



Decolorization and degradation of reactive orange 16 by *Bacillus stratosphericus* SCA1007

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Abstract

Efficient bacterial strain was isolated from the dye contaminated area and identified as *Bacillus stratosphericus* SCA1007 based on 16S rRNA gene sequence (GenBank under accession number KY992944). This isolate was selected based on its potential to efficiently decolorize reactive orange 16 dye which is extensively used in textile industries. Various culture conditions like dye concentration, temperature, pH, salinity, and additional nitrogen source were optimized in the present study. The optimal conditions for decolorization of reactive orange 16 was found to be: dye concentration 150 mg/L, pH 7, temperature 35 °C, and yeast extract as nitrogen source. The isolate was also resistant to 4% saline culture condition. Decolorization and degradation of dye were confirmed through UV–visible spectroscopy, Fourier transform infrared (FTIR) and liquid chromatography-mass spectrometry analysis (LC–MS). Toxicity studies were performed on *Escherichia coli* and *Vigna radiata* to confirm the non-toxic nature of the degraded metabolites. This is the first study demonstrating complete decolorization of reactive orange 16 dye by *Bacillus stratosphericus* SCA1007 at high salinity within 10 h of incubation under optimized conditions.

Introduction

The growth of polluting industries is causing tremendous stress on the environment and the ecological system. Textile industry is one of the most polluting industries (Pritchard and Costa 1991). Textile processing industry

uses a large amount of chemicals dissolved in water which ultimately makes its way into the environment leading to manifold increase in land and water pollution. Synthetic dyes are one such class of chemicals. Apart from textile industry, these are extensively utilized in leather tanning, paper industries, agriculture research, food technologies, photo electrochemical cells, light harvesting arrays, and hair colors (Popli and Patel 2015). Textile industry alone produces 2,80,000 tonnes of dyes which are released every year all around the world (Elango et al. 2017). Once the processing of product is done, discharge of colored effluent is mixed with water streams. This mixing of highly colored effluent leads to water pollution (Ojekunle and Lateef 2017). Appearance of color in the water is the main visible indication of water being polluted (Ali 2010). The wastewater enters agricultural land, which has serious implications for living organisms. Moreover, synthetic dye reduces light penetration through water, thereby reducing photosynthesis carried out by hydrophytes, thus disturbing the food chain of aquatic eco system (Yaseen and Scholz 2018).

Dyes containing azo group are the most versatile class of man-made dyes which are applied widely because of their ease of use, varied color and cost efficiency (Tomczak and Gorecki 2012). They are highly mutagenic and carcinogenic in nature (Pillai 2017). The azo bonds are resistant to

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breakdown, resulting in accumulation in the environment. Therefore, it is essential to develop efficient and economical solution for textile effluents (Park et al. 2007). Among the azo dyes, monoazo dye reactive orange 16 is an extensively used textile dye (Kapdan and Oztekin 2003). It is brilliantly colored and easily dissolves in water which makes it even more challenging for conventional wastewater treatment methods to remediate dye.

Numerous physicochemical techniques are applied for the remediation of dye effluents, which include chemical oxidation, adsorption, coagulation, precipitation, electrolysis, bleaching, membrane filtration, and ozonation (Geetha and Velmani 2015). The major disadvantages of all these methods are that they are expensive, less efficient and lead to production of sludge, which creates further pollution (Sharma and Roy 2015). Therefore, biological methods are considered an attractive solution for mineralization of dyes (Bhatia et al. 2017), as these are environmental friendly and cost-effective (Megha et al. 2015). Generally, dye decolorization is the result of two processes either by adsorption or degradation. During adsorption, parent structure of dye remains unchanged as dyes are being adsorbed onto microbial cell surfaces, whereas, in case of degradation, naive structure of the dye is changed or broken down to smaller fragments or converted to CO₂, H₂O and salts of inorganic origin (Zhou and Zimmermann 1993).

Several taxonomic groups of microbes like bacteria, fungi, and yeast algae can decolorize and sometimes mineralize reactive orange 16. These are *Pseudomonas* sp., *Nocardiopsis alba*, *Micrococcus luteus*, *Mycobacterium* sp., *Bacillus flexus*, *Fundalia trogii*, and *Irpex lacteus* (Kalyani et al. 2009; Ali 2010; Shobana and Hamgam 2012; Saha and Rao 2019). It is found that bacterial decolorization is normally faster as compared to fungal decolorization. The present study aims to isolate and characterize a bacterium which is able to decolorize and degrade reactive orange 16.

Material and methods

Chemicals

The chemicals used in the study were of analytical grade. Reactive orange 16 was obtained from Sigma-Aldrich, Bangalore, India. Mineral salt medium (MSM) [in g/L: potassium dihydrogen phosphate 1.0, sodium chloride 1.0, magnesium sulfate heptahydrate 0.5, calcium chloride 0.1, sodium hydrogenphosphate 1.0, yeast extract 4.0, agar (solid media)] and nutrient media were retrieved from HiMedia, Mumbai, India.

Isolation, screening, and identification of dye decolorizing bacteria

Soil samples were sourced from areas of Jharkhand (23.3441° N, 85.3096° E) and Bihar (25.5941° N, 85.1376° E) where dyeing is prevalent and were serially diluted in 0.9% (w/v) saline solution, following which, sample was spread over nutrient agar plates (Habib et al. 2018). Morphologically distinct colonies were selected and purified on nutrient agar slant. Pure colonies were kept at 4 °C for further use.

Screening was carried out in mineral salt medium agar plates containing reactive orange 16 dye to isolate bacteria with decolorizing ability for reactive orange 16 dye (25 mg/L). Bacterial colonies, showing clear zones, were identified for further studies. Initial experiments were performed to study the dye decolorization efficiency of each isolated bacteria, using flask (250 mL) containing 100 mL mineral salt medium along with dye (150 mg/L). Freshly grown bacterium was used to inoculate the media. The bacterial isolates which showed the best potential for decolorization of reactive orange 16 were screened to be used for further investigations (Zin et al. 2020).

