# Biodegradation of Crystal Violet dye using potential bacteria *(Enterobacter* sp. CV-S1) isolated from textile effluent (#23440)



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2

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# Biodegradation of Crystal Violet dye using potential bacteria (*Enterobacter* sp. CV-S1) isolated from textile effluent

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Industrial effluent containing textile dyes is regarded as a major environmental concern in the present world. Crystal Violet is one of the vital textile dyes of triphenylmethane group, widely used in textile industry, known for its mutagenic and mitotic poisoning nature. Bioremediation, especially through bacteria is becoming an emerging and important sector in effluent treatment. This study aimed to isolate and identify a unique bacterial strain from textile effluent which enables to degrade Crystal Violet dye at a significant level. Newly identified bacteria *Enterobacter* sp. CV–S1 confirmed by 16s rDNA sequencing, was found as a potential bioremediation biocatalyst in the aerobic degradation/de-colorization of Crystal Violet dye. Complete decolorizing efficiency was observed in mineral salt medium containing up to 150 mg/l of Crystal Violet dye by 10% (v/v) inoculums of *Enterobacter* sp. CV-S1 tested under 72 hours of shaking incubation at temperature 35°C and pH 6.5. The efficiency of degrading triphenylmethane dye by this isolate, minus the supply of extra carbon or nitrogen sources in the media highlights the significance of larger-scale treatment of textile effluent.

# Biodegradation of Crystal Violet Dye Using Potential Bacteria (*En* ) Isolated from Textile Effluent

i suggest either of the following as the title
1. Characterisation of crystal violet degrading bacteria from textile industry effluents
2. Biodegradation of crystal violet dyes by bacteria isolated from textile industry effluents.

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sk biswas should be removed

the abstract should be restructured and the method explicitly stated.

this study aimed to isolate and identify crystal violet degrading bacteria from industrial effluents with potential use in bioremediation.

this is a conclusion.

**Abstract**

Industrial effluent containing textile dyes is regarded as a major env in the present world. Crystal Violet is one of the vital textile dyes of triphe idely used in textile industry, known for its mutagenic and mitotic poisonin ation, especially through bacteria is becoming an emerging and important se ment. This study aimed to isolate and identify a unique bacterial strain from textile effluent which enables to degrade Crystal Violet dye at a significant level. Newly identified *Enterobacter* sp. CV–S1 confirmed by 16s rDNA sequencing, was found as a potential bioremediation biocatalyst in the aerobic degradation/de-colorization of Crystal Violet dye. Complete decolorizing efficiency was observed in mineral salt medium containing up to 150 mg/l of Crystal Violet dye by 10% (v/v) inoculums of *Enterobacter* sp. CV-S1 tested under 72 hours of shaking incubation at temperature 35°C and pH 6.5. The efficiency of degrading triphenylmethane dye by this isolate, minus the supply of extra carbon or nitrogen sources in the media highlights the significance of larger-scale treatment of textile effluent.

## 1. Introduction

The textile industry plays a vital role in the global economy as well as in our daily life, concurrently turn into one the main source of environmental pollution in the world in terms of quality and quantity (Mondal et al. 2017). The textile industry consumes a larger volume of water in which almost ninety percent appears as wastewater. Textile wastewater contains the different type of dyes as the major pollutant which is not only recalcitrant but also imparts intense color to the waste effluent. The chemical reagents used in the textile se chemical composition ranging from inorganic to organic molecules. Inappropriate disposal of textile wastewater causes serious environmental problems that affect the aquatic organism adversely (Subhathra et al. 2013).

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cite more ref.

