**11**).

glucoside (**11**) (44). The reaction is illustrated in Figure 6. The yield was 8% (43). The biological activity of the glycosidic aloe-emodin is still not known and should be considered in the future research.

Glucoyslation by Beauveria bassiana

Another successful fungus in transforming anthraquinone by means of glucosylation was *Beauveria bassiana* ATCC 7159 (55,56). Biotransforming ability of *Beauveria bassiana* was not limited to glucosylation, but it might also perform hydroxylation, oxidation and methylation (38,57–65). *Beauveria bassiana* initially opted for hydroxylation prior to glucosylation (66). The reaction is shown in Figure 7. The C-2 entity of 1-aminoanthraquinone (**12**) underwent hydroxylation prior to glycosylation, forming a metabolite called 1-amino-2-(4'-*O*-methy*l-2β-O*-d-glucopyranosyloxy)anthraquinone (**13**). The reaction took 7 days under optimum conditions of 150 rpm and 25 °C, with a remarkable yield of 72%. The metabolite was a good anti-inflammatory and antioxidant agent (67).

Besides that, *Beauveria bassiana* had the ability to selectively choose 2-amino group of 1,2-diaminoanthraquinone (**14**) over 1-amino group to be glucosylated (55,66). The biotransformed compound is called 1-amino-2-(4′-*O*-methyl-2*β*-*N*-d-glucopyranosylamino) anthraquinone (**15**). The reaction took 7 days under optimum conditions of 150 rpm and 25

°C, with a yield of 67% (44). The reaction is illustrated in Figure 8. 1,2-diaminoanthraquinone is an additive and a sensor of nitric oxide (NO) (68). However, none of the glycosylated 1,2-diaminoanthraquinone bioactivity has so far been evaluated. Both examples showed the preference of *Beauveria bassiana* for C-2 *N*- glycosylation of aminoanthraquinone instead of C-1 *N*-glycosylation, presumably due to steric hindrance at the latter position. If C-2 was absent of heteroatom substitution, the hydroxylation process would have preceded (55,56).

Meanwhile, biotransformation of hydroxyanthraquinone such as 1,8-dihydroxyanthraquinone (chrysazin) (**16**) and 1,2-dihydroxyanthraquinone (alizarin) (**17**) by *Beauveria bassiana* produced 8-hydroxy-1-(4′-*O*-methyl-1*β*-*O*-d-glucopyranosyloxy) anthraquinone (**18**) and 1-hydroxy-2-(4′-*O*-methyl-2*β*-*O*-d-glucopyranosyloxy) anthraquinone (**19**), respectively within 7 days. The former yielded 46% while the latter yielded 63%. This *O*-glucosylation process preferred C-2 over C-1, similarly as aminoanthraquinone previously explained. This concept was valid if both C-1 and C-2 carried hydroxyl groups. Again, this may be due to steric hindrance factor at C-1 hydroxyl group (55,56). The optimum conditions for both of the anthraquinone derivatives were 150 rpm and 25 °C (44). The reactions are shown in Figures 9 and 10.

Chrysazin was known for its carcinogenicity (47) while alizarin showed a good antioxidant activity (69). In addition to that, alizarin had good cytotoxicity profile

against some cancer cells such as osteosarcoma and breast carcinoma cells (70). It also had immunosuppressant activity (71). However, none of the biotransform glycosidic compounds of these anthraquinones have been evaluated for their pharmacological potential.

Another example was rhein (**20**), with a chemical formula of 1,8-dihydroxy-3-carboxyl anthraquinone. In the presence of *Beauveria bassiana*, hydroxylation at C-7 occurred prior to glycosylation at the same position, producing a compound called 7-*O*-(42-methoxy-*β*-d-glucopyranoside) aloe-emodin (**21**). The reaction is shown in Figure 11. The parent compound had been identified as a good antifeedant, anticancer and antifungal agent (45,72–74). However, the biological activity of the resultant glycosylated rhein has not yet been evaluated.
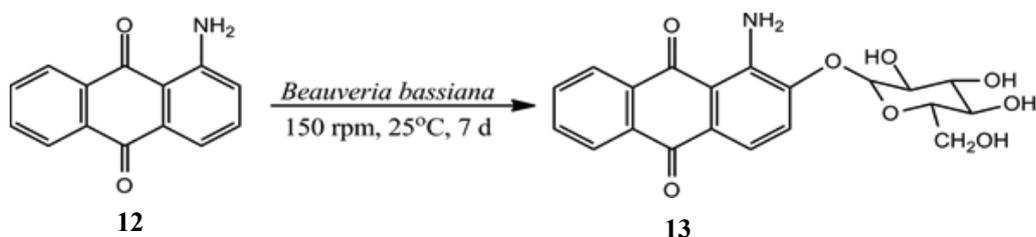

Figure 7: Glucosylation of 1-aminoanthraquinone (**12**) into 1-amino-2-(4'-O-methyl-2β-O-d-glucopyranosyloxy) anthraquinone (**13**).
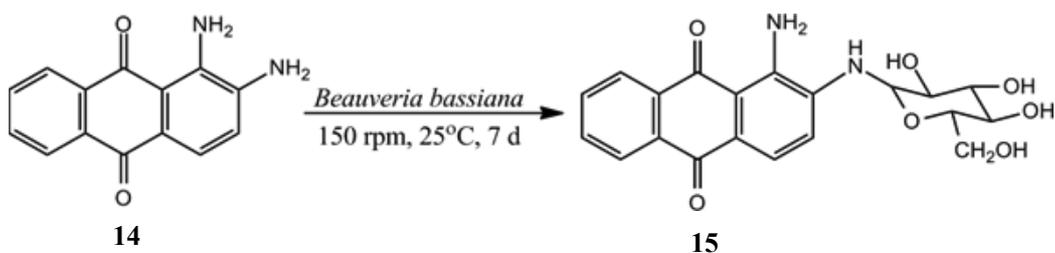

Figure 8: Glucosylation of 1,2-diaminoanthraquinone (**14**) into 1-amino-2-(4′-O-methyl-2β-N-d- glucopyranosylamino) anthraquinone (**15**).

