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Decolorization of Remazol Brilliant Violet 5R and Procion Red MX-5B by Trichoderma Species

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ABSTRACT: Industrial wastewater including dye waste disposal, has been released in a massive amount and is difficult to degrade, especially synthetic dyes. In this study, 10 different types of fungi were isolated from a decayed wood in UTM forest and were labelled as S1-S10. Two dyes were chosen for this study, which were Procion Red MX-5B (PRMX5B) and Remazol Brilliant Violet 5R (RBV5R). These fungi were screened for their ability to decolor both dyes and further tested for their ability to decolor the dyes in liquid medium under several parameters; carbon and nitrogen sources, initial pH value, temperature, and agitation. S1 decolorized PRMX5B efficiently with the addition of glucose (45%), ammonium nitrate (61%), pH 3 (69%), temperature 37°C (49%), and agitation 100 rpm (69%), whereas S2 decolorized efficiently with the addition of glucose (60%), ammonium nitrate (49%), pH 3 (70%), temperature 37°C (46%), and agitation 100 rpm (74%). S1 demonstrated efficient decolorization of RBV5R with the addition of glucose (80%), ammonium nitrate (62%), pH 3, temperature 37°C (75%), and agitation 100 rpm (90%), whereas S2 demonstrated efficient decolorization with the addition of glucose (52%), ammonium nitrate (67%), pH 3, temperature 37°C (75%), and agitation 100 rpm (71%).The percentage of decolorization of dyes was measured by using a UV-Vis spectrophotometer. These fungi were then identified using the 18sr RNA method. Based on macroscopic and microscopic characteristics and a polygenetic tree, fungi S1 belong to *Trichoderma koningiopsis* and fungi S2 belong to *Trichoderma atroviride*.

KEYWORDS: Decolorization; filamentous fungi; *Trichoderma koningiopsis*; *Trichoderma atroviride*

1. Introduction

Dye is a natural or synthetic substance used to change or add any colour to something. This is widely used around the world, with over 10,000 different dyes used in industry and over 7 x 105 tonnes of synthetic dyes produced annually [1]. Moreover, the consumption of synthetic dyes keeps increasing proportionally to the product demand. Thus, the untreated dye waste that is discharged into the rivers, lakes, or ponds will lead to water pollution. This is because the presence of very small amounts of dyes, such as at a low concentration of less than 1mg/l, is

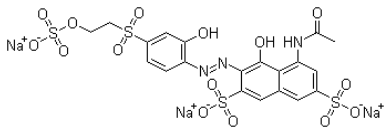
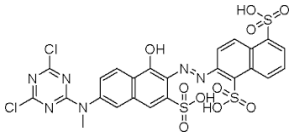
highly visible and immediately affects the ecosystem [1,2]. There are several ways to decolorize dyes that include physical, biological, and chemical processes such as membrane filtration and absorption, and electrocoagulation. Adsorption is a well-known equilibrium separation process and an effective method for water decontamination applications. Although the removal of dye using chemical methods can give positive results, the accumulation of concentrated sludge creates a disposal problem. Another responsibility problem occurs. Compared to biological methods, this method was the lowest-cost alternative. This is because it is commonly applied to treat industrial waste due to its ability to accumulate and degrade different pollutants [3,4]. The most recognisable contaminant in a waste stream, except all the rubbish is colour. This is due to the fact that even with a low concentration of synthetic dyes in water (1 ppm), they are highly visible and thus have an impact on the environment.It prevents the aesthetic quality of surface water from being affected and affects the aquatic ecosystem because light penetration is reduced. This will reduce the photosynthesis process among the aquatic life because of the less light penetration. Thus, it is very important to create an effective treatment to overcome and reduce the colour in effluent wastes [2,4]. The objectives of this study are to screen, isolate, and identify fungi screened from nature that have the ability to decolorize RBV5R and PRMX5B, and to investigate the effect of different parameters on the decolorization process.