The wastewater produced from the textile, dye and dyestuff industries is a complex combination of various inorganic and organic materials. Dyes commonly have a synthetic origin and complex aromatic molecular structures which create them more stable and more difficult to biodegrade. The textile industries consume the largest amount of dyestuffs, at nearly 60-70% of over 10000 synthetic dyes is estimated to be over $7\times10^5$ metric tons which include a wide range of activities from the preparation of the raw material to pretreatment as well as dyeing and finishing of textile material (Jana et al. 2015; Sriram et al. 2013). Due to the wide range of dyes, fibers, process auxiliaries and final products during the dyeing processes, ample amount ( about 10–90%) of dye-stuffs that do not bind to the fibers were released into the sewage treatment system or the environmental water (Abadulla et al. 2000; Zollinger 2003). Dye wastes represent one of the most awkward groups of pollutants because they easily may recognize by naked eyes and are non-biodegradable (Mojsov et al. 2016). Triphenylmethane dyes are synthetic compounds widely used in various industries and their removal from efflu o their higher degree of structural complexity (Morales-Álvarez et al. 201 complex mixture in textile effluent directly indicates the water has been polluted, and this highly colored effluent is forthrightly responsible for polluting the receiving water. As a textile dye effluent disposal in aqueous ecosystems leads adverse impact in terms of chemical oxygen demand (COD) and high biological oxygen demand (BOD), and their metabolites lead to toxic, carcinogenic, mutagenic effect to flora and fauna which ultimately cause severe environmental problems worldwide (Mittal et al. 2005; Sharma et al. 2009).

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move to another paragraph and recast the sentence

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Due to their toxic, mutagenic and carcinogenic properties as well as their [illegible]e-coloration of natural waters, the release of dyes and their metabolites into the environment is highly concerned (Khadijah et al. 2009). Thus, precise attention should be taken into consideration on the utilization of dyes industrially. Inadequate method has been reported yet to decolorize textile effluents economically. For the removal of synthetic dyes from the water bodies, a number of physicochemical methods, such as filtration, specific coagulation, use of activated carbon and chemical flocculation, have been used (Olukanni et al. 2006; Verma et al. 2012). Using these expensive physiochemical methods, vast amounts of sludge is produced, which resulted into the secondary level of land pollution (Shah 2013). For this [illegible] economically inexpensive and eco-friendly removal techniques of the polluting dyes are requisite. As a potential alternative, the biological process including several taxonomic [illegible] microbes such as bacteria, fungi, yeast together with algae have been recognized growin[illegible] due to their cost-effectiveness, less sludge producing ability, and eco-friendly (Kalyani et al. 2009). Bacteria from different trophic groups can achieve a higher degree of dye-degradation and can process a complete mineralization of dyes under optimal conditions (Asad et al. 2007). Recently, microbial degradation of textile effluent has been reported as an economical and eco-friendly than physiochemical methods (Shah 2013).

Thus present study focused on the decolorizing capal[illegible] industrially important Crystal Violet (CV) dye. The [illegible] associated with isolation, growth characterization and identification of Crystal Violet dye degrading bacteria as well as determination of the optimum environmental conditions for degradation.

## 2. Materials and methods

### 2.1. Sample collection

The untreated water and sludge samples of textile effluent [illegible] two local thread dyeing plants of Kumarkhali, Kashia, Bangladesh. Four samples, named as water-1, water-2, sludge-1 and sludge-2, were collected from stagnant textile effluents from drainage canal. The color, pH and temperature of the samples were measured and recorded. The samples were collected in sterile plastic bottles, brought to the laboratory and kept at 4°C in refrigerator for preservation within 24 hours of sampling.

*the environment is a source of concern.*

*recast. There is urgent need for economically inexpensive and ecofriendly removal techniques.*

*the present study aimed to isolate and characterise crystal violet degrading bacteria from textile industry effluents for potential use in industrial bioremediation process.*

*state the name and locations of teh dyeing plants*

not necessary. remove.