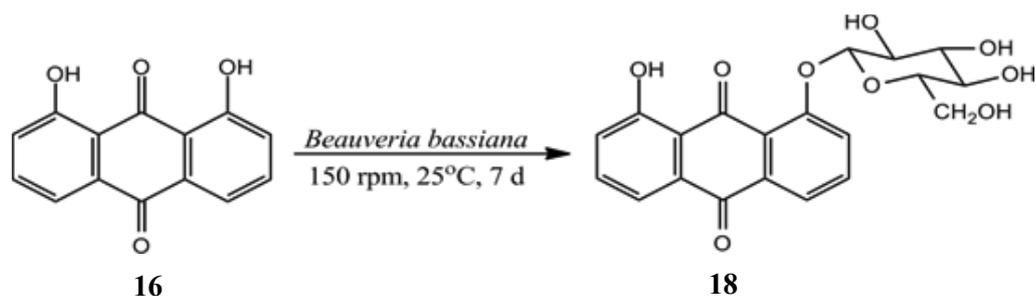

Figure 9: Glucosylation of chrysazin (**16**) into 8-hydroxy-1-(4′-O-methyl-1β-O-d-glucopyranosyloxy) anthraquinone (**18**).
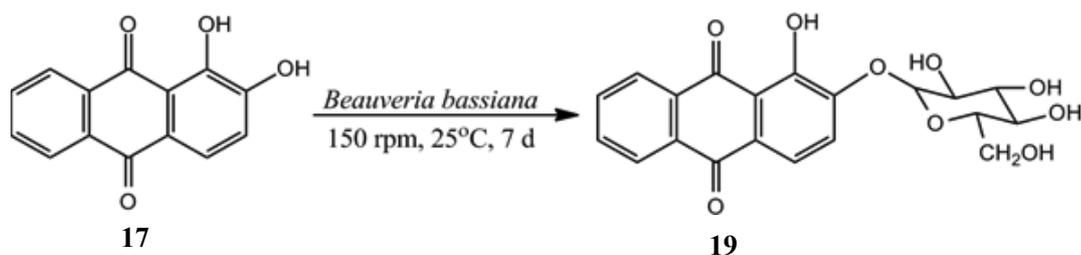
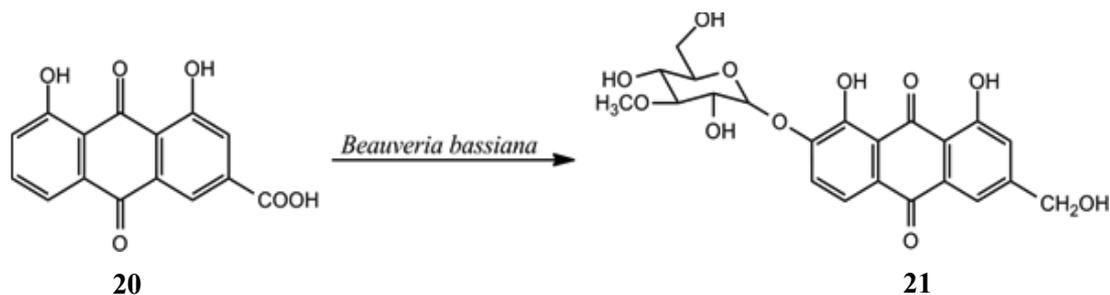Figure 10: Glucosylation of alizarin (**17**) into 1-hydroxy-2-(4′-O-methyl-2β-O-d-glucopyranosyloxy) anthraquinone (**19**).

Figure 11: Glycosylation of rhein (**20**) into 7-O-(42-methoxy-β-d-glucopyranoside) aloe-emodin (**21**).

A recent study had successfully identified a pair of gene encoded glycosyltransferase- methyltransferase (GT-MT) enzymes in *Beauveria bassiana*. GT enzymes could regio- and stereospecifically yield *O*- and *N*-glucosides. Consequently, the resulting glucosides would undergo methylation by MT enzyme to afford 4-*O*-methylglucosides. A simple example could be seen in the methyl glucosylation of 2-hydroxyanthraquinone (**22**) and aminoanthraquinone. The former would introduce sugar moiety to the OH or NH_2 group of hydroxyanthraquinone and aminoanthraquinone to produce 2-hydroxyanthraquinone glycoside (**23**) or aminoanthraquinone glycoside, whilst the letter would introduce a methyl group to the OH group of the sugar moiety of the resultant glycoside of 2-hydroxyanthraquinone or aminoanthraquinone to produce the methylglucosylated 2-hydroxyanthraquinone (**24**) or methyl glucosylated aminoanthraquinone. The methyl glucosylated compounds were proved to exhibit higher water solubility and better abstinence against glycoside hydrolysis (38). The proposed biotransformation of **22** is illustrated in Figure 12.

Glucosylation by Bacillus licheniformis

Another recent study used uridine diphosphate (UDP)-glucose donated by indigenous *E. coli* BL21 (DE3) strain. Glycosyltransferases (YjiC) from *Bacillus licheniformis* DSM13 were then expressed into the *E. coli* to glucosylate three different anthraquinone derivatives, viz., anthraflavic acid (**25**), 2-amino-3-hydroxyanthraquinone (**26**) and alizarin (**27**). Generally, in the *E. coli* strain, glucose was transformed to glucose-6-phosphate by hexokinase enzyme. Then, phosphoglucomutase enzyme transformed glucose-6-phosphate to glucose-1-phosphate. By the action of glucose 6-phosphate uridyltransferase, glucose-1-phosphate was converted to UDP-α-D-glucose. Then, YjiC helped to transfer UDP-α-D- glucose into the anthraquinone derivatives, resulting in their respective glucosylated compounds, viz., anthraflavic acid 2-*O*-glucoside (**28**), 2-amino-3-*O*-glucosyl- anthraquinone (**29**) and alizarin 2-*O*-glucoside (**30**). The reactions for 23, 24 and 25 are illustrated in Figures 13, 14 and 15, respectively. With the optimum glucose supplement of 4%, the production yield (85%) was achieved within 48 h. Since the anthraquinone derivatives were known to suppress the proliferation of cancer cell, the aglycones and glucosylated metabolites were tested for their cytotoxic activities on the growth of gastric carcinoma (AGS), cervical carcinoma (Hela) and hepatocarcinoma (HepG2) cell lines (5). The percentage of cancer cell growth is tabulated in *Table 2*.