2. Materials and Methods

2.1.Chemicals and Dyes

RBV5R and PRMX5B were purchased from Sigma-Aldrich Co., Ltd. (USA) at the highest purity available. Table 1 shows their chemical structure and their properties. Merck (Darmstadt, Germany) purchased malt extract agar and glucose. All the other chemicals were purchased from Qrec Co. Ltd (New Zealand).

Table 1. Properties of RBV5R and PRMX5B

Properties	RBV5R	PRMX5B
Structure		
Another name	Reactive Violet 5 (RBV 5R)	Reactive Red 2
Molecular formula	$C_{20}H_{16}N_3Na_3O_{15}S_4$	$C_{19}H_{10}Cl_2N_6Na_2O_7S_2$
Molecular weight	735.59 gmol-1	615.34 gmol-1
Melting point	>360 °C	>300°C
Function	Usually used for cotton, silk and linen dyeing and printing, also suitable for discharge printing	Mainly used for cotton, wool, silk, also can be used to knot dyeing. Its storage stability is poor

2.2.Microorganisms and Growth Conditions

Ten different types of fungi were collected from the areas of Universiti Teknologi Malaysia, Johor Bahru, Malaysia. These fungi were stored in the refrigerator to preserve their growth. The selected fungi were cultured in malt extract agar and incubated for 7 days. Two agar plugs were cut from the fungus mycelium that grows in agar medium and placed into a conical flask

to study the decolorization of dye. The flasks were incubated for 4, 8 and 12 days, and the decolorization was monitored using a UV/VIS Spectrophotometer. All experiments were performed in duplicate to obtain a valid result. Agar powder (2 g) and yeast extract (2 g) were weighed and added to 40mL of distilled water. The solution was then sterilized together with the petri dish and another apparatus for 60 minutes. After that, chloramphenicol (3 mg) was added into the solution [5]. The sterilized petri dish was placed in laminar flow and 20 mL of malt extract solution was poured into each petri dish under fluorescent light. Then, the selected fungi are taken using a cork borer before being placed on solidified agar medium. Then the petri dish was sealed with parafilm [5].

2.3.Batch studies

Glucose (0.8 g) and yeast (0.8 g) were added to 40 mL of distilled water in the conical flask before being autoclaved for 60 minutes. After that, chloramphenicol (12 mg) was added and the selected fungi were cut from fully grown ME agar medium before being added into conical flasks. The mouths of the conical flask were covered with aluminium foil. Various parameters were conducted in this study on the decolorization of dye, such as carbon sources (glucose, fructose and galactose), nitrogen sources (yeast extract, ammonium nitrate and ammonium sulphate), pH (3,4,5 and 6), temperature (20°C, 27°C and 37°C) and agitation (100 rpm). The decolorization was monitored using a Nanocolor UV/VIS Spectrophotometer at 4, 8, and 12 days. The liquid culture was filtrated and centrifuged at 4000rpm for 30 minutes. After that, the supernatant was collected for further tests. Decolorization was monitored by scanning the absorbance between 400nm and 800nm using a Nanocolor UV/VIS Spectrophotometer. The maximum absorption of RBV5R was recorded at 558.1nm while PRMX5B was at 533.1nm. The decolorization percentage of RBV5R and PRMX5B was calculated as follows:

$$\text{Decolourization (\%)} = \frac{C_0 - C_f}{C_0} \times 100\%$$

Where C_0 is the absorbance of the dye as the initial experiment and C_f is the absorbance of the dye after decolorization.

3. Results and Discussion

3.1.Identification of fungi

Table 1. Decolorization of test dye on solid medium by fungi screened from nature.