## 2.2. Dyes, chemicals and microbiological media

Crystal Violet (CV) dye, belongs to Triphenylmethane group was procured from local thread dyeing plant of Kumarkhali, Kushtia, Bangladesh. The components (g/l) of dye decolorizing microbiological mineral salt (MS) medium were $K_2HPO_4$ (2), $(NH_4)SO_4$ (0.5), $KH_2PO_4$ (0.2) and $MgSO_4$ (0.05). The ingredients (g/l) of trace element (TE) solution were $FeSO_4$ (0.4), $MnSO_4$ (0.4), $ZnSO_4$ (0.2), $CuSO_4$ (0.04), KI (0.3), $Na_2MoO_4$ (0.05) and $CoCl_2$ (0.04) where the enrichment medium consisted of MS medium with glucose (0.1%), yeast extract (0.05%), peptone (0.5%) and NaCl (0.5%). In addition, nutrient broth and nutrient agar medium were used for the maintenance of the culture. Nutrient agar medium was used to maintain the stock cultures for screened bacterial isolates routinely and stored at 4°C temperature (Kaur et al. 2010).

recast and summarize. see Hamid et al. 2015.

## 2.3. Bacterial isolation

All four samples (untreated textile effluents) were used to isolate dye decol modified enrichment culture techniques as stated by Shah (2013). Steps involved enrichment, isolation and screening of dye decolorizing bacteria were (i) 1 ml of each sample was first diluted with 9 ml of sterilized water in a test tube separately. (ii) The stock was kept in static condition for few minutes to allow it to precipitate (iii) 1 ml supernatant from each diluted sample was transferred into each single test tube containing 9 ml enrichment medium (iii) A required amount of crystal violet dye solution was added into the stock to adjust the concentration 100 mg/l and was incubated to observe dye decolorization. (v) The species showing remarkable decolorization within 24 to 72 hours were streaked on 2% enrichment agar medium containing 100 mg/l of crystal violet dye (vi) Colonies of bacteria those exhibited a clear decolorization zone around them on enrichment agar medium were picked and cultured for 24 hours in MS medium containing 1 ml/l TE solution and 0.02 – 0.05% sucrose.(vii) An individual colony was then reintroduced into 9 ml enrichment medium containing 100 mg/l of Crystal Violet dye and was incubated to observe dye decolorization by individual bacteria. (viii) 10% of overnight cultured isolates were inoculated into 10 ml MS medium supplemented with 1ml/l TE solution and 100 mg/l crystal violet dye. (ix) The mixture was incubated to observe any decolorization activity. (x) 2 ml of the sample was then removed aseptically and centrifuged for 10 minutes at 10,000 rpm. (xi) The supernatant was used to determine the decolorization percentage of the added dye. (xii) Isolates exhibited that most decolorizing efficiency were selected and preserved (in nutrient agar up to 1 month and in 50% glycerol up to 6 months) for further studies.

159 2.4. Bacterial growth determination

160 In order to determine the effect of pH on bacterial grov teria CV-S1 was
161 cultured in nutrient broth. A twenty four hours observation mperate using 10
162 ml MS medium containing 10% (v/v) inoculums and 50 mg/l Crystal Violet dye of varying pH
163 (6.00, 6.50, 7.00, 7.50, 8.00 and 8.50) . To determine the optimum temperature, degradation assay
164 was performed from 30 to 40°C temperature using same stock condition at pH 6.50.

This is repeated under environmental parameters optimization.

165

166 *2*.5 DNA extraction and quality analysis

167 The genomic DNA extraction was performed using modified CTAB method presented by
168 Winnepenninckx et al. (Winnepenninckx et al. 1993) and the quality of DNA was analyzed
169 through Gel electrophoresis in 1% agarose gel.

as described by

170

171 2.6. 16S rDNA sequencing for bacterial identification

what do you mean by 16S DNA?

remove the name of the centre.