Anthraflavic acid (**25**) was biotransformed to anthraflavic acid 2-*O*-glucoside (**28**) (see Figure 13). The resultant glycone of anthraflavic acid displayed comparable inhibitory activity as its aglycone for all three cancer cell lines. The second anthraquinone derivative was

2-amino-3-hydroxyanthraquinone (**26**) which upon glycosylation produced 2-amino-3-*O*-glucosyl-anthraquinone (**29**) (Figure 14). The inhibitory effect of the resultant glycoside towards the proliferation of AGS was comparable to its parent compound. However, both of them showed slightly similar cytotoxic activities against HeLa and HepG2 cells growth. The last derivative was alizarin 2-*O*-glucoside (**30**) which showed the highest inhibitory effect than alizarin (**27**), anthraflavic acid 2-*O*-glucoside and 2-amino-3-*O*-glucosyl-anthraquinone against all three-cancer cells growth. Since it possessed the greatest cytotoxic activities, chemical structure of alizarin-*O*-glucoside was further elucidated by using 1D and 2D NMR spectral analyses which characterized it to be alizarin 2-*O*-*β*-D-glucoside (see Figure 15). From these results, it could be concluded that alizarin 2-*O*-glucoside exhibited positive inhibitory effect than other metabolites (5).
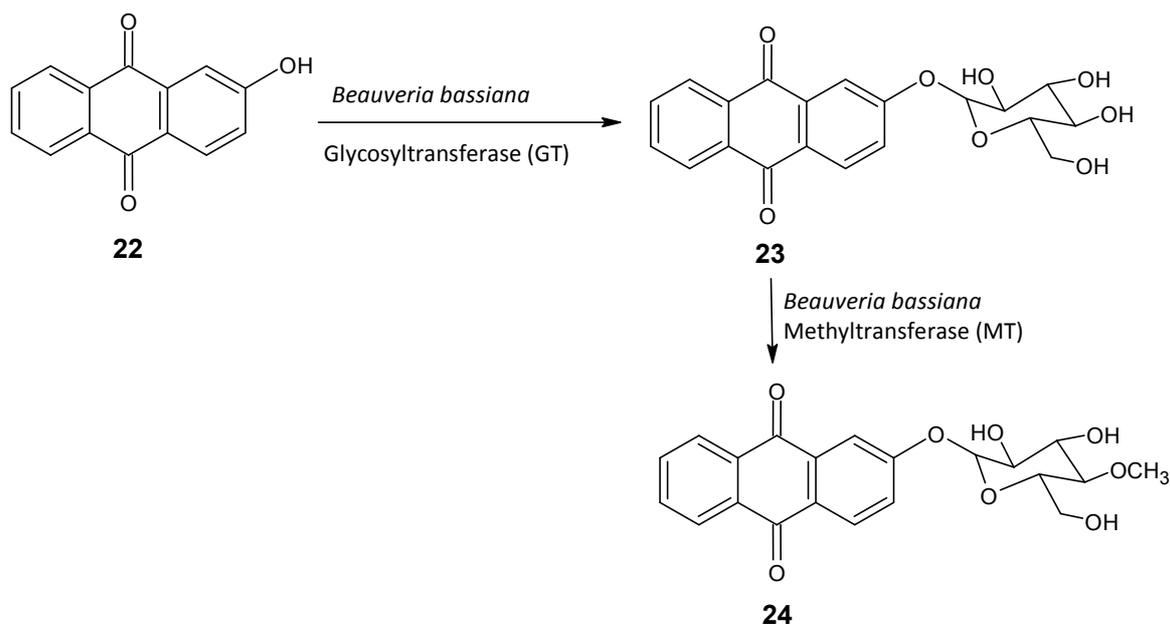

Figure 12: The proposed action of GT-MT enzyme by Beauveria bassiana towards the formation of 2-hydroxyanthraquinone glycoside (**23**) and methylglucosylated 2-hydroxyanthraquinone (**24**).
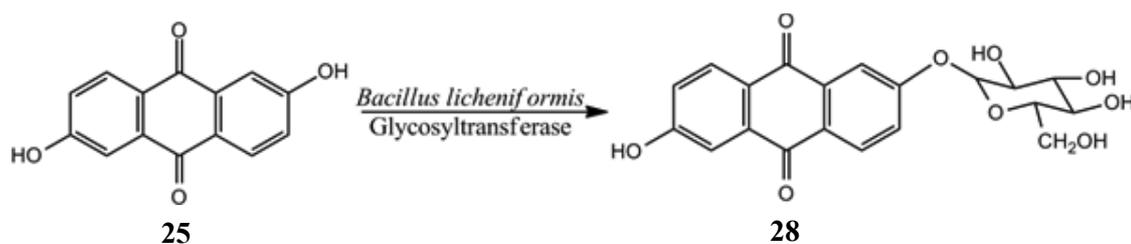

Figure 13: Glucosylation of anthraflavic acid (**25**) into anthraflavic acid 2-O-glucoside (**28**).

Table 2 : The percentage of cancer cell growth after being subjected to 100 µM anthraquinone derivatives and their metabolites.