Biochemical, morphological, and molecular characterizations were performed to identify the selected bacterium. Gram reaction, motility, shape, and endospore staining were the basis of morphological characterization (Breed et al. 1957). Following biochemical tests were done, namely starch hydrolysis, gelatinase, lipase, carbohydrate fermentation, catalase, oxidase, indole test, methyl red, voges-proskauer, nitrate reduction, hydrogen sulfide production, and citrate utilization. Genus was identified in accordance to Bergey's Manual of Determinative Bacteriology (Breed et al. 1957). Bacterial genomic DNA was isolated from the suspended pellet using Zymo Research Fungal/Bacterial DNA Micro-Prep™ following the standard protocol prescribed by the manufacturer. DNA samples were subjected to PCR amplification of 16S rRNA gene using the universal primers pA (5'-AGAGTTTGATCCTGGCTCAG-3') and pH (5'-AAGGAGGTGATCCAGCCGCA-3'). The PCR amplification conditions were carried as described by Yadav et al. (2016). 16S rRNA gene sequencing (Xcelris Labs, Ahmedabad, India) was to identify species of selected bacterium isolate. Consensus sequences were aligned using ClustalW. Basic local alignment search tool (BLAST) of the National Center for Biotechnology Information (NCBI) was used to study the homology of sequences. Neighbor joining method was used to construct phylogenetic tree by the aid of Molecular Evolutionary Genetics Analysis (MEGA) software version 6.06. Under accession numbers KY992944, 16S rRNA gene sequences of isolate SCA1007 were submitted to GenBank. Isolate SCA1007 was deposited at culture collection

facility of ICAR-National Bureau of Agriculturally Important Microorganisms (NBAIM), Mau Uttar Pradesh, India.

Decolorization assay

Decolorization was studied in Erlenmeyer flasks (250 mL) having MSM (100 mL) amended with reactive orange 16 dye. Four percent of freshly grown *Bacillus stratosphericus* SCA1007 was inoculated in each flask. Absorbance of cells was recorded at $\lambda = 600$. Inoculum size was maintained (1.50×10^6 CFU/mL) and was left for incubation at 35 °C for 24 h. Reactive orange 16 dye was examined for absorbance (A) maximum (λ_{\max}) (493 nm) using spectrophotometer UV-1800 Shimadzu. Two milliliters of reaction medium were removed and centrifuged at consistent intervals of 0 h, 24 h, 48 h, and 72 h respectively. Absorbance of supernatant was recorded at 493 nm. Medium without dye and inoculum was used as blank. All experiments were carried out in triplicate and standard deviation was calculated. The bacterial isolates which showed maximum decolorizing ability were used for subsequent investigations (Karim et al. 2018).

The decolorization capacity showed by different isolates was measured in terms of percentage decolorization (Dos et al. 2007) and calculated as follows:

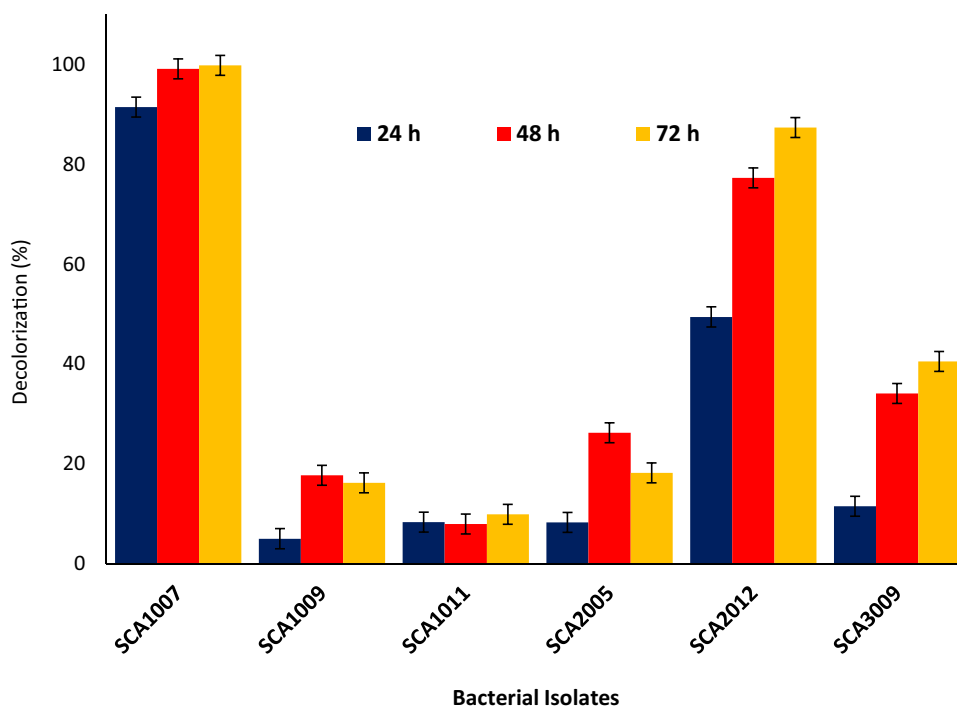
$$\%Decolorization = \left(\frac{A - B}{A} \right) \times 100$$

where,

A = Absorbance of media before incubation

B = Absorbance of media after decolorization

Fig. 1 Percentage decolorization of reactive orange 16 by bacterial isolates after 24 h, 48 h, and 72 h of incubation



Optimization of culture conditions for efficient decolorization

One variable at a time method was used to check the impact of different factors on decolorization of reactive orange 16 by *Bacillus stratosphericus* SCA1007 was studied. Experiments were performed at different concentration of dye ranging from (25–400 mg/L), pH (4–11), temperature (5–55 °C), NaCl (1–7%), and shaking/static. Buffers like sodium hydroxide (1 mol/L) and hydrochloric acid (1 mol/L) was used to maintain lower and higher pH. Data represent are means of three replicates.

Degradation analysis

Degraded metabolites obtained after 10 h of incubation was extracted by mixing the product with equal volume of ethyl acetate. The extracted product was dried over anhydrous sodium sulfate and evaporated to dryness in a rotary vacuum evaporator. Fourier transform infrared (FTIR) analysis of the extracted product was done using PerkinElmer 783 spectrophotometer in the mid IR region of 500–4500 cm^{-1} (Gao et al. 2020).