Species	Decolorization (%)
S1	98.5
S2	97.5
S3	ND
S4	ND
S5	17.5
S6	12.6
S7	16.2
S8	10.5
S9	56.5
S10	63.2

Ten different types of fungi were collected from the areas of UTM and labelled as S1-S10. Two samples were selected among these 10 different fungi based on their ability to decolorize

PRMX5B and RBV5R (Table 1). Two samples (S1 and S2) were sent to 1st Base for identification and phylogenetic trees for both selected fungi were obtained. The Basic Local Alignment Search Tool (BLAST) was used to determine the similarity between the two. Figure 1 and Figure 2 show the mycelia and polygenetic tree of an isolated fungi strain.

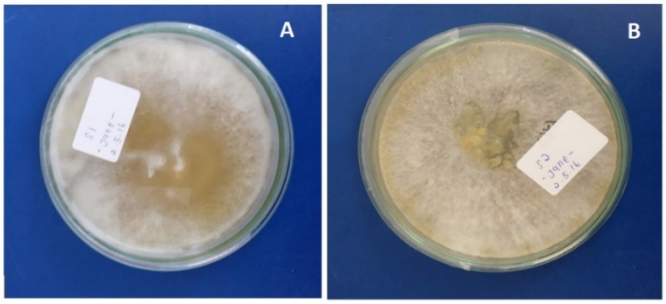


Figure 1. Mycelia of *Trichoderma koningiopsis* (A); *Trichoderma atroviride* (B)

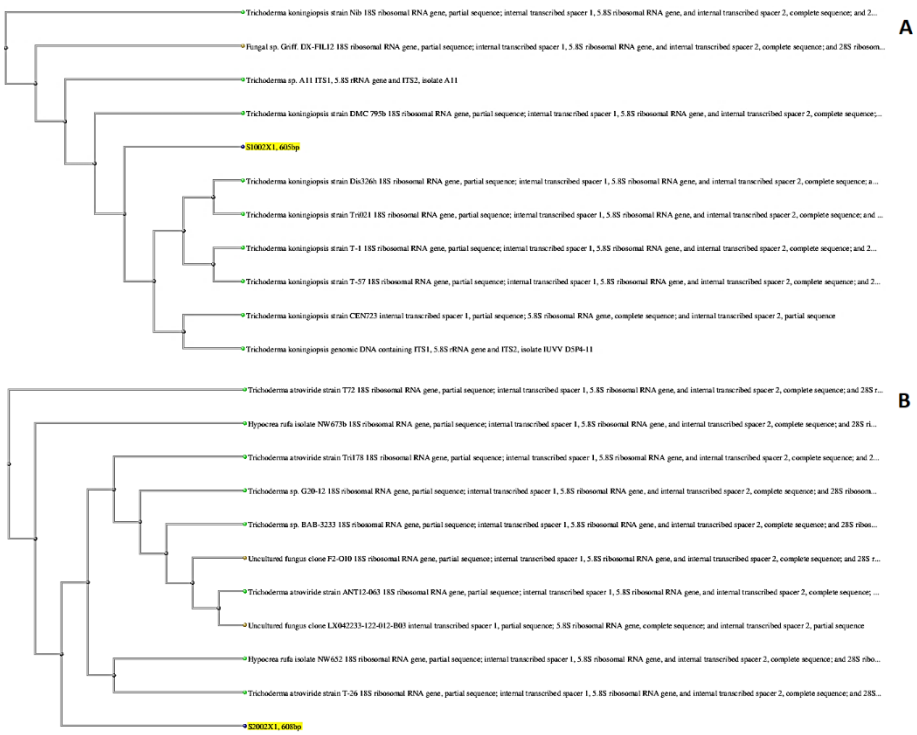


Figure 2. Phylogenetic tree of isolated fungi strain: *Trichoderma koningiopsis* (A); *Trichoderma atroviride* (B).