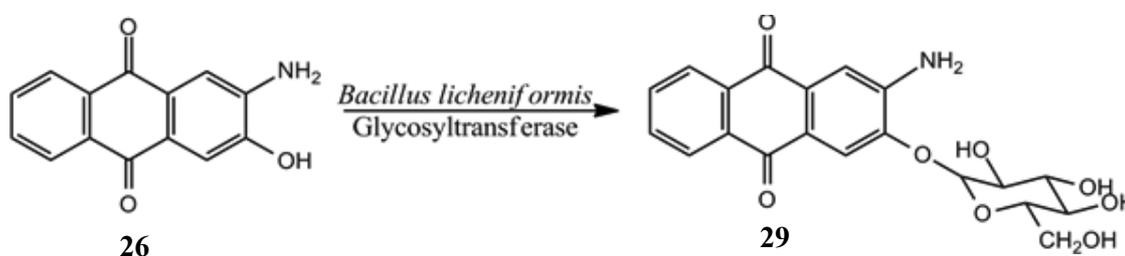| Compounds	AGS (cell growth %)	HeLa (cell growth %)	HepG2 (cell growth %)
Anthra flavic acid (**25**)	~20%	>60%	~60%
Anthraflavic acid 2- *O*-glucoside (**28**)	>20%	~40%	>30%
2-amino-3-hydroxyant hraquinone (**26**)	>40%	~30%	>40%
2-amino-3-*O*- glucosyl- anthraquinone (**29**)	~20%	>20%	>40%
Alizarin (**27**)	~60%	~90%	>60%
Alizarin 2-*O*-glucoside (**30**)	<10%	~10%	~10%

Figure 14: Glucosylation of 2-amino-3-hydroxyanthraquinone (**26**) into 2-amino-3-O-glucosyl- anthraquinone (**29**).
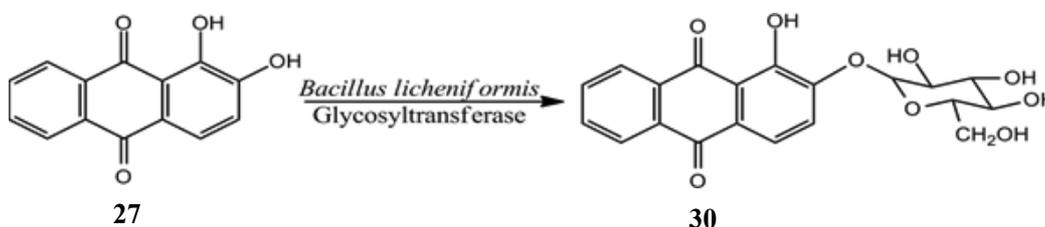

Figure 15: Glucosylation of alizarin (**27**) into alizarin 2-O-glucoside (**30**).

MICROBIAL TRANSFORMATION:

BIODEGRADATION

Anthraquinone-based dyes are known to have bulky aromatic chemical structure which makes them more recalcitrant and obstinate in the environment (75,76). Nevertheless, they are inexpensive, have good hydrophilicity and are varied in colours (77–79). Since most of them possess toxicity including phytotoxicity, genotoxicity and cytotoxicity, (75,77,80) and have been reported to cause environmental pollution (16). Hence, a microbial biodegradation process was performed to detoxify the anthraquinone-based dyes. The dyes were treated with microbes in the wastewater before it was released into the environment. Biodegradation was among the top choice of treatment due to its low cost, environmental friendly as well as its general applicability (77), unlike physico-chemical treatment which was inefficient, had high limitations, needed high operational cost and created secondary pollution (81–83). Biodegradation is one of the examples to prove that microbial biotransformation is not only done to add a new functional group, but it could also help to remove the

existence functional group in the parent compound.

Oxidative biodegradation

1,4-dihydroxyanthraquinone also known as Pigment Violet 12 (**31**) is one of the dyes which is widely used in the industry. It was treated with *Coriolus versicolor* IFO30388 to reduce its toxicity towards the environment. *C. versicolor* has several oxygenase enzymes such as ligninase, peroxidase, Mn peroxidase and lactase which are considered to be responsible for the oxidation process (84–86). Pigment Violet 12 upon oxidation formed lactone intermediates (**32, 33**) which then underwent hydrolytic cleavage at carbon-oxygen bond to form phthalic acid (**34**) which was subsequently degraded via decarboxylation to form benzoic acid (**35**) (86). Thus, the toxicity of Pigment Violet 12 was scaled down and it was safe to release into the environment. The reaction is shown in Figure 16.

Another example was shown by *Bjerkandera fumosa* 137 and *Pleurotus ostreatus* D1. Both of them belong to ligninolytic fungi which could produce versatile peroxidase enzymes (87,88). Versatile peroxidase from *Bjerkandera fumosa* 137 (VPBF) and versatile peroxidase from *Pleurotus ostreatus* D1 (VPPO) were able to catalyse the degradation process of anthraquinone (**1**) by mean of breaking the aromatic ring structure or by polymerisation (87,89), thus decolourising the compound. The

decolourisation process happened very rapidly (87). The process involved was oxidation of the aromatic compound directly by the peroxidase enzyme or aided by chelation with Mn^{3+}. In the polymerisation reaction, Mn^{2+} was ready to donate an electron to become Mn^{3+}, while hydrogen peroxide (H_2O_2) acted as the electron acceptor. Then, Mn^{3+} chelated with the compound and polymerised. Indirectly, precipitate was formed (87,90). The reaction is simplified in Figure 17. The activity of VPBF was stimulated by the addition of Mn^{2+} in the form of $MnCl_2$ or $MnSO_4$, with the latter reported to exhibit greater affinity. Contrary to VPBF, VPPO's catalytic activity was barely dependent to Mn^{2+}, but significantly dependent on the presence of H_2O_2 (87,91,92). Nonetheless, generally, VPPO was said to be more effective in catalysing direct decolourisation.