Liquid chromatography-mass spectrometry (LC-MS/MS) analysis

Monitoring of UV-vis spectra and FTIR gave information about change in the structures of parent dyes. To identify dye metabolites, analysis was performed using LC-MS/

MS. Extracted metabolites were re-suspended in HPLC grade methanol. The mobile phase comprised of acetonitrile, ammonium formate (2 mM), and formic acid (0.1%) with a flow rate of 0.4 mL/min. The purity was checked by a UV detector at 450 nm. Elution was executed in an isocratic mode and with 15 min run time. LCMS was carried out by the Thermo Scientific, USA, equipped with C18 column. Mass spectra was obtained using an ion trap mass spectrometer fitted with an electronic spray (ESI, Thermo Scientific, USA) interface operated in a positive ionization mode (100–1000 m/z) with a spray voltage of 5 kV, capillary voltage 12 V, capillary temperature 275 °C, sheath gas at 30 AU, and auxiliary gas at 5 AU. The instrument was HPLC coupled with MS (LTQ XL system (linear ion trap)). Data was recorded using Xcalibur software (Asses et al. 2018).

Table 1 Morphological and biochemical characterization of isolates in accordance with Bergey's Manual of Determinative Bacteriology

Morphological characterization	Strain SCA1007
Gram stain (G+/G-)	G (+)
Spore formation	Spore forming
Motility	Motile
Colony characterization on agar plate	
Cell shape	Rod
Size	≥ 1 µm
Pigmentation	White
Form	Irregular
Margin	Entire
Elevation	Raised
Growth in broth media	Uniform fine turbidity
Growth in agar slants	Echinulate
Optical characteristics	Translucent
Biochemical test	
Starch hydrolysis	Negative
Gelatin hydrolysis	Negative
Lipid hydrolysis	Positive
Glucose fermentation	Negative
Lactose fermentation	Negative
Sucrose fermentation	Positive
Catalase activity	Negative
Oxidase test	Positive
Nitrate reduction	Negative
Indole test	Negative
Methyl red test	Negative
Voges-Proskauer test	Positive
Citrate utilization	Negative
Hydrogen sulfide production	Negative

Toxicity Study

The phytotoxicity as well as microbial toxicity was performed. The phytotoxicity test was performed by petri dish method. The test was carried out with 150 mg/L concentration of reactive orange 16 dye and its degraded metabolites by dissolving in 10 ml of distilled water. The sterile petri dish containing a double layered of Whatman no. 3 filter papers were prepared. Three dishes were prepared: dish soaked with water (control), dish soaked with dye solution and dish soaked with degraded metabolites. The healthy seeds of *Vigna radiata* were selected and surface sterilized with 1.2% sodium hypochlorite solution then washed thoroughly with deionized water. The sterilized seeds were soaked in respective solution for 1 h and then transferred to soaked petri dish (10 seeds on each petri dish). To maintain the moisture, seeds were water very alternate day. Length of root, shoot and germination rate (%) were calculated after 7 days of incubation (Shahi and Sapkota 2018; Haque et al. 2021). Experimental setup contained distilled water as control, dye, and its degraded metabolite. Growth inhibition zone of *Escherichia coli* was measured after 24 h of incubation at 35 °C.

$$\text{Germination}(\%) = (\text{seed germinated} \div \text{Total seeds}) \times 100$$

Results and discussion

Isolation and screening of dye decolorizing bacteria

Nineteen bacterial isolates were obtained, because of their ability to grow on and decolorize reactive orange 16 dye. Of these, fourteen were Gram negative and five were Gram positive in nature. The Gram-negative bacteria resists

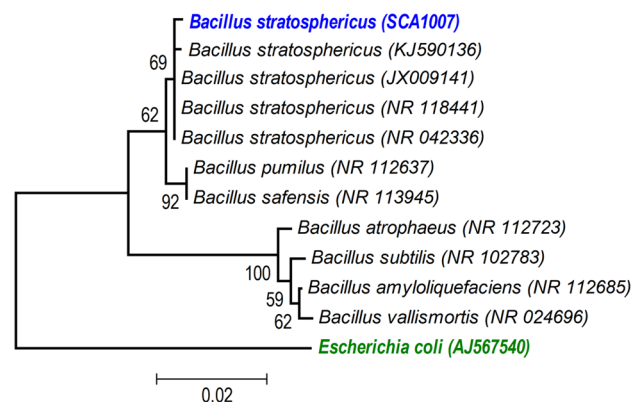


Fig. 2 Phylogenetic tree of a SCA1007 constructed by MEGA 6.01 software using the neighbor joining (NJ) method. Numbers at nodes indicate levels of bootstrap values (%) based on an NJ analysis of 1,000 replicates

Fig. 3 UV/visible spectra of reactive orange 16 **a** before and **b** after decolorization by *Bacillus stratosphericus* SCA1007