172 Partial sequence of 16S rDNA was carried by using Applied Biosystem 3130 at the Centre for
173 Advanced Research in Science (CARS), in Dhaka University, Bangladesh. The bacteria-specific
174 forward primer F27 (AGAGTTTGATCCTGGCTCAG) and reverse primer R1391
175 (GACGGGCGGTGTGTRCA) were used to amplify 16S rDNA fragments in PCR. The recipe of
176 a total of 25 µl of reaction mixture was dd$H_2O$ 14.75µl, $MgCl_2$ (25mM) 2µl, buffer (10×) 2.5 µl,
177 dNTPs (10mM) 0.5µl, Taq DNA Polymerase (5u/µl) 0.25µl, DNA template 1µl, forward primer
178 (10µM) 2 µl and reverse primer (10µM) 2 µl. The PCR amplification was performed by Swift™
179 Minipro Thermal Cycler (Model: SWT-MIP-0.2-2, Singapore) using the following program:
180 Denaturation for 5 minutes at 95°C, followed by 40 cycles of 40 seconds of denaturation at the
181 same temperature, annealing for 60 seconds at 65°C and elongation at 72°C for the first 2
182 minutes and followed by a final extension for 10 minutes. The sequence generated from the
183 automated sequencing of PCR amplified 16S rDNA was analyzed through NCBI BLAST
184 (http://www.ncbi.nlm.nih.gov) program to ascertain the possibility of a similar organism through
185 alignment of homologous sequences and the required corresponding sequences that were
186 downloaded. The evolutionary history was inferred using the Neighbor-joining method which
187 was performed on the Phylogeny.fr platform through online based software: Muscle (v3.7),
188 Gblocks (v0.91b), PhyML program (v3.0 aLRT) and TreeDyn (v198.3) (Dereeper et al. 2010;
189 Edgar 2004).

which of the isolates was molecularly identified and why?

190

191 2.7. Bacterial inoculums preparation

remove

192 The bacterial inoculum was prepared in a 50 mL sterile MS medium containing TE solution
193 together with 0.02-0.05% sucrose at temperature 35°C, pH 6.50 in 24 hour of shaking incubation
194 at 120 rpm.

195

196 2.8. Environmental parameters optimization

see comments under bacterial growth determination.

197 To optimize various environmental parameters (pH, t ye concentration and
198 inoculum size) for decolorization of Crystal Violet dye under 120 rpm shaking incubation 10 ml
199 of decolorizing medium (MS medium supplemented with TE solution +Crystal Violet dye) was
200 placed in 50 ml test tube. The mixture was inoculated with the 24 hours incubated bacterial
201 culture. Uninoculated crystal violet dye solutions were kept as control. Each experiment was
202 performed in triplicate and the mean values were recorded. To finalize optimum pH for dye
203 decolorization different pH gradients 6.00, 6.50, 7.00, 7.50, 8.00 and 8.50 were fixed using 1N
204 HCl and 3N NaOH. To determine the effect of temperature on decolorization, three temperature
205 gradients 30°C, 35°C and 40°C were used. To detect the effect of initial dye concentration, media
206 of different dye concentrations 50 mg/l to 200 mg/l were used while 8, 9 and 10% (v/v) of 24
207 hours incubated inoculums were inoculated for dye decolorization.

this method was not referenced.

208 2.9. Assay of dye degradation/decolorization

209 The rate of decolorization expressed as a percentage was determined by observing the reduction
210 of absorbance at absorption maxima (λ max). The uninoculated MS medium supplemented with
211 respective dye was used as a reference. 2 ml of reaction mixture was kept at different time
212 intervals, and the samples were centrifuged at 10,000 rpm for 10 min to separate biomass. The
213 concentration of dye was determined by absorbance at 660 nm. The measurement of absorbance
214 was done by the Photo-electric colorimeter (AE-11M, China). The color removal efficiency was
215 stated as the percentage ratio based on the following equation (Chen et al. 2003):

$$\text{Dye Degradation (\%)} = \frac{\text{Initial OD} - \text{Final OD}}{\text{Initial OD}} \times 100$$

216

217 **3. Results and discussion**

218 3.1. Physical characterization of textile effluent

The observation of physical characters of the collected textile effluent samples had revealed a high load of pollution indicators. The effluent colors of 3 samples were black due to a mixture of different chemicals and dyes and the rest was turquoise blue due to the fact that only turquoise dye was used in the dyeing process. The pH of the tested samples was slightly acidic to neutral. Temperature of the collected sample were around 18ºC due to winter season. Physical characters of textile effluent may vary due to the mixing of different types of organic and inorganic compounds derived from different environmental conditions. Chikkara and Rana had observed the colour and smell of textile effluent sample which was black and pungent respectively at pH