The first example was Alizarin Red S (AR, **36**). It possessed two hydroxyl groups at C-1 and C-2, and sodium monosulphonate derivative at C-3. After being subjected to VPBF or VPPO, it underwent direct oxidation and broke down its aromatic ring. Therefore, its red colour decolourised completely with the absence of precipitate formation. As compared to Basic Blue 22 (BB22, **37**), Acid Blue 62 (AB62, **38**) and Reactive Blue 4 (RB4, **39**), the substituents at the aromatic ring of AR was the shortest, enabling it to accessibly move towards the enzymes (87). The chemical structures of all four anthraquinone dyes are shown in Figure 18.
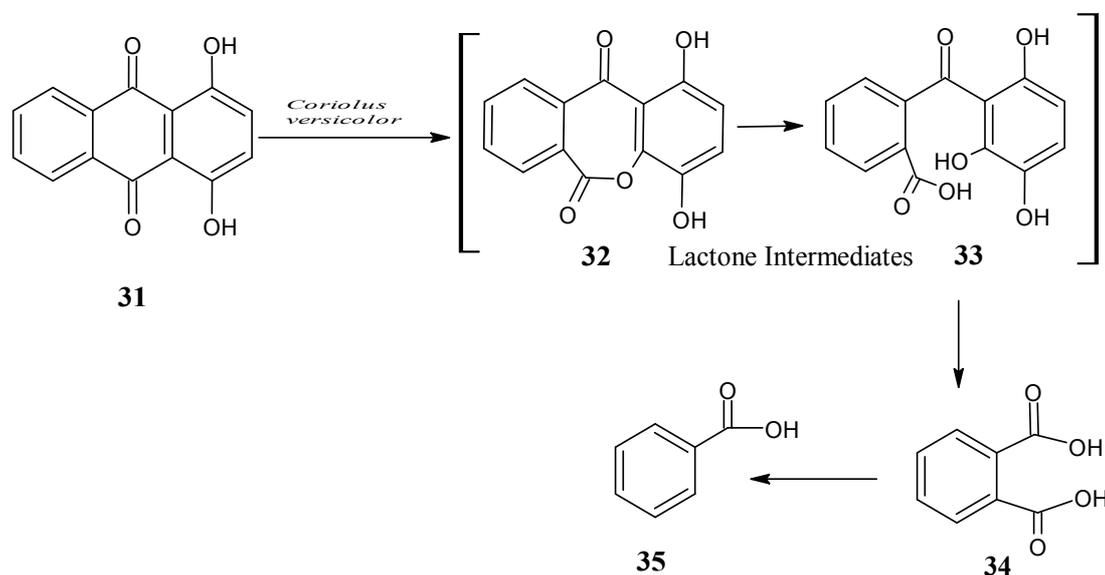

Figure 16: The biodegradation pathway of Pigment Violet 12 (**31**) in the presence of Coriolus versicolor IFO30388.
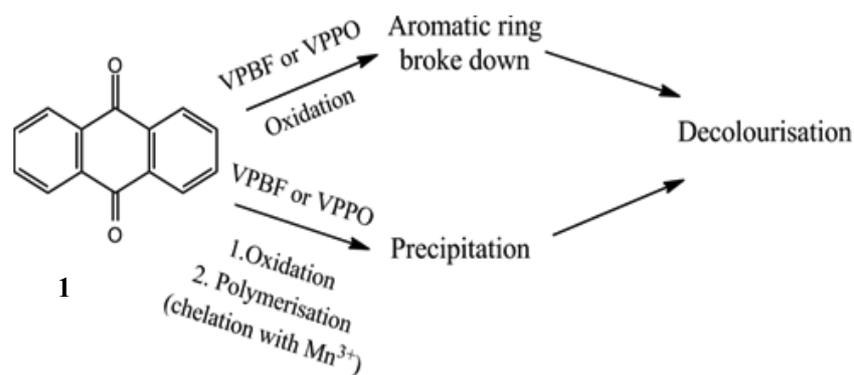
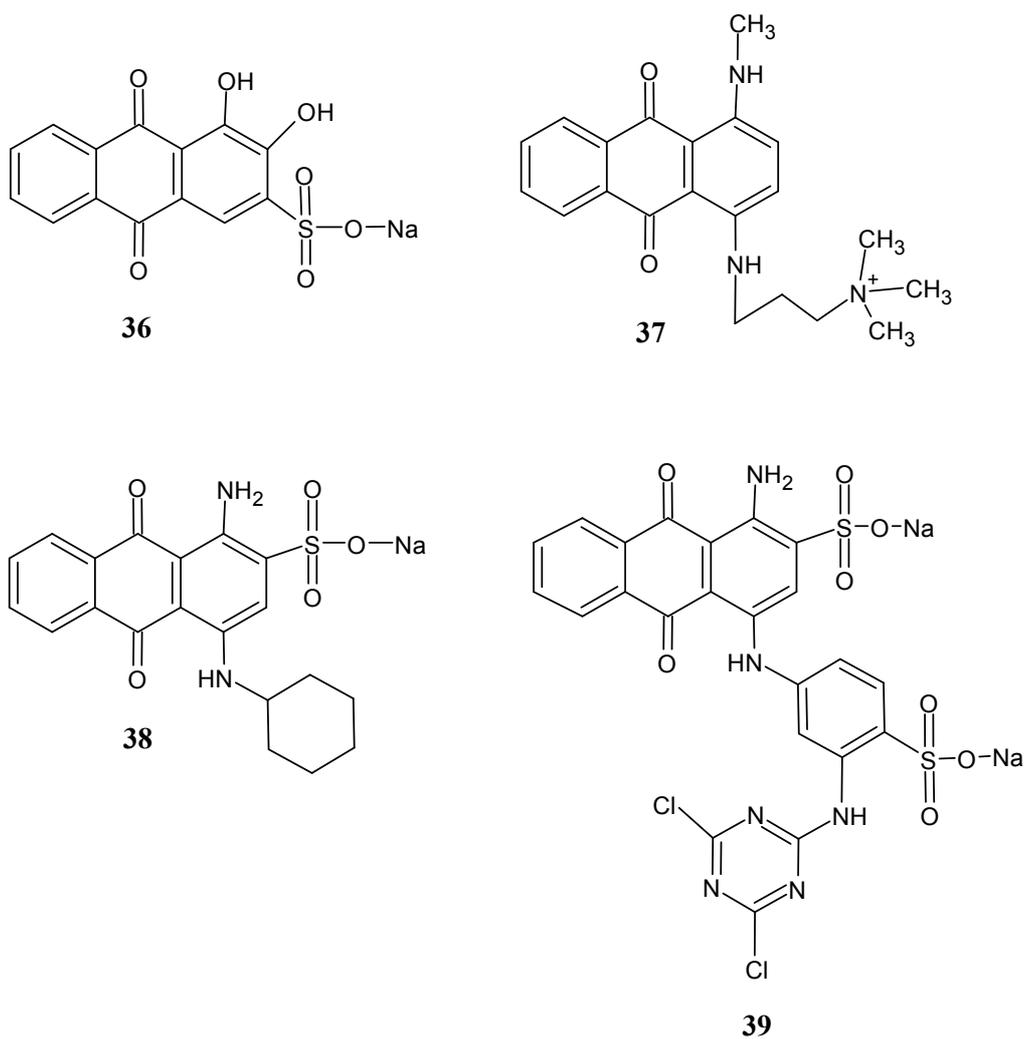Figure 17 : The simplified biodegradation pathway of anthraquinone dye (**1**) by VPBF and VPPO.