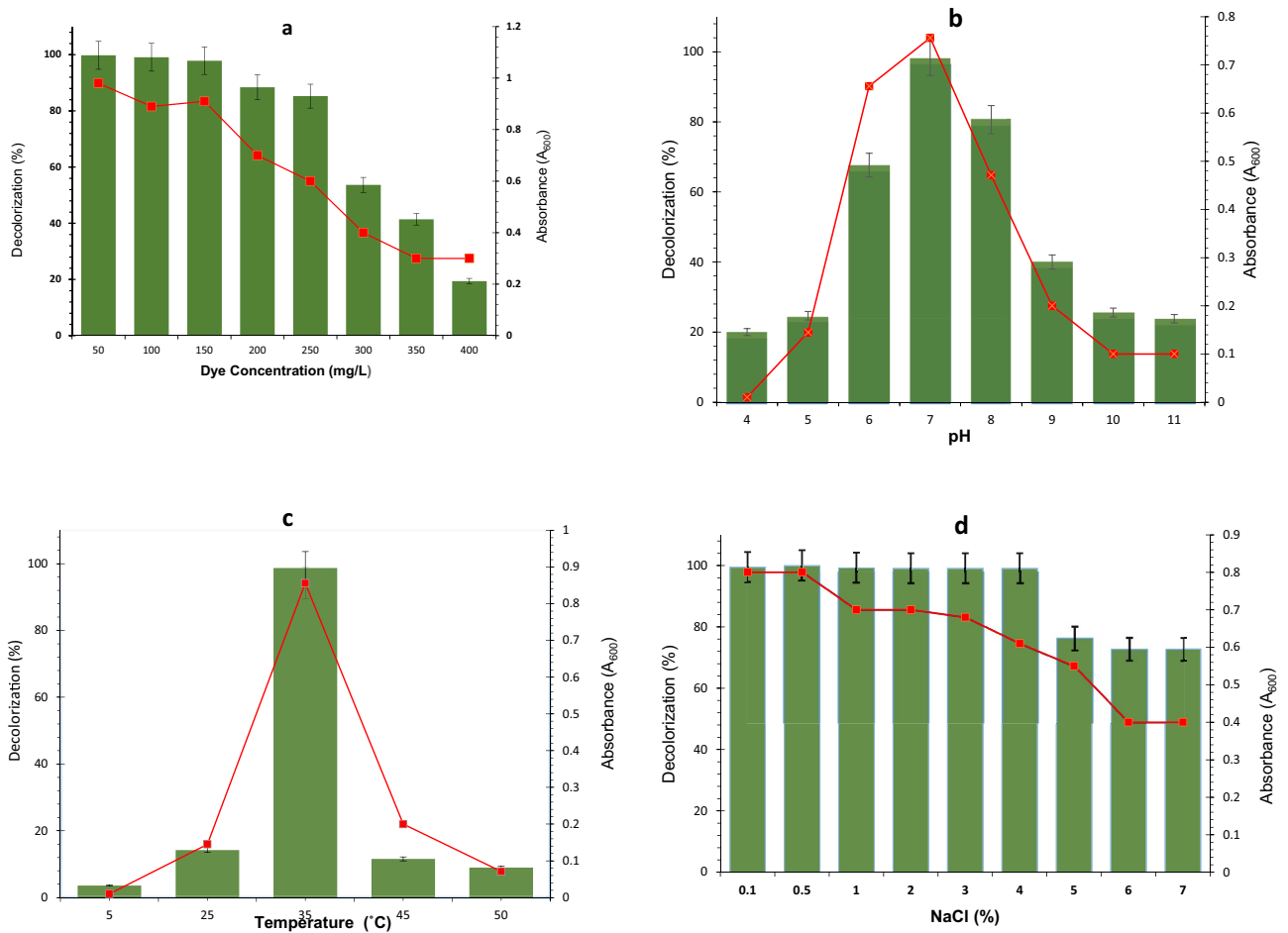
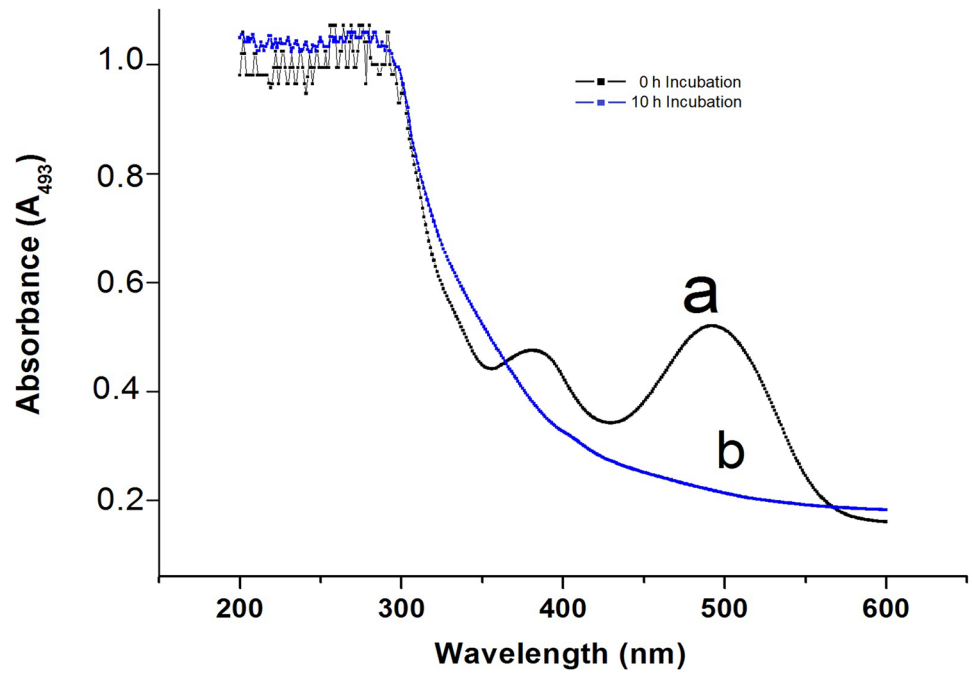


Fig. 4 **a–d** Decolorization of synthetic dye at different **a** initial concentration, **b** pH, **c** temperature, and **d** concentration of NaCl (%) of reactive orange 16 by *Bacillus stratosphericus* SCA1007

harsh environment due to changes in the outer membrane such as mutation in porins, modified hydrophobic properties etc. (Breijeh et al. 2020). Based on the clear zone formation in plate assay containing reactive orange 16 dye (25 mg/L), six isolates were screened which showed decolorization potential. Efficiency of decolorization by each selected isolate was checked by preliminary batch experiments. Bacterial isolate was inoculated to media and incubated for 24 h, 48 h, and 72 h at 35 °C. Absorbance (A) was measured at 493 nm. Among the isolates, maximum decolorization efficiency was observed in case of SCA1007 (Fig. 1).

The bacterial isolate SCA1007 was initially identified according to Bergey's Manual of Determinative Bacteriology (Table 1). Further identification of the strain was done based on 16S rRNA gene sequencing. Consensus sequence was aligned using clusterW. Basic local alignment search tool (BLAST) of the National Center for Biotechnology Information (NCBI) was used to study the homology of sequences. These sequences were selected based on maximum identity score and their alignment was done using clustalW. A phylogenetic tree was constructed using MEGA 6.06 software. (Fig. 2). Analysis of 16S rRNA gene sequence showed closest similarity with *Bacillus stratosphericus*. The 16S rRNA gene sequence of SCA1007 has been submitted GenBank under the accession number KY992944 (<https://www.ncbi.nlm.nih.gov/nucleotide/KY992944>).