3.2.Batch studies

Figure 3A and 3B show *Trichoderma koningiopsis* and *Trichoderma atroviride* have the highest percentage of PRMX5B decolorization, which are 45% and 60% with glucose as a carbon source after 12 days of incubation. Other carbon sources such as fructose and galactose also show an increased decolorization within the incubation days. It can be observed that with fructose acting as another carbon source, *Trichoderma koningiopsis* and *Trichoderma atroviride* decolor the dye up to 32% and 46%, while using galactose, both *Trichoderma koningiopsis* and *Trichoderma atroviride* decolor the dye up to 42%. While for Remazol Brilliant Violet 5R, Figure 3C shows *Trichoderma koningiopsis* decolor dye using glucose, fructose, and galactose up to 80%, 78%, and 75% respectively within 12 days of incubation.

Figure 3D shows *Trichoderma atroviride* also has the highest percentage of decolorization with glucose as its carbon source, which is 52%, 40% (fructose) and 46% (galactose) after 12 days of incubation. Trichoderma koningiopsis and Trichoderma atroviride took glucose as a carbon source as well as nutrients for their growth. This is because glucose has a simpler structure that makes it easier to degrade and most readily usable by fungi. In addition, glucose gives high laccase activity [6,7].

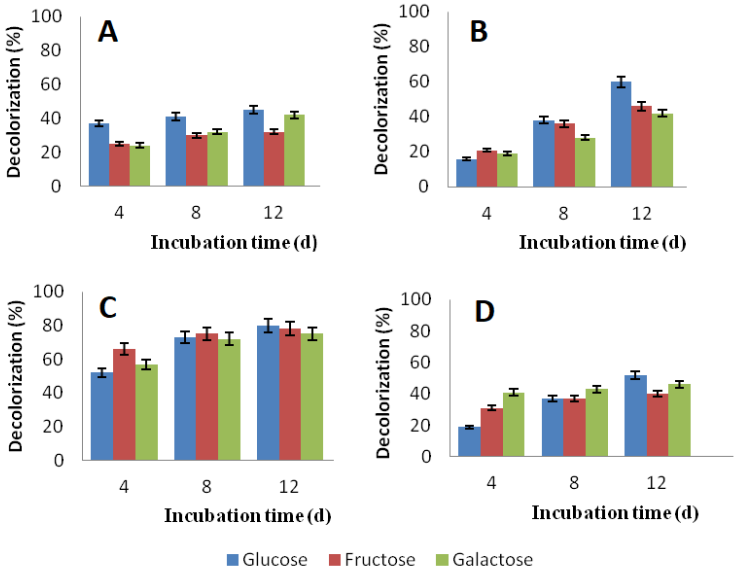


Figure 3. Effect of carbon source in decolorization of PRMX5B by *Trichoderma koningiopsis* (A), and *Trichoderma atroviride* (B); and RBV 5R by *Trichoderma koningiopsis* (C) and *Trichoderma atroviride* (D).

Two different species of fungi, *Trichoderma koningiopsis* and *Trichoderma atroviride,* were chosen to investigate their ability to decolor PRMX5B and RBV5R dye using three nitrogen sources such as yeast extract, ammonium nitrate and ammonium sulphate for 12 days of incubation. Figure 4 shows the percentage of decolorization of PRMX5B and RBV5R dye by *Trichoderma koningiopsis* and *Trichoderma atroviride*. For PRMX5B dye, *Trichoderma koningiopsis* and *Trichoderma atroviride* have the highest percentage of decolorization, which are 40% and 45% with yeast extract as a nitrogen source after 12 days of incubation. Other nitrogen sources such as ammonium nitrate and ammonium sulphate also show an increased decolorization within the incubation days given. It can be observed that with ammonium nitrate acting as another nitrogen source, *Trichoderma koningiopsis* and *Trichoderma atroviride* decolor the dye up to 61% and 49%, while using ammonium sulphate, both *Trichoderma koningiopsis* and *Trichoderma atroviride* decolor the dye up to 46% and 38%. While *Trichoderma koningiopsis* decolorized dye using yeast extract, ammonium nitrate and ammonium sulphate up to 60%, 62%, and 51% respectively within 12 days of incubation, *Trichoderma atroviride* also has the highest percentage of decolorization with yeast extract as their nitrogen source, which are 56%, 67% (ammonium nitrate) and 58.6% (ammonium sulphate) after 12 days of incubation. From the data obtained, it shows that ammonium nitrate has the highest percentage of decolorization for nitrogen source for *Trichoderma koningiopsis* and *Trichoderma atroviride* between both dyes compared to yeast extract and ammonium sulphate. This is because forest soils contain ammonium and nitrate, and it's assumed that ammonium is the major form of inorganic nitrogen that is taken up by fungi and other soil

microorganisms [8]. Both these fungi have the ability to decolor the dyes and use ammonium nitrate as a nutrient because it was collected in the natural environment.

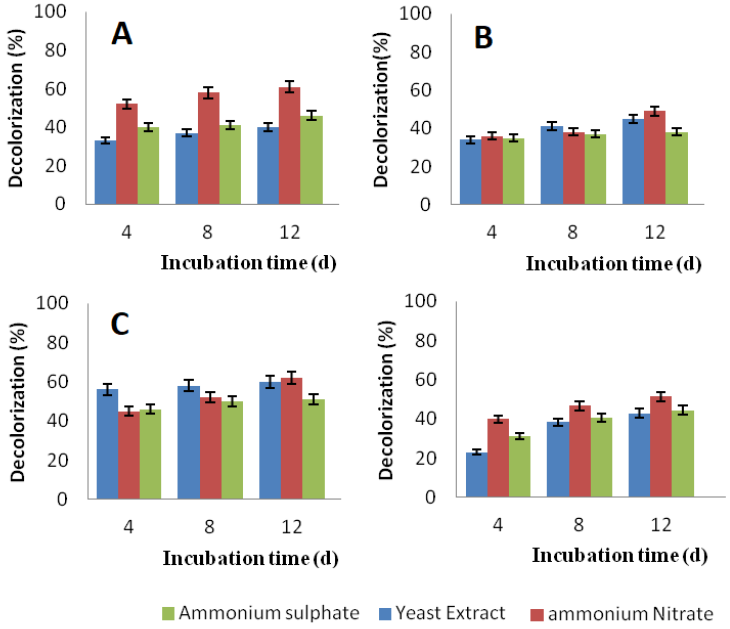


Figure 4. Effect of nitrogen source in decolorization of PRMX5B by *Trichoderma koningiopsis* (A) and *Trichoderma atroviride* (B); and RBV5R by *Trichoderma koningiopsis* (C) and *Trichoderma atroviride* (D).

Figure 5A and 5B show the percentage of decolorization of PRMX5B by *Trichoderma koningiopsis* and *Trichoderma atroviride* at different values of pH (pH 3, pH 4, pH 5, and pH 6). For *Trichoderma koningiopsis*, after 4 days of incubation, the initial pH at pH 3, pH 4, pH 5 and pH 6 showed the decolorization of PRMX5B at 57%, 52%, 34% and 28%, while for *Trichoderma atroviride*, the decolorization of PRMX5B was at 64% (pH 3), 46% (pH 4), 33% (pH 5) and 27% (pH 6). After 12 days of incubation, the initial pH at pH 3, pH 4, pH 5 and pH 6 showed decolorization of PRMX5B at 69%, 61%, 43% and 30% respectively for *Trichoderma koningiopsis*. For *Trichoderma atroviride*, decolorization of PRMX5B was at 70% (pH 3), 53% (pH 4), 44% (pH 5) and 34% (pH 6). Next, Figure 5C and 5D showed the percentage of decolorization of RBV5R by *Trichoderma koningiopsis* and *Trichoderma atroviride* at different values of pH. For *Trichoderma koningiopsis*, after 4 days of incubation, the initial pH at pH 3, pH 4, pH 5 and pH 6 showed the decolorization of RBV5R at 61%, 67%, 64% and 61%, while for *Trichoderma atroviride*, the decolorization of RBV5R was at 47% (pH 3), 52% (pH 4), 31% (pH 5) and 39% (pH 6). After 12 days of incubation, the initial pH at pH 3, pH 4, pH 5 and pH 6 showed decolorization of RBV5R at 71%, 73%, 70% and 67% respectively for *Trichoderma koningiopsis*. For *Trichoderma atroviride*, decolorization of RBV5R was at 60% (pH 3), 58% (pH 4), 42% (pH 5) and 50% (pH 6). From the data obtained, it can be concluded that the optimum pH value for both *Trichoderma koningiopsis* and *Trichoderma atroviride* is at pH 3. This is because the optimum pH was between pH 3 and pH 5, which was suitable for fungal growth and enzymatic production. The chemistry of both molecules and fungal biomass was influenced by the initial pH value of the dye solution [9].

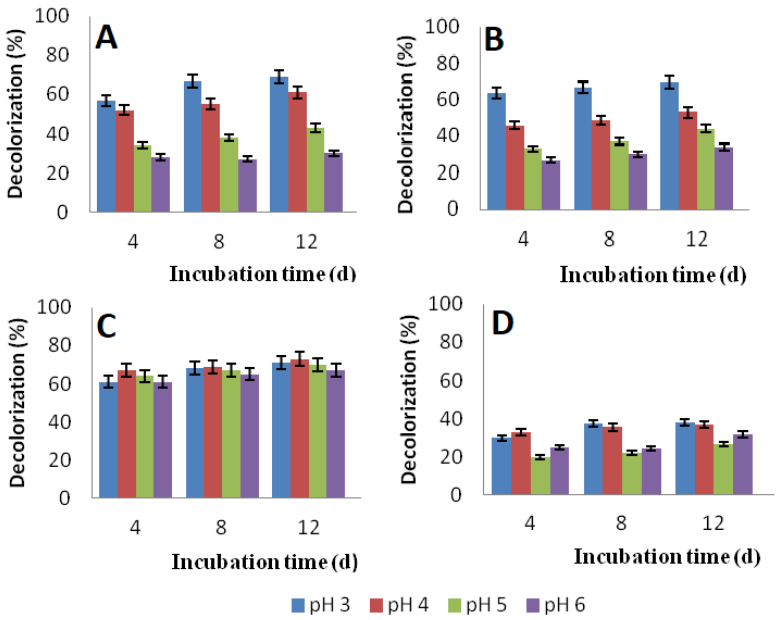


Figure 5. Effect of pH in decolorization of PRMX5B by *Trichoderma koningiopsis* (A) and *Trichoderma atroviride* (B); and RBV5R by *Trichoderma koningiopsis* (C) and *Trichoderma atroviride* (D).

Figure 6A and 6B show the percentage of PRMX5B decolorization at three different temperatures, 20°C, 27°C and 37°C. Both *Trichoderma koningiopsis* and *Trichoderma atroviride* decolorized dye at a temperature of 20°C were 12% and 14%, respectively, after 4 days of incubation. The percentages keep increasing after 8 days and 12 days of incubation. For *Trichoderma koningiopsis*, the percentage of decolorization was 19% (8 days) and 27% (12 days), while for *Trichoderma atroviride*, it was 18% and 21% respectively. Trichoderma koningiopsis decolorizes dyes up to 20% (4 days), 28% (8 days), and 40% (12 days) at 27°C, whereas Trichoderma atroviride decolorizes dyes 11%, 24%, and 38% at 20°C, 27°C, and 37°C, respectively.*Trichoderma koningiopsis* and *Trichoderma atroviride* show 49% and 46% after being incubated for 12 days. While *Trichoderma koningiopsis* decolorized dye up to 66% and *Trichoderma atroviride* decolorized dye at 60% within 4 days of incubation at 37°C. After 12 days, the percentage of decolorization at 37°C increased to 75% (*Trichoderma koningiopsis*) and 72% (*Trichoderma atroviride*). While for temperature at 20°C after 12 days, the results obtained show that 48% (*Trichoderma koningiopsis*) and 33% (*Trichoderma atroviride*) of decolorization of dye, and for temperature at 27°C, *Trichoderma koningiopsis* and *Trichoderma atroviride* can decolor dye up to 69% and 49% respectively within the same time of incubation. It can be concluded that the optimum for both *Trichoderma koningiopsis* and *Trichoderma atroviride* to decolor dye over 45% was at 37°C. As the temperature increased, the decolorization of the dye increased. This may be because the solubility of the enzyme increased at high temperatures and then enhanced the enzymatic activity of the fungus to degrade dye. It shows that warmer conditions are the most suitable for the growth of the microbial cell [10-12].