Figure 18 : The chemical structures of Alizarin Red S (AR, **36**), Basic Blue 22 (BB22, **37**), Acid Blue 62 (AB62, **38**) and Reactive Blue 4 (RB4, **39**).

Table 3 : The percentage of anthraquinone dyes degradation for several types of microbes.

Type of anthraquinone dye	Microbes	Anthraquinone dyes degradation (%)
RBBR (40)	*Bacillus* sp. NWODO-3	72.12
	Bacillus sp. MABINYA-1	70.45
	Bacillus sp. MABINYA-2	4.91
	Bacillus sp. FALADE-1	1.19
	Lysinibacillus sphaericus JD1103	50.00
CBB (41)	*Funalia trogii* ATCC 200800	98.00
	Streptomyces griseosporeus SN9	81.00

BB22 has 2 N-containing substituents at C-1 and C-4 where the latter is containing a long chain group. Upon interaction with VPBF or VPPO, its dark blue colour was partially changed to red- brown, concomitantly with the precipitation process. Unlike AR, BB22 favoured polymerisation reaction, instead of aromatic rings breakdown (87,93). AB62 has N- substituent with cyclohexane at C-1, sulphonic acid at C-3 and amine group at C-4. Similar with BB22, its dark blue colour turned to red-brown and precipitate was formed upon reaction with VPBF or VPPO, indicating polymerisation reaction had taken place (87,93). On the other hand, RB4 possesses amine substituent at C-1, sodium monosulphonate substituent at C-2, and large and complex substituent at C-4. Similar to BB22 and AB62, its dark blue colour was also partially changed to red-brown and formed precipitate after interacting with VPBF or VPPO. This had occurred due to a polymerisation reaction (87,93).

Aside from that, the *Bacillus* species which belonged to ligninolytic bacteria was also capable in degrading anthraquinone dye. *Bacillus* sp. NWODO-3 and *Bacillus* sp. MABINYA-1 were more potent in biodegrading Remazol Brilliant Blue R (RBBR, 40) than *Bacillus* sp. MABINYA-2 and *Bacillus* sp. FALADE-1, amounting 72.12±0.38%, 70.45±0.0%, 4.91±0.36% and 1.19±0.0%, respectively. The degradation of RBBR was performed by peroxidase enzymes produced by the bacteria through enzymatic oxidation process (81). Another report showed the biodegradation capacity of ligninolytic *Serratia* sp. which was able to produce oxidative enzymes such as laccase and lignin peroxidase. *Serratia liquefaciens* sp. PT01 degraded RBBR more efficiently than *Serratia* sp. JHT01 (94). Expression of laccase and lignin peroxidase could also be seen in ligninolytic *Lysinibacillus sphaericus* JD1103. In aerobic conditions, it could degrade 50.00±0.10% RBBR within 72 h, indicated by the intensity of colour changes (95). A comparable study had reported the action of lignin peroxidase produced by *Streptomyces griseosporeus* SN9 on RBBR and Cibacet Brilliant Blue BG (CBB, 41). In 4 min of reaction, 47% of CBB had already been degraded. Its percentage increased to 69% after 1 h and 81% after 48 h, whilst RBBR was 18% and 55%, respectively (96). When reacted with *Funalia trogii* ATCC 200800, about 92-98 % of RBBR was biodegraded after 10 h, optimally in less glucose concentration (97). The results are tabulated in Table 3.

Biodegradation through dehalogenation

In another study, RB4 (39) was subjected to aerobic bacterial granules (ABGs) to be biodegraded. According to Chaudhari *et al*. due to its recalcitrance and obstinacy, degradation of anthraquinone dyes was most appropriately performed by aerobic bacterial granules. Upon degradation, 11 different phyla of aerobic bacteria were obtained and detected which were *Clostridia, Actinobacteria, Proteobacteria, Firmicutes, Bacteroides, Candidate division* TM7*, Chloroflexi, Thermi, Acidobacteria, Spirochaetes, Gemmatimotnadetes* and *Tenericutes*. Among all of them, *Proteobacteria* was the predominant phyla in degrading RB4 with the relative abundance of 68.07%. *Proteobacteria* had tolerance characteristics as well as potential to degrade RB4, indicated by the notable increase of *α-, β-, ε-* and *γ-proteobacterial* communities in the suspended culture during the biodegradation process (75). Using of ABGs to degrade anthraquinone dyes was the most appropriate choice from the economic and technical advantages

perspectives (75,98). The change of colour of RB4 was the indicator whether the reaction was successful or not.