Decolorization analysis

UV–visible spectroscopy was used to confirm the decolorization of reactive orange 16. If color removal is through biodegradation, two things could happen: disappearance of visible light absorbance peak or appearance of new peak (Chen et al. 2003; Patil et al. 2010). The absorbance peak (at 0 h) of dye reduced within 10 h of incubation (Fig. 3). Culture, which was utilized for decolorization of reactive orange 16 was pelleted through centrifugation (at 10,000 rpm for 15 min). Absence of color in the pellet of *Bacillus stratosphericus* SCA1007 showed that decolorization was due to degradation of compound, not through adsorption. Laccase enzyme been produced by *Bacillus stratosphericus* SCA1007 which is confirmed by appearance of reddish-brown zone around bacteria. We believe laccase enzyme may attribute to bioconversion of reactive orange 16 (Kiiskinen et al. 2004; López et al. 2006).

Optimization of culture conditions

In order to examine the potential of *Bacillus stratosphericus* SCA1007 to decolorize dye, it was exposed to varying dye concentrations (25–400 mg/L). At concentrations above 150 mg/L, the decolorization percentage was inversely

proportional to dye concentration (Fig. 4a). This is because, on increasing dye concentration, toxicity of dyes increases. This leads to restriction of metabolic activity which in turn inhibits cell growth (Shobana and Hangam 2012). Thus, dye concentration plays an important role in the choice of microbes utilized for removal of dyes from effluent, as high concentration can decrease the decolorization capacity due to the toxic nature of dyes (Khehra et al. 2006a).

The effect of pH on dye decolorization efficiency of *Bacillus stratosphericus* SCA1007 was checked, over a wide

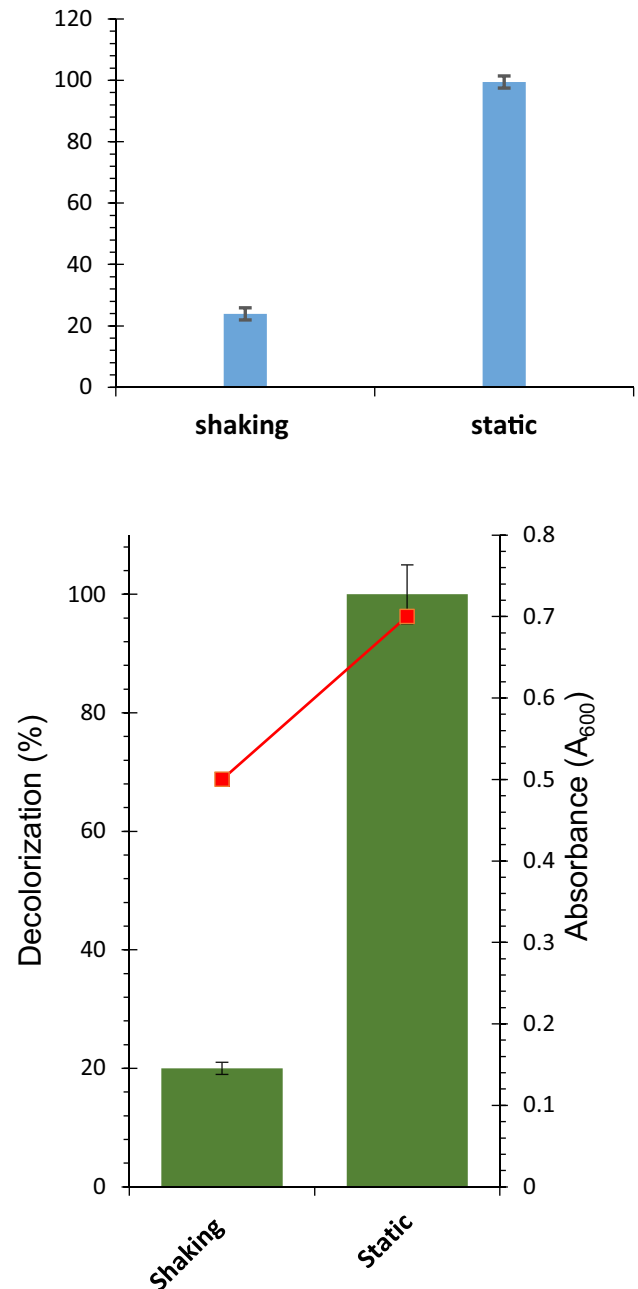
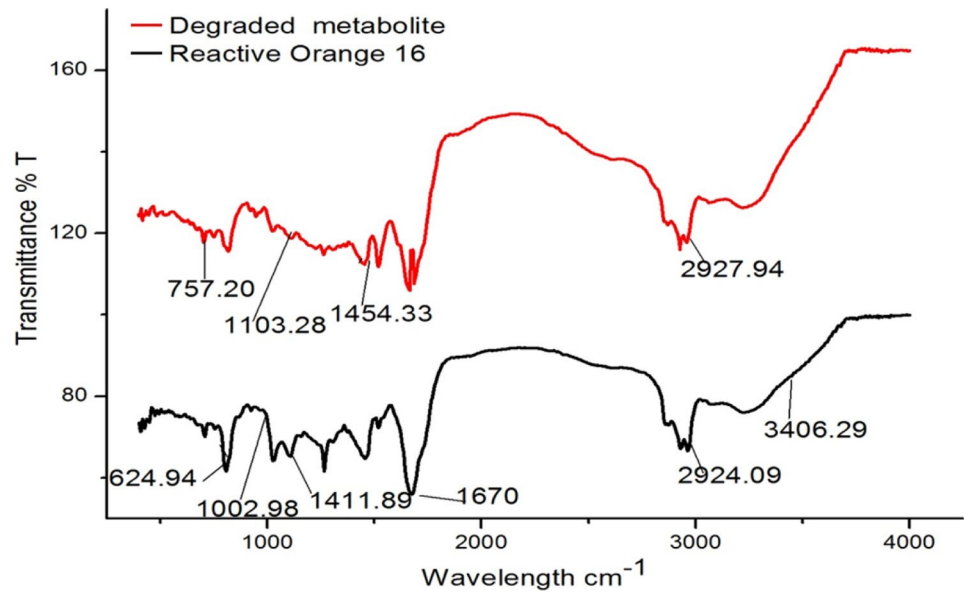


Fig. 5 Decolorization of reactive orange 16 by *Bacillus stratosphericus* SCA1007 under shaking/static conditions

Fig. 6 FTIR spectra of **a** degraded product and **b** reactive orange 16 dye, respectively



range of pH 4–11. Increase in decolorization percentage from pH 4 to 7 was observed. The optimum pH for decolorization of reactive orange 16 was found to be pH 7, at which complete decolorization of dye was recorded within 10 h of incubation (Fig. 4b). Mostly, azo dye decolorizing microbes have been reported to degrade dye at pH near neutral. *Alcaligenes aquatilis* showed maximum decolorization of Synozol Red at pH 7 (Ajaz et al. 2019). Reduced pH has a negative impact on decolorization efficiency of bacteria. Negative impact is due to formation of protonated azo dye at low pH. This alteration in chemical structure of dye hinders with bacterial decolorization (Deng et al. 2008).

To study the impact of temperature on dye decolorization, a range of temperatures from 4 to 55 °C was investigated. In this experiment, the initial dye concentration and the pH were fixed at 150 mg/L and 7 respectively. Results showed that the impact of temperature on decolorization was prominent over the examined range. The dye decolorization increased in proportion to the increase in temperature up to a maximum of 35 °C. On raising the temperature to 40 °C and above, there was drastic decrease in decolorizing potency of microorganisms. Thus, 35 °C was found to be optimal temperature for decolorization of reactive orange 16 (Fig. 4c). The result obtained is in accordance with a previous study which showed that 35 °C is the optimum temperature for maximum decolorization of reactive orange 16 by *Pseudomonas* sp. (Patel et al. 2013). Denaturation of catabolic enzymes might be the cause of decrease of metabolic activity at higher temperature (Chang and Lin 2001). In another study by a Masarbo et al. 2018, *Bacillus* sp. strain AK1, *Lysinibacillus* sp. strain AK2, and *Kerstersia* sp. strain VKY1 were able to decolorize sulfonated azo dye (Methyl

Orange) at 35 °C within 18 h of incubation. Ambient temperature for maximum decolorization of reactive red 198 by consortia consisting of *Enterococcus faecalis* and *Klebsiella variicola* was found to be 37 °C. On further increasing the temperature to 40 °C, there was decline in decolorization percentage (Eslami et al. 2019).

The dye effluents contain high amounts of salt dissolved in it (Cui et al. 2014). The effect of salinity on the decolorization capacity of bacterial isolate was checked. The experiment was conducted using different concentrations of NaCl in a range of 0.1–7%. It was found that SCA1007 was tolerant to a wide range of salinity. Maximum decolorization of dyes was observed for up to 4% of salt concentration after 10 h of incubation (Fig. 4d), followed by decrease in decolorization. Similar results have been reported, where *Bacillus fusiformis* strain KMK5 could decolorize Acid orange 10 effectively within 48 h in 0.5–3% salt concentrations (Kolekar et al. 2008). In another study, the percentage decolorization using *Klebsiella* sp. was maintained above 80% at 1–4% salt concentrations at 48 h (Cui et al. 2014). A decline in decolorizing efficiency by microorganisms was probable due to increased salt concentration, which causes plasmolysis of cells which ultimately leads to decline in cellular activity (Wood 2015). *Bacillus stratosphericus* SCA1007 could efficiently decolorize reactive orange 16 over a wide range (1–4%), unlike in case of *Comamonas acidovorans* MTCC 3364 which showed tolerance up to 2% of sodium chloride (Rudakiya and Pawar 2013).

The decolorization of reactive orange 16 in static and shaking culture conditions was studied. The decolorization potential of SCA1007 was adversely affected in shaking condition. Highest decolorization was achieved under static culture condition within 10 h of incubation under optimized

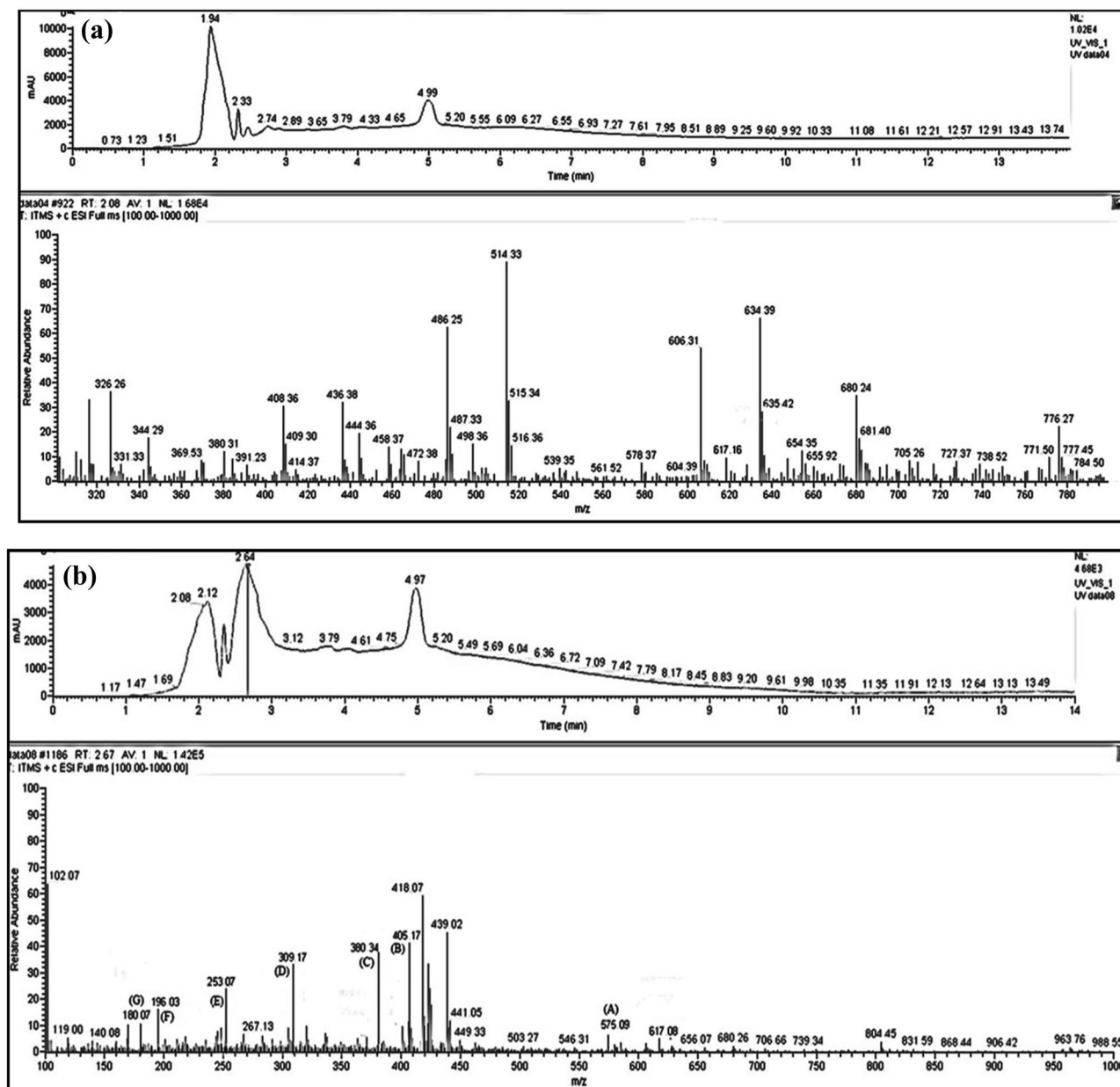
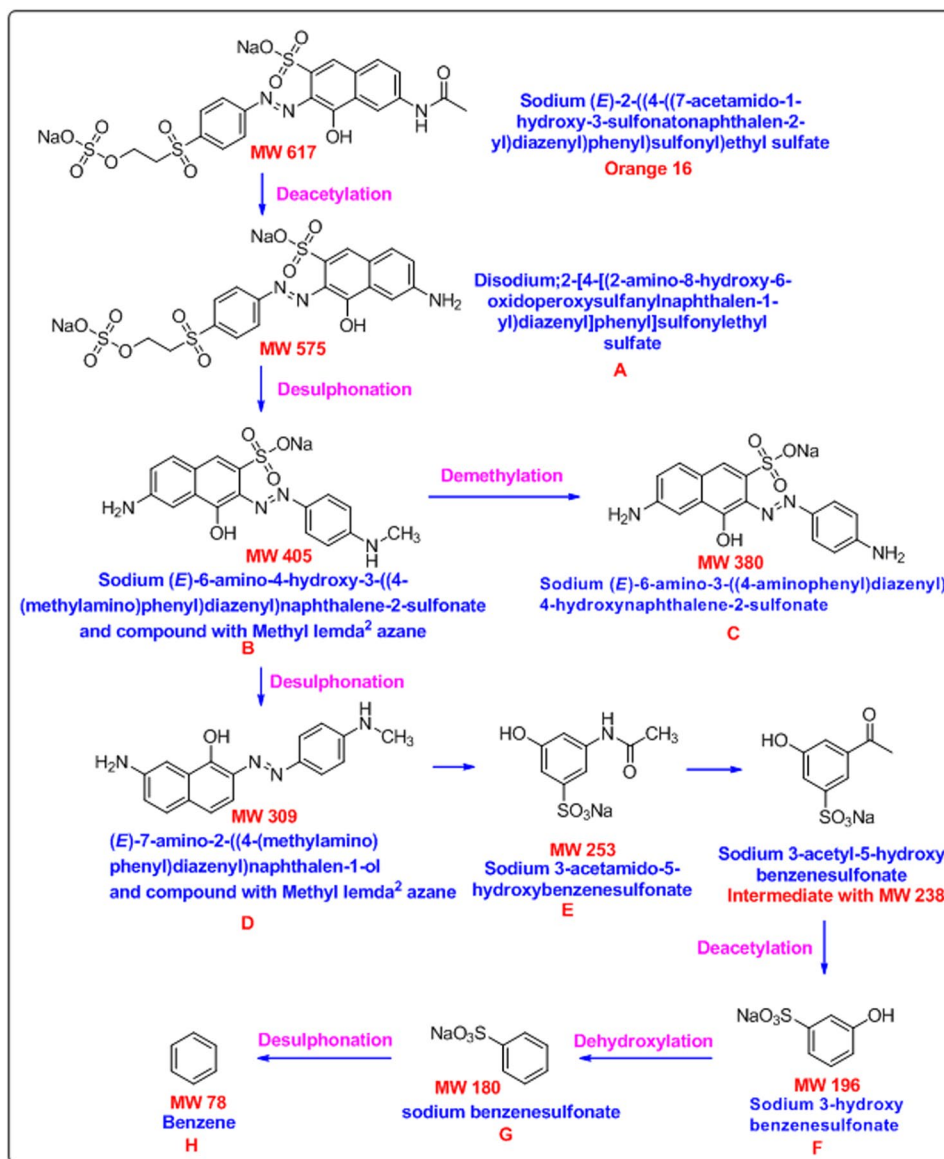


Fig. 7 LC–MS/MS spectrum of (a) reactive orange 16 and (b) representative mass spectra of selected peak of degraded products formed during the decolorization process by *Bacillus stratosphericus* SCA1007

culture condition (Fig. 5). Similarly, *Nocardiosis alba* showed decolorization of reactive orange 16 when incubated under static conditions. On the other hand, under shaking condition, culture grew well with almost negligible decolorization (Shobana and Hangan 2012). *Alcaligenes aquatilis* showed maximum decolorization of Synozol Red 6HBN at 37 °C, pH 7 under static culture condition (Ajaz et al. 2019). This may be because of the competition between oxygen

and azo compounds for the reduced electron carriers under aerobic condition (Mabrouk and Yusef 2008). Other authors have also reported that for efficient color reduction, shaking, and vigorous aeration should be avoided (Chen et al., 1999; Khehra et al., 2006a, b). Aerobic process inhibits dye reduction process. This is because the electron released due to oxidation of electron donors by the cells are preferred to reduce oxygen instead of azo dye (Yoo et al. 2001).

Fig. 8 Proposed biodegradation pathway of reactive orange 16 using *Bacillus stratosphericus* SCA1007 with the identification of different degradation intermediates by LC–MS/MS. The compound in brackets was not found



Degradation analysis

The FTIR spectral of control dye (reactive orange 16) and its degraded products by *Bacillus stratosphericus* SCA1007 were compared. The spectral of reactive orange 16 dye exhibited peak at 624 cm^{-1} for C–C stretching. Bending of C–N cm^{-1} was indicated at 664 cm^{-1} . The peak at 837 cm^{-1} represents S=O stretching. Bending of C–CH₃ was displayed at 1002 cm^{-1} . A peak at 1049 cm^{-1} represents C=O stretching. A peak at 1411 cm^{-1} showed stretching vibration of the benzene ring. N=N stretching was displayed by 1670 cm^{-1} . Peak at 2924 cm^{-1} indicates presence of CH₃ and CH₂ groups and peak at 3406 cm^{-1} indicates N–H stretching. A slight change in IR spectra of peaks of degraded products as compared to control dye was observed. The peak at 757 cm^{-1} showed C–N stretching. Bending of C–H was

indicated by 1103 cm^{-1} . Stretching of C–OH and N=O was indicated by 1302 cm^{-1} and 1452 cm^{-1} , respectively. The peak at 2959 cm^{-1} indicates C–H stretching. Similar FTIR results were observed for reactive orange 16 dyes degradation by bacteria (Telke et al. 2009) (Fig. 6).

Liquid chromatography- mass spectrometry analysis (LC–MS)

It was observed from the LC–MS/MS spectra of both dye and its biodegraded metabolites that there has been change in the pattern. The several compounds obtained after dyes decomposition at different m/z ratios confirmed the biodegradation of dyes by *Bacillus stratosphericus* SCA1007 (Fig. 7). Reactive orange 16 having molar mass 617 g/mol was biodegraded by *Bacillus stratosphericus* SCA1007. During the process of degradation, acetyl

Fig. 9 Toxicity study of dyes (a) reactive orange 16 and its degraded metabolite on germination of *Vigna radiata* seeds



Table 2 Phytotoxicity of reactive orange 16 and its degradative product

Test Parameter	water	Reactive orange 16 dye	Degradative product of reactive orange 16
Germination %	100	70	80
Length of root (cm)	3.35 ± 0.23	1.5 ± 0.19	2.63 ± 0.36
Length of shoot (cm)	13.68 ± 0.34	6.35 ± 0.15	11.15 ± 0.37

*The mean was calculated with the standard deviation (\pm), which was statistically analyzed by Student's *t* test (with > 95 percentage confidence level; $p < 0.05$)

group having *m/z* 43 was removed which leads to the formation of disodium;2-[4-[(2-amino-8-hydroxy-6-oxidoperoxysulfonyl)naphthalen-1-yl]diazanyl]phenyl]sulfonylethylsulfate (A) 575 (*m/z*). At this point desulfonation takes place which gives rise to sodium (E)-6-amino-4-hydroxy-3-((4-(methylamino)phenyl)diazanyl)naphthalene-2-sulfonate (B) 405 (*m/z*). Further reaction was followed by two processes (i) removal of methyl group which gives an intermediate sodium (E)-6-amino-3-((4-aminophenyl)diazanyl)4-hydroxynaphthalene-2-sulfonate (C) 380 (*m/z*) and (ii) desulfonation which leads to formation of 7-amino-2-(*p*-tolylidiazanyl)naphthalen-1-ol compound with methyl-12-azane (1:1) (D) 309 (*m/z*). After this step removal of aniline group, addition of sulfur and sodium and deamination reaction takes place which gives an intermediate sodium 3-acetamido-5-hydroxybenzenesulfonate (E) 253 (*m/z*). This step is followed by removal of ammonia and acetyl group by formation of sodium 3 hydroxy 5 acetylbenzene sulphonate (F) 196 (*m/z*). Further removal of -OH from (F) 196 (*m/z*) leads to formation of benzene (G) 180 (*m/z*) (Fig. 8). Similar degradation pattern has been observed by Soni et al. (2015).

Toxicity study

The release of effluent from textile industry in the form of treated and untreated wastewater affects the soil fertility, hence agricultural productivity. It is necessary to evaluate the toxicity of the dye before and after degradation because environmental health criteria require for both the removal

and detoxification of pollutants. Since dyes are harmful to environment, it is important to check the microbial as well as phytotoxicity of dye and its degraded products. Phytotoxicity study was performed on *Vigna radiata* towards dyes and their degraded products by calculating seed germination (%). Germination of seeds was more with degraded metabolite and distilled water, as compared to dyes, indicating a less toxic effect of degradation products as compared to dyes. The present study revealed that biodegradation of reactive orange 16 by *Bacillus stratosphericus* SCA1007 led to detoxification and production of less toxic metabolites, thus suggesting bio-treated wastewater containing dye could be used for agricultural purpose (Fig. 9) (Table 2). For analysis of microbial toxicity, the inhibition zone was found to be (4 mm) with control dyes (reactive orange 16), while its degraded metabolite displayed no growth inhibition.

Conclusion

The study concludes that *Bacillus stratosphericus* SCA1007 can be utilized effectively for complete decolorization of reactive orange 16 at a dye concentration of 150 mg/L within 10 h at pH 7 incubated at 35 °C. The bacterial isolate could also tolerate salinity as high as 4%. It was also observed that dye decolorization by the isolated strain was by biological degradation. This is the first report of reactive orange 16 dye degradation using *Bacillus stratosphericus* SCA1007. Dye

degradation property of *Bacillus stratosphericus* SCA1007 can be further used as a basic tool for bioremediation of various dyes in textile effluents.

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Author contribution Conceptualization, methodology, original draft preparation, and review and editing: Kriti Akansha. Liquid chromatography-mass spectrometry analysis (LC-MS): Manish Kumar. Figures: Ajar Nath Yadav. Supervision: Shashwati Ghosh Sachan and Debashis Chakraborty.

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Code availability Not applicable.

Declarations

Ethics approval Not applicable.

Consent to participate Not applicable.

Consent for publication Not applicable.

Conflict of interest The authors declare no competing interests.

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