Figure 7A and 7B show the percentage of decolorization of PRMX5B by *Trichoderma koningiopsis* and *Trichoderma atroviride* at two different conditions, with agitation and static growth. For *Trichoderma koningiopsis*, after 4 days of incubation, the decolorization of PRMX5B at static and shaking conditions was 18% and 25%, while for *Trichoderma*

atroviride, the decolorization of PRMX5B was at 10% (static growth) and 24% (with agitation).

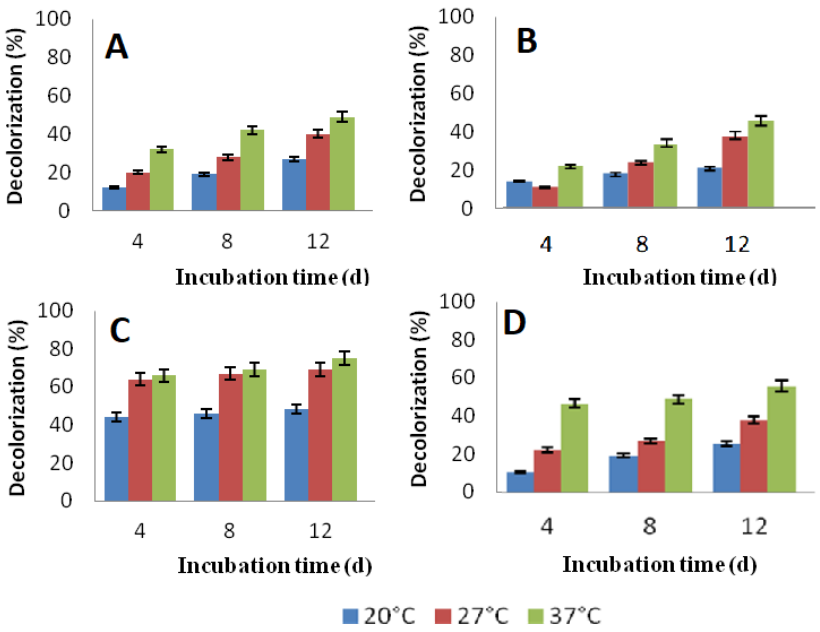


Figure 6. Effect of temperature in decolorization of PRMX5B by *Trichoderma koningiopsis* (A) and *Trichoderma atroviride* (B); and RBV5R by *Trichoderma koningiopsis* (C) and *Trichoderma atroviride* (D).

After 12 days of incubation, both fungi showed a higher percentage of decolorization of dye in the shaking state compared to the static state, which was 69% (*Trichoderma koningiopsis*) and 74% (*Trichoderma atroviride),* while without agitation, *Trichoderma koningiopsis* and *Trichoderma atroviride* decolorized dye up to 42% and 58%, respectively. Figure 7C and 7D show the percentage of decolorization of RBV5R by *Trichoderma koningiopsis* and *Trichoderma atroviride* at two different conditions, with agitation and static growth. For *Trichoderma koningiopsis*, after 4 days of incubation, the decolorization of RBV5R at static and shaking conditions was 51% and 59%, while for *Trichoderma atroviride*, the decolorization of PRMX5B was 36% (static growth) and 42% (with agitation). After 12 days of incubation, both fungi showed a higher percentage of decolorization of dye in the shaking state compared to the static state, which was 90% (*Trichoderma koningiopsis*) and 71% (*Trichoderma atroviride),* while without agitation, *Trichoderma koningiopsis* and *Trichoderma atroviride* decolorized dye up to 79% and 42%, respectively. The results obtained can conclude that agitation (100 rpm) enhanced the decolorization of dye within the time of incubation. This is because agitation may have increased the distribution of nutrients and oxygen to the fungus, thereby promoting enzyme secretion and fungal growth. The decolorization of these synthetic dyes under static growth conditions was lower compared to with agitation conditions. This is because the growth of mycelia increased, which are rich in amino hydrophobins (which absorb the water-soluble dye) [13,14].

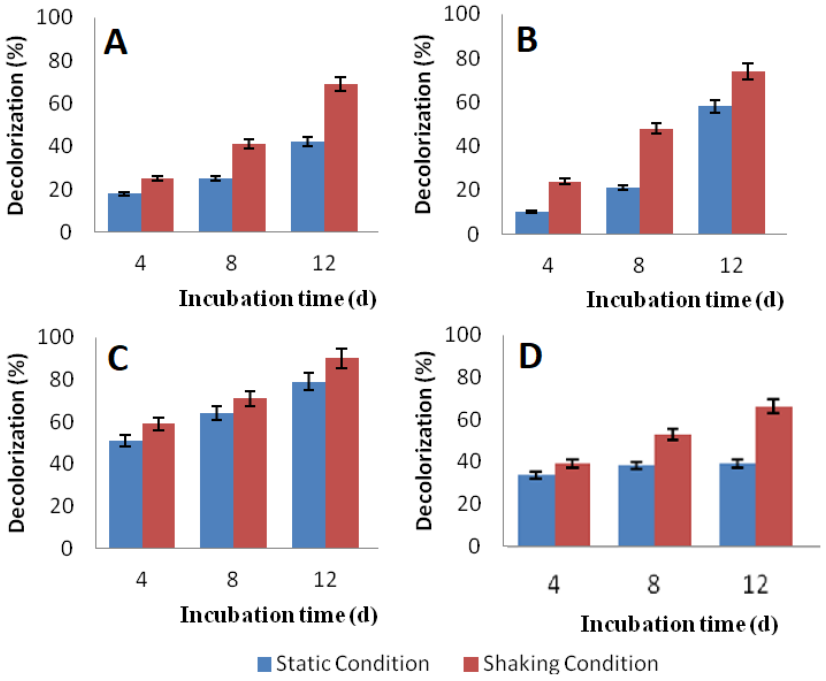


Figure 7. Effect of temperature in decolorization of PRMX5B by *Trichoderma koningiopsis* (A) and *Trichoderma atroviride* (B); and RBV5R by *Trichoderma koningiopsis* (C) and *Trichoderma atroviride* (D).

4. Conclusions

Based on the phylogenetic tree, fungi S1 and S2 were identified as *Trichoderma koningiopsis* and *Trichoderma atroviride,* respectively. Both fungi were able to decolorize RBV 5R and Reactive Red 2 under several parameters given, such as carbon and nitrogen sources, pH, temperature, and agitation. This study can be improved by applying the same method to different types of dyes; studying the effect of other parameters such as metal ions, salinity, and surfactant tween 80; also, the condition of fungal growth should be monitored strictly to enhance the secrection of ligninolytic enzymes.

Acknowledgements

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Conflicts of Interest

The authors declare no conflict of interest.

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