The degradation process involved dehalogenation of **39** at C-4 to 4-amino-9,10- dihydro-9,10-dioxoanthracene-2-sulphonic acid (**42**) and 2-(4,6-dichloro-1,3,5-triazin-2-ylamino)- 4-aminophenol (**43**). 4-amino-9,10-dihydro-9,10-dioxoanthracene-2-sulfonic acid was further degraded to 1-aminoanthracene-9,10-dione (**44**) before mineralisation process took place. Meanwhile, 2-(4,6-dichloro-1,3,5-triazin-2-ylamino)-4-aminophenol directly underwent mineralisation process (75). The mineralisation process might have involved degradation pathways of naphthalene, benzoate and amino-benzoate (75,99). The reaction is illustrated in Figure 19. The optimum environment for RB4 degradation was in static condition (75,100-101), pH 3.0-11.0 and temperature of 20-60 °C (75). Maximum decolourisation of RB4 could be seen at static condition rather than in shaking condition (101). At pH 3.0-11.0, about 52-72% of RB4 was decolourised, while 48 to 72 % of RB4 was decolourised at 20-60 °C (75). However, it was said that the maximum decolourisation happened at 55 °C (75,102).

The degradation process was meant to detoxify the anthraquinone dyes since these dyes were known to be phytotoxic, cytotoxic and genotoxic in nature (75). Some literature revealed that the toxicity of anthraquinone dyes towards human cells and microbes were worse than azo dyes (75,103-104). The phytotoxicity included 76% reduction of chlorophyll-a which was considered a considerable amount that might have affected the biosynthesis process of *Triticum* sp. (75,105-106). Nonetheless, non-phototoxicity characteristics were observed in the metabolites (75). In terms of cytotoxicity, RB4 could reduce 46.7% of HaCat (human keratinocyte) and 55.0% of FHM (fish epithelial: connective tissue and muscle) cell viabilities. However, the metabolites showed 96% HaCat and 100% FHM cell lines survival. For genotoxicity, a remarkable RB4-induced DNA damage in lymphocytes, HaCat and FHM were reported. Fortunately, the metabolites showed significant reduction in genotoxicity. Thus, it can be concluded that the metabolites were free from having any phytotoxic, cytotoxic and genotoxic properties (75).
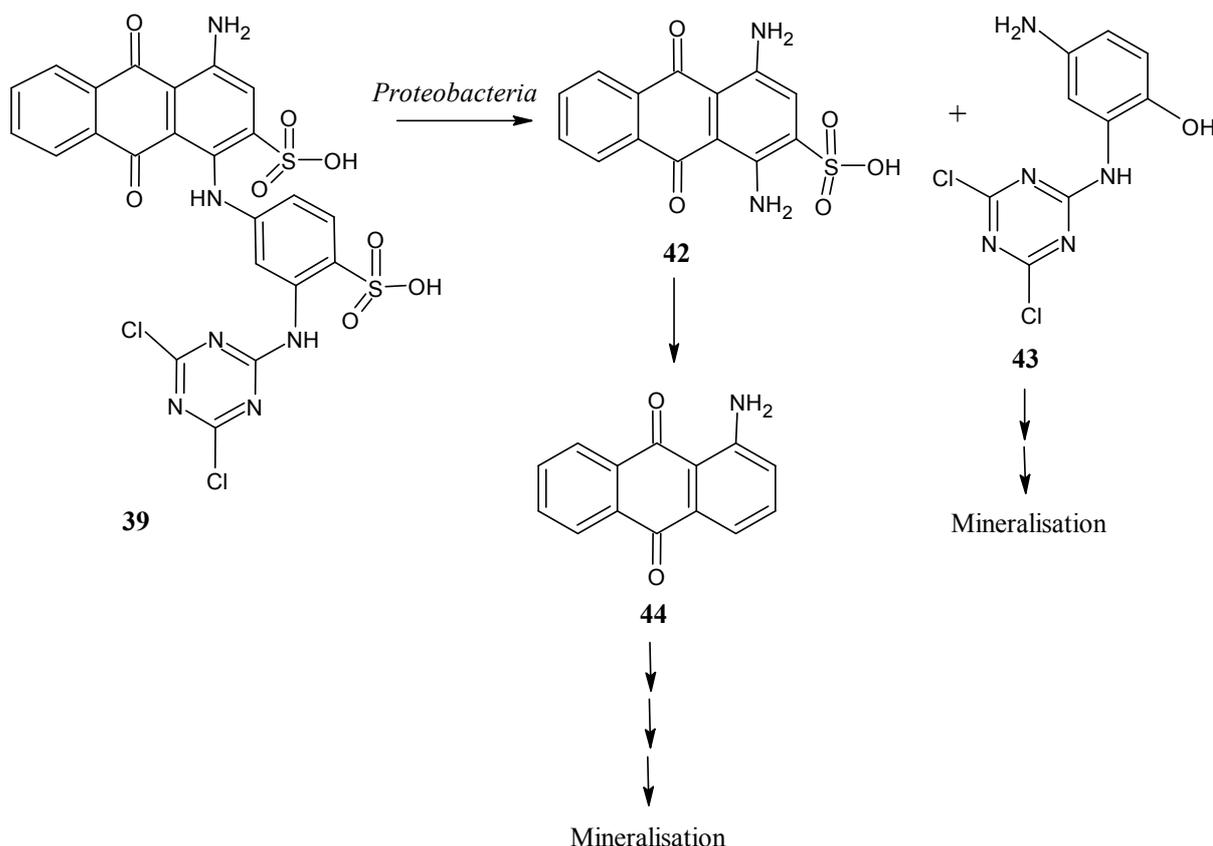

Figure 19: The biodegradation of RB4 (**39**) by Proteobacteria.

Biodegradation through hydrolysis

Another study was conducted to biodegrade RBBR (**40**), which is another example of anthraquinone dyes (77). Similar to RB4 (**75**) and other toxic anthraquinone dyes, RBBR also possesses phytotoxicity properties (77,80). Enzymatic reduction or enzymatic hydrolysis was a crucial step in removing the chromogenic groups from the anthraquinone backbone (77,107). *Bacteroidetes* (Gram-negative bacteria) and *Firmicutes* (mostly Gram-positive bacteria) were the prominent and dominant species in

degrading RBBR by hydrolysis reaction. The products formed consisted of formamide (**45**), dibutyl phthalate (DBP) (**46**), 2-ethylhexyl hydrogen phthalate (**47**) and diisobutyl phthalate (DIBP) (**48**). The parent compound and its resultant biotransformed products are shown in Figure 20. However, it was said that the phthalate compounds; DBP, 2-ethylhexyl hydrogen phthalate and DIBP were endocrine disruptors. They could seriously threaten human health and be quite toxic to aquatic organisms. The effect could occur even at low concentration since they were water-soluble and stable in aqueous environment (77,108).
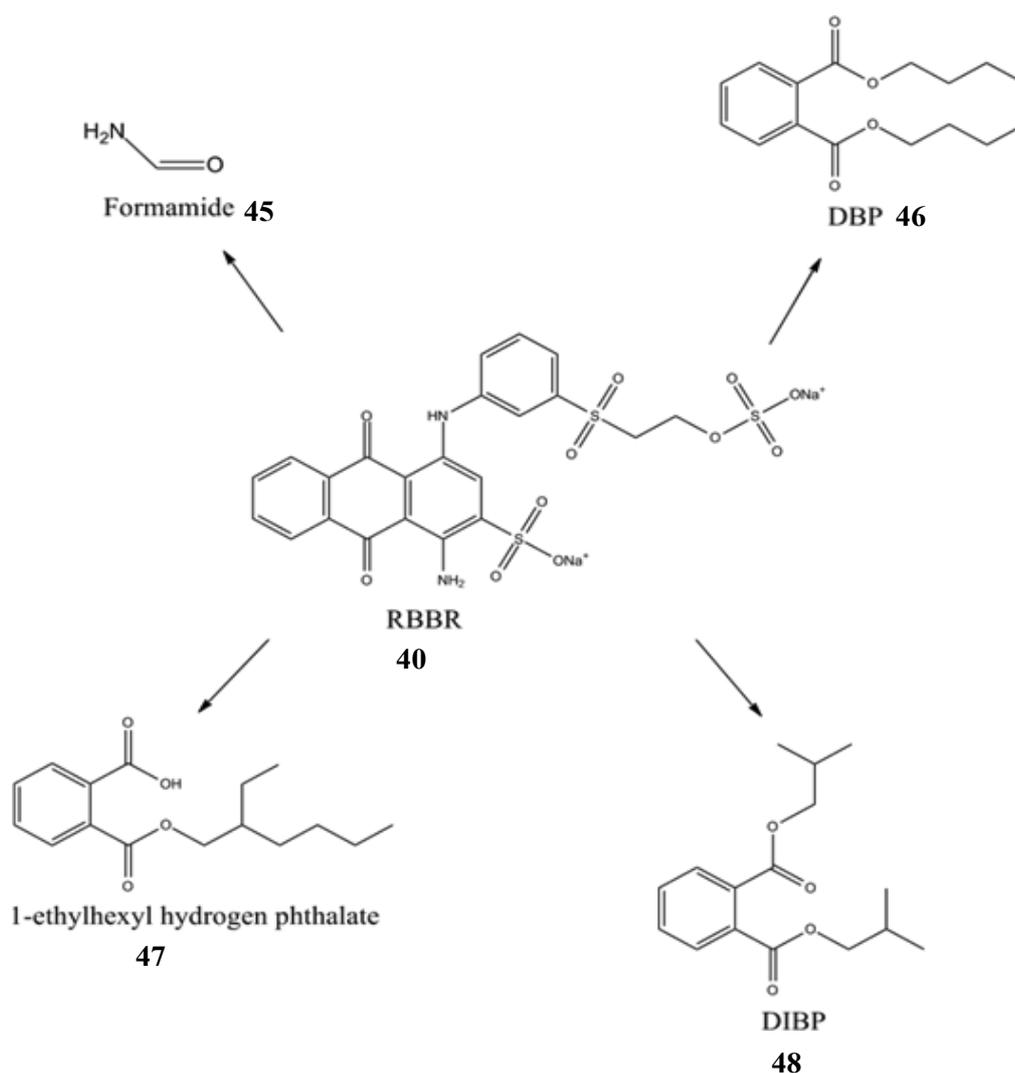

Figure 20: The biodegradation of RBBR (**40**) by Bacteroidetes and Firmicutes to formamide (**45**), dibutyl phthalate (DBP) (**46**), 2-ethylhexyl hydrogen phthalate (**47**) and diisobutyl phthalate (DIBP) (**48**).

Conclusion

In conclusion, microbial biotransformation of anthraquinone may involve some reactions such as dehalogenation, hydrolysis, oxidation and glycosylation. Upon biotrasformation, some products showed better or new biological activities than the parent compounds. The instance can be seen in chrysophanol and chrysophanol-8-*O*-glucoside, where chrysophanol had shown good antimicrobial and anti-inflammatory activities, while its glycosylated product showed good antiplatelet and anticoagulant activities. However, not all studies did the evaluation on the biological activities of the biotransformed compounds. Therefore, future researchers may further evaluate new biological activities of the biotransformed compounds and proceed in conducting *in-vivo* study to evaluate their pharmacodynamics profile. Moreover, microbial biodegradation process has also been successfully applied to detoxify the anthraquinone-based dyes possessing phytotoxicity, genotoxicity and cytotoxicity deleterious effects. Biodegradation has proved that microbial biotransformation is not only done to add a new functional group to enhance desirable bioactivity, but it could also help to remove the existence functional group in the parent compound that may be responsible for the manifestation of any toxicity. Even though microbial glycosylated compounds had been shown to demonstrate better pharmacokinetic profile in *in-vitro* studies than the parent compounds, generalisation of results through *in-vivo* studies has not been done properly. Hence, we may expect a high probability of getting positive results in an *in- vivo* study if an *in-vitro* study was successful. Thus, the biotransformed products of biological active anthraquinones may provide lead for the discovery of future safe drugs to treat various chronic ailments.

Acknowledgements

Conflict of Interest

There are no conflicts of interest.

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