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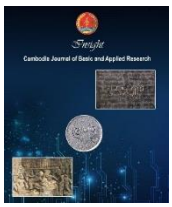

Biodecolorization of azo dye using *Bacillus subtilis* strain CKCC isolated from compost residues

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សង្ខិត្តន័យ

ថ្នាំជ្រលក់ពណ៌អេហ្សូ (Azo dyes) គឺជាក្រុមថ្នាំជ្រលក់ពណ៌គីមីសំយោគមួយប្រភេទ ដែលត្រូវបានប្រើប្រាស់ក្នុងវិស័យឧស្សាហកម្ម និងផលិតកម្មផ្សេងៗយ៉ាងច្រើនក្នុងទូទាំងពិភពលោក។ ថ្មីៗនេះ កម្រិតសារធាតុពុលនៃក្រុមថ្នាំជ្រលក់ពណ៌អេហ្សូបានធ្វើឲ្យអ្នកស្រាវជ្រាវមានចំណាប់អារម្មណ៍យ៉ាងខ្លាំង។ កាកសំណល់រាវដែលចេញពីដំណើរការនៃផ្នែកវាយនកម្ម សម្បូរទៅដោយសារធាតុពុលដែលមានផលប៉ះពាល់ធ្ងន់ធ្ងរនិងអាចបង្កជាជម្ងឺទៅលើអង្គត់ហ្សែន និងជម្ងឺមហារីកជាដើម។ ដើម្បីកាត់បន្ថយនូវការប្រឈមមុខនឹងបញ្ហាធំៗទាំងនេះ អ្នកស្រាវជ្រាវបានសិក្សាទៅលើដំណើរការបំបែកសារធាតុពុលតាមបែបជីវសាស្ត្រ ដោយដាក់សារធាតុពុលនៃកាកសំណល់វាយនកម្ម (Dye) ទាំងនោះឲ្យស្ថិតក្រោមសកម្មភាពបំផ្លាញរបស់អតិសុខុមប្រាណ។ អត្ថបទនេះបានបង្ហាញអំពីការកាត់បំបែកពណ៌អេហ្សូ ទៅតាមបែបជីវសាស្ត្រ (Biodecolorization) ដែលគ្មានផលប៉ះពាល់ដល់បរិស្ថាន និងអាចជាជម្រើសល្អជាងវិធីបន្សាបតាមបែបគីមីរូប។ ការសិក្សានេះផ្តោតសំខាន់លើការពិនិត្យរក និងញែកចេញពីសំណល់ដីកំប៉ុសនូវអតិសុខុមប្រាណដែលពូកែបំបែកសារធាតុពុលនៃថ្នាំជ្រលក់ពណ៌អេហ្សូ។ ក្នុងការសិក្សាស្រាវជ្រាវដំណាក់កាលដំបូង គេអាចញែកបាននូវអតិសុខុមប្រាណដែលអាចបំបែកសារធាតុពុលនៃថ្នាំជ្រលក់ពណ៌អេហ្សូក្នុងមជ្ឈដ្ឋានដុំវិញខ្លួនរបស់វា ដោយសកម្មភាពអង់ហ្ស៊ីម។ ដោយផ្អែកទៅលើការវិភាគនៃអង្គត់ហ្សែន ១៦S rRNA អតិសុខុមប្រាណនេះមានលក្ខណៈស្រដៀងគ្នាទៅនឹងអតិសុខុមប្រាណប្រភេទ *Bacillus subtilis* strain CKCC រហូតដល់ ៩៩,៣៥%។ ដោយដាក់ឲ្យស្ថិតក្នុងលក្ខខណ្ឌល្អបំផុត (សីតុណ្ហភាព ៣៧.០ អង្សាសេ, កម្រិត pH ៧.០, ស្ករគ្លុយកូស និង yeast extract (0.5% *w/v*), កូរ រយៈពេលមួយនាទីឲ្យបាន ២០០ ដុំ, និងទុករយៈពេល ៩៦ ម៉ោង) ចំពោះកំហាប់ដំបូងនៃថ្នាំជ្រលក់ពណ៌អេហ្សូ ២០.០ mg/l គេទទួលបានបរិមាណនៃការកាត់បំបែក

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៨៩,០៦ ± ១,០៩%។ បើប្រៀបធៀបនឹងលទ្ធផលនៃការសិក្សាដែលគ្រាន់តែទុកឲ្យស្ងៀមដោយមិនកូរ គេទទួលបានកម្រិតនៃការកាត់បំបែក ៧៨,៩៥ ± ២,៥១%។ អ្វីដែលគួរឱ្យចាប់អារម្មណ៍ក្នុងការសិក្សានេះ គឺអតិសុខុមប្រាណថ្មីប្រភេទ *Bacillus subtilis* strain CKCC អាចកាត់បំបែកសារធាតុពុលនៃការកសំណល់រាវរ៉ាយនក័ណ្ឌបានទាំងពីរលក្ខខណ្ឌ (ការទុកស្ងៀមមិនកូរ និងការកូរឲ្យរំលែជាជុំរយៈពេលមួយនាទី)។ ដូច្នេះ អតិសុខុមប្រាណថ្មីប្រភេទ *Bacillus subtilis* strain CKCC ត្រូវបានគេចាត់ទុកថាជាប្រភពអតិសុខុមប្រាណដ៏វិសាស្ត្រដ៏មានសក្តានុពលមួយក្នុងការបន្សាបសារធាតុពុលក្នុងការកសំណល់រាវរ៉ាយនក័ណ្ឌ ដោយសារវាមានសមត្ថភាពការកាត់បំបែកសារធាតុពុលនៃថ្នាំជ្រលក់ពណ៌អេហ្សូ ទាំងក្នុងលក្ខខណ្ឌត្រូវការអុកស៊ីសែន និងមិនត្រូវការអុកស៊ីសែន។

ABSTRACT

Azo dyes are commonly used synthetic dyes found in various industrial applications globally. Recently the toxicity of these dyes has attracted the attention of researchers. The effluent from textile processes involving these dyes can have lethal consequences due to their genotoxicity, mutagenicity, and carcinogenicity. Concerns about secondary pollution from the physio-chemical treatment of textile effluents has driven research into biological treatment processes where azo dye effluent undergoes bacterial degradation. Biodecolorization has been presented in this paper as an eco-friendly alternative to physio-chemical treatment. This study focuses on the screening and isolation of effective bacteria for the decolorization of azo dyes from compost residues. Primary screening was able to isolate bacterial strains that demonstrate decolorization of azo dye effluent due to enzymatic activity surrounding each colony. 16S rRNA gene sequence analysis identified that this isolated strain had 99.35% similarity with the *Bacillus subtilis* strain CKCC. Under optimal treatment conditions (37.0℃; pH of 7.0; agitated at 200 rpm, fed with a 0.5% *w/v* yeast extract), a decolorization efficiency of 89.06 ± 1.09% was achieved after 96.0 h for an initial azo dye concentration of 20.0 mg/l. This compares with an efficiency of 78.95 ± 2.51% under static conditions. The *B. subtilis* CKCC strain has significant potential as a bioresource for effluent treatment in the textile due to its capacity to degrade azo dyes under both aerobic and anaerobic conditions.

1. Introduction

Natural dyes, made from plant extracts, insects, marine species, and minerals have been used since the Roman Empire (Kadolph, 2008). Blue and yellow dyes from plants have been in common use for at least 40,000 years (Mani et al., 2019). The first commercial synthetic dye, mauveine, was discovered in 1856 by the British chemist William Henry Perkin who attempted to replicate a red dye from the rubia root produced by French chemist Pierre Robique (1780-1840) in 1826 (Dave et al., 2015). Synthetic azo dyes are polycyclic aromatic compounds obtained via the oxidation of anthracene. They are used extensively in the manufacture of dyes for use in the textile and paper industries (Ito, 2013). The word azo comes from azote, the French word for nitrogen. It is represented by the nomenclature R_1-N=N-R_2, where -N=N- is an azo bond, and R_1 & R_2 are either aryl or alkyl compounds (Chung, 2016). In azo dyes, azo bonds are substituted with benzene or naphthalene, which may contain different functional groups. For instance, chloro (-Cl), methyl (-CH_3), nitro (-NO_2), amino (-NH_2), hydroxyl (-OH) and carboxyl (-COOH) groups produce different types of synthetic azo dyes (Dave et al., 2015). Azo dye effluent has been known to produce genotoxic, mutagenic, and carcinogenic health impacts (Mahajan et al., 2019). For instance, benzedine, a byproduct of the production of azo dyes is well known as a carcinogen of the human bladder (Gičević et al., 2020).

Industrialization from the 19th century coincided with increased levels of pollution from textile industry effluent (Evans et al., 2012). Today, there are more than 100,000 types of commercial dyes, including at least 3,000 types of azo dyes used in the textile, paper, and leather industries, globally (Boonsong & Paksamut, 2018). Azo dyes have become popular in the textile industry as they are resistant to physicochemical, and biological degradation from exposure to sweat (Saratale et al., 2011). In 2013, it was reported that approximately 1.3 million tons of synthetic azo dyes are released each year in effluent from the industry globally (Singh et al., 2015) due to between 10 and 15% of dyes not being fixated during the dying process (Senthilkumar et al., 2014). Pollution from these dyes has become concentrated in low- and middle-income countries such as India, Indonesia, Thailand, and Vietnam, where large quantities of textiles are produced (Gupta, 2009). In 2011, it was estimated that between 17 and 20% of all industrial wastewater pollution stemmed from the textile industry (Kant, 2011), with a typical concentration of 16-20 mg/l (Guadie et al., 2017). In previous studies, the efficacy of physicochemical treatment methods including chemical oxidation, adsorption, coagulation and flocculation, electrochemical processes, membrane filtration, and the use of nanomaterials to treat this waste have been studied (Shanker et al., 2017; Ramakrishna & Viraraghavan, 1997). However, technical constraints related to the economic feasibility of these approaches; and the secondary pollution caused by sludge production limit the usefulness of these methods (Thakur et al., 2012).

Textile effluent from dye manufacturing contains acids, alkalis, salts, and metal ions, with concentrations of up to 15 to 20% (Ali, 2010). High salinity levels in effluent present challenges for the development of biological treatment methods, as many bacteria are intolerant of these conditions (Manu & Chaudhari, 2003). However, some treatment processes involving bacteria, fungi, yeast or algae that can decolorize textile effluents by cleaving azo bonds via enzymatic processes (Telke et al., 2015; Singh et al., 2015) under either anaerobic or aerobic conditions. Enzymatic oxidation via laccases or azoreductase is effective in decolorizing a wide range of azo dyes (Chacko et al., 2011). Ligninolytic enzymes produced by fungal decomposition, such as laccase, lignin peroxidase, manganese peroxidase, versatile peroxidase has also demonstrated high decolorization

efficiency (Gold & Alic, 1993). While there are also some limitations in the use of fungi derived enzymes (Banat et al., 1997), many potential enzymes for use in the treatment of dye effluent have been reported (Karam & Nicell, 1997). Biodecolorization is a potentially eco-friendly alternative to physio-chemical treatment methods. The objective of this study is to isolate bacterium with the potential to decolorize azo dyes from agricultural compost residues and to determine optimal growth conditions (pH, temperature, and the addition of nutrient supplements) for the degradation of azo dyes.

2. Material and methods

2.1 Chemicals and medium

Reactive red 120 (RR120), a pure synthetic azo dye used in industry (λ_{max} = 537 nm), and Azure B (AB), an indicator dye (λ_{max} = 650 nm) were purchased from Sigma-Aldrich, USA. Nutrient broth medium containing 0.5% (*w*/*v*) peptone, 0.5% (*w*/*v*) NaCl, 0.15% (*w*/*v*) meat extract, and 0.15% (*w*/*v*) yeast extract; Berg's mineral salt medium (MS) at pH 7.0 containing 0.2% (*w*/*v*) $NaNO_3$, 0.05% (*w*/*v*) K_2HPO_4, 0.02% (*w*/*v*) $MgSO_4.7H_2O$, 0.002% (w/v) $MnSO_4.H_2O$, 0.002% (*w*/*v*) $FeSO_4.7H_2O$, and 0.002% (*w*/*v*) $CaCl_2.2H_2O$ were also purchased. Agar (2%, *w*/*v*) was supplemented for the preparation of an agar medium. All chemicals were analytical grade.

2.2 Isolation and screening of the bacteria

A total of 450 x 10 g compost residue samples was collected from various sites in Suphanburi province, Thailand. The samples were maintained at a temperature below 37℃ while being transferred to the laboratory. Then azo dye decolorizing bacteria were cultured in the nutrient broth medium. The culture was screened in 10 mL test tubes (pH 7.0) and inoculated with the compost residue samples, supplemented with Azure B at a concentration of 20 mg/l. The samples were then incubated at 37℃, agitated at 100 rpm for 24 h. Potential Azure B samples were then removed using an aseptic serial dilution by a sterile NaCl solution (0.85% *w*/*v*) and spread on NB agar plates containing Azure B at a concentration of 20 mg/l then incubated at 37℃. Single, morphologically different colonies demonstrating a clear halo zone was isolated from the samples. A culture of each strain was preserved in 50% glycerol stock and stored at (-20℃). The dye removal efficiency of each pure isolate was then verified in a liquid nutrient broth medium, with an Azure B indicator dye (20 mg/l). An equal initial cell inoculation (OD = 600 nm) was performed. 100 mL of cell starter 5% (*v*/*v*) was inoculated in a 500 mL Erlenmeyer flask and agitated at 200 rpm. After 72 h, the most effectively decolorized samples were selected based on the color of the indicator dye.

2.3 Strain identification and phylogenetic analysis

Pure colonies of the decolorization bacteria were isolated before Genomic DNA was extracted using a DNeasy Blood & Tissue Kit (QIAGEN, Germany). Then, the 16S rRNA gene was amplified using PCR (Bio-Red, T100TM, Thermal cycle) and universal eubacteria specific primers 8F (5' AGAGTTTGAT CCTGGCTCAG-3') and 1492R (5'GGTTACCTTGTTACGACTT-3'). The PCR procedure was performed under standard conditions following the instructions of the manufacturer. PCR amplifications were conducted on 50 µl samples with a T100TM Thermal Cycler at 95℃ for an initial denaturation of 30 s over 30 cycles; annealing at 50℃ for 30 s; and then extension at 68℃ for 1.30s. The final elongation was conducted at 68℃ for 5 min, before cooling at 10℃. The PCR products were then purified using a PCR Purification Kit (QIAGEN, Germany). Sequencing data were then derived using the BioEdit Sequence Alignment Editor Program and compared with the NCBI GenBank online database using BLASTn. The phylogenetic relationship of the sequence was constructed using the neighbor-joining method and a phylogenetic tree was created using MEGA-10 software.

2.4 Biodecolorization efficiency analysis

Experiments were conducted using an equal initial optical density (OD) with a cell inoculation of 5% (*v*/*v*). To evaluate the decolorization efficiency, 1 ml of the culture and control samples without cell inoculation were aseptically collected from the Erlenmeyer flask daily and centrifuged (Kubota 5922, Japan) at 8,000 rpm for 5 min. The supernatant samples were measured at the relevant wavelength for each sample using a UV-Vis spectrophotometer (Shimadzu Model UV-160, Japan). The dye decolorization efficiency was calculated using the decolorization efficiency equation:

$$\text{Decolorization efficiency (\%)} = \frac{\text{OD initial - OD degraded}}{\text{OD initial}} \times 100$$

Where, OD initial = Absorbance before cell inoculation; and OD degraded = Absorbance after cell inoculation.

2.5 The effect of physicochemical treatment on biodegradation and biodecolorization efficiency

The effect of a nutrient supplement, as a source of carbon and organic nitrogen, on the decolorization efficiency was studied. A glucose and yeast extract were evaluated at concentrations between 0.3 and 0.5%, *w*/*v*. Both static and shaking culture conditions were applied, with effects of both pH and temperature on decolorization analyzed. To determine the robustness of each treatment, they were applied to a range of initial dye concentrations from 20 mg/l to 60 mg/l. Cell growth (CFUs/ml) and decolorization efficiency (% removal) were calculated daily for four days. The results of the experiments were analyzed and presented as mean values with a standard deviation and standard error.

3. Results and discussion

3.1 Screening and isolation of azo dye decolorizing bacteria

In total, 130 out of 450 test tubes demonstrated positive dye removal and were selected as part of a primary screening (data not shown). Following ten sub-cultures, a further 120 test tubes were excluded. As shown in **Table 1**, 17 morphologically pure bacterial colonies were isolated from these 10 test tubes. These were selected based on a defined clear zone on agar substrate and high removal efficiency (**Fig.1**), indicating bacteria with enzymatic degradation capacity (Chen et al., 2003). Isolates with an absence of a clear zone were rejected.

The bacterial strain selected demonstrated a clear zone index of 1.56 ± 0.11 (data not shown) and a decolorization efficiency of 91.78 ± 0.50%. Hereafter this is referred to as strain CKCC. The diameter of the clear zone was used to indicate whether primary hydrolysis of a multifunctional enzyme had occurred. The microbial ligninolytic enzymes used to degrade the dies included azoreductase, laccase, manganese peroxidase and lignin peroxidase. The enzymatic system applied in this study will be the focus of a later study.

Table 1: Morphologically pure colonies derived from primary screening.

N	Strain	Colony shape	Color	Clear zone	Removal efficiency
1	B26	Irregular	Gray	Appears as a clear	77.13%
2	B35	Circular	Gray	Appears as a clear	72.94%
3	B35.1	Circular	Gray	Appears as a clear	51.32%
4	B40	Circular	Gray	Appears as a clear	61.94%
5	B41	Circular	Gray	Appears as a clear	55.23%
6	CKCC	Circular	White	Clearly visible	91.78%
7	G3	Circular	White	Clearly visible	86.34%
8	G7	Circular	White	Clearly visible	85.89%
9	PP26	Circular	White	Appears as a clear	83.33%
10	PP34	Circular	White	Appears as a clear	59.65%
11	PP39	Circular	White	Appears as a clear	49.64%
12	PP45	Circular	White	Appears as a clear	50.45%
13	PP45S	Circular	White	Appears as a clear	50.95%
14	R23	Circular	White	Appears as a clear	69.95%
15	R23.1	Irregular	White	Appears as a clear	49.48%
16	R39	Circular	White	Appears as a clear	69.75%
17	R39.1	Circular	Orange	Appears as a clear	70.85%

3.2 Identification and phylogenetic analysis

The partial sequence of the 16S rRNA gene revealed that strain CKCC possessed a 99.35% similarity with *Bacillus subtilis* X-c. The phylogenetic tree indicated that strain CKCC belonged to the *Bacillus* genera (**Fig.2**). Many species in the *Bacillus* genus including *B. subtilis*, *B. cereus* and *B. coagulans* have been studied as biodecolorization agents, isolated from different natural sources (Khan et al., 2013). *Bacillus* strains have also been of interest to other applications such as biotechnology, bioremediation, and biorefinery (Yusupova et al., 2020). Strain *B. subtilis* is assessed as have a Level 1 biosafety risk (non-infectious bacteria). It is not considered pathogenic to humans, animals or plants. Strain *B. subtilis* is tolerant of multiple environmental stresses and has been applied as a probiotic for both humans and animals. The Bacillus genus has also been applied to azo dye decolorization aerobic, facultatively anaerobic, static, and anoxic conditions (Saranraj et al., 2018). This suggests that the *Bacillus* species has high potential as a bio-resource for the novel and improved treatment of effluent from the textile industry.

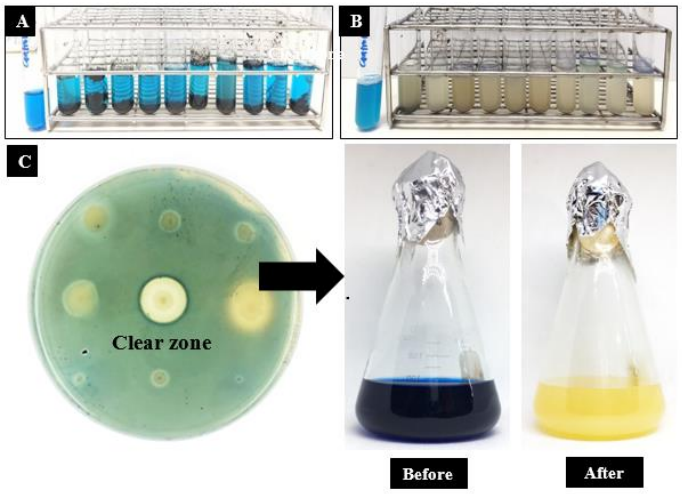

Fig. 1. Primary screening of the samples (**A**) Initial inoculation of the compost residue samples; (**B**) After 24 h of inoculation; (**C**) Clear zone constructions on Azure B agar medium, indicative of a pure isolate, followed by Azure B liquid removal after 72 h.

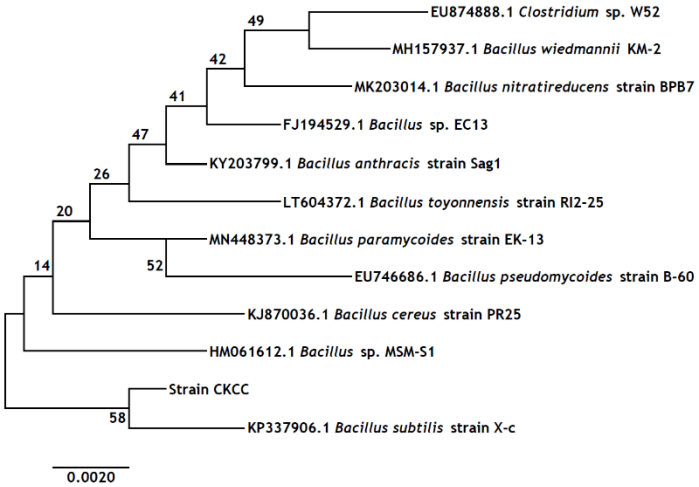

Fig. 2. The phylogenetic identification of *B. subtilis* strain CKCC.

3.3 Effects of physicochemical on decolorization efficiency

In this study, the performance of *B. subtilis* strain CKCC in the biodegradation of azo dyes was shown to be enhanced under optimal conditions. After 96 h of incubation, the decolorization efficiency of the B. subtilis strain CKCC was highest under agitation at 200 rpm and a neutral pH. Under these conditions, the removal efficiency for the azo dye (RR120) and indicator dye (Azure B) at an initial concentration of 20 mg/l was found to be 89.06 ± 1.09% and 91.78 ± 0.50%,

respectively (Fig.3). Under static conditions, the degradation efficiency of the azo dye was only 78.95 ± 2.51% (data not shown). Under aerobic conditions, cell growth was slow over the first 24 h of incubation but increased over the next 48 h, before reaching a stationary phase and then rapidly declining after 72 h, where it reached a death phase after 96 h (Fig.4).

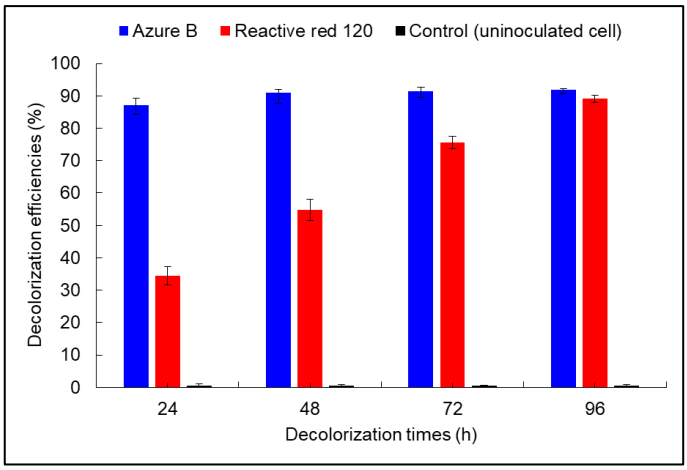

Fig. 3. The decolorization efficiency of *B. subtilis* strain CKCC strains under optimal conditions.

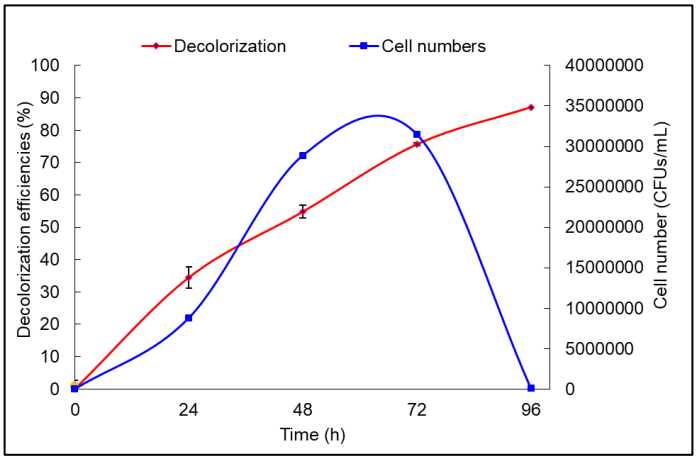

Fig. 4. The association between cell growth and decolorization efficiency.

The application of microbial enzymes for the decolorization of azo dyes is an eco-friendly technology. In this study, a novel indicator plate method was developed as an effective method for screening bacteria with the capacity to degrade azo dyes in both anaerobic and aerobic conditions. A mechanism by which ligninolytic enzymes may be applied to the degradation of azo dyes was determined. Natural microbes produce extracellular enzymes with the capacity to break down the complex chemical structures that cause azo dye effluent to be toxic. The growth rate of the screened bacteria in response to organic nutrient availability was analyzed to consider the effect on the oxidation of azo dye structures (Mani et al., 2019; Hatvani & Mécs, 2002). The enzyme activity involved in the decolorization pathways was found to be more effective within a culture medium containing high amounts of organic nutrients (Buswell et al., 1995). In particular, yeast extracts were found to be the most effective nitrogen source for enhancing decolorization efficiency (Moosvi et al., 2005). In contrast, inorganic nitrogen sources were found to be unavailable for supporting bacterial growth, leading to low decolorization efficiency (Li et al., 2019). Strain CKCC also required oxygen to progress metabolic pathways via the transfer of electrons as previously reported by Reddy et al. (2016).

Bacterial strains such as *Bacillus*, *Citrobacter*, *Pseudomonas*, *Clostridium*, *Staphylococcus*, and *Micrococcus* have been reported to grow under aerobic conditions but not produce the enzymatic activity that enables a high decolorization efficiency under low oxygen, static or anaerobic conditions (Wang et al., 2009). In contrast, the *B. subtilis* strain CKCC demonstrated facultative anaerobic behaviors, capable of supporting aerobic respiration pathways, before converting to microaerophilic behaviors depending on the availability of oxygen. This is significant as in previous reports, only a small number of bacterial species have been identified with the potential to degrade the azo dyes under aerobic conditions (Lin & Leu, 2008), including *Bacillus*, *Sphingomonas*, *Pseudomonas* and *Listeria* (Kuberan et al., 2011). Efficient azo dye decolorization via anaerobic or microaerophilic pathways using the *B. subtilis* strain CKCC requires more complex organic nutrient sources (Singh et al., 2017); neutral pH conditions (Wang et al., 2009; Moosvi et al., 2007); and moderate temperatures for optimal cell growth to occur (Asad et al., 2007). In addition, where there are high initial dye concentrations (60 mg/l), toxicity has been found to directly inhibit cell growth, as outlined in previous investigations (Ray et al., 2016). This is due to the influence of azo dyes on the enzymatic systems responsible for elimination pathways (Pearce et al., 2003). This is in line with other studies that have shown that the aromatic compounds within complex azo dye structures strongly impact decolorization efficiency (Saratale et al., 2011).

To demonstrate that the toxic biodegradation products were not generated by secondary pollution, the presence of a clear zone construction in the isolates was used to indicate ligninolytic enzyme activity, using an indicator dye as a substrate (Chen et al., 2003). Phytotoxicity assessments were used to demonstrate that the ligninolytic enzymes that produce bacterium in textile effluent significantly reduced toxicity during decolorization (Bharagava et al., 2018). This is aligned with a previous study, which found that the phytotoxicity of by-products of bio-decolorization was less toxic than the initial dye (Nouren et al., 2017). Another study found that plant growth-promoting bacteria (PGPB) screened from *Bacillus* sp. strain MR-1/2 both demonstrated a high decolorization efficiency for azo dyes while facilitating plant growth (Shahid et al., 2018). The present study demonstrated that the *B. subtilis* strain CKCC possesses a high decolorization efficiency when treating azo dye RR120 effluent. The capacity to degrade azo dyes via different metabolic pathways is of particular interest for practical applications in the field.

4. Conclusion

The Azo dye is used significantly in the textile sector for coloring fabrics. High concentrations of it are released into aqueous environments through textile effluents. Annually,

worldwide, approximately 280,000 tons of textile wastewater containing dyes are discharged into the environment. Therefore, its removal from textile wastewater and effluents is necessary. In this present research, a newly isolated azo dye decolorization agent was identified to have a 99.35% similarity with *B. subtilis* strain CKCC based on a 16S rRNA gene sequence analysis. Under optimal conditions (37.0℃; pH 7.0; agitated at 200 rpm), azo dye RR120 with an initial concentration of 20.0 mg/l was degraded by 89.06 ± 1.09% after 96 h. This isolated strain demonstrated high decolorization efficiency and potential commercial viability for azo dye effluent treatment in the textile industry. The improved performance of the *B. subtilis* strain CKCC is attributed to its microaerophilic characteristics, which indicate a capacity to tolerate different environmental conditions. This provides significant advantages for the eco-friendly alternative treatment of colored azo dye textile effluents. Thus, it is recommended that FT-IR and HPLC analysis of azo dye structures before and after breakdown and a phytotoxicity assessment of culture residues in *B. subtilis* strain CKCC following treatment is conducted in future studies. This may help to better understand azo dye degradation pathways by this approach. Different oxidoreductive enzymes involve different reaction pathways for azo dye decolorization. Identifying new eco-friendly options, such as the *B. subtilis* strain CKCC are required for practical application. Further, the sequencing of these genes may be of interest for other purposes. The use of novel biocatalysts for azo dye degradation requires more research to realize commercial benefits from these results.

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported. All authors have read and approved the final, published version of the manuscript.

Credit authorship contribution statement

CHEM Chanchao: Conceptualization, Research methodology, Investigation, Data analysis, Manuscript preparation, Review and editing. PASON Patthra: Resources and Analytical tools. WAEONUKUL Rattiya: Resources and analytical tools. RATANAKHANOKCHAI Khanok: Supervision. TACHAAPAIKOON Chakrit: Conceptualization, Supervision, Review and editing.

All authors have read and agreed to the published version of the manuscript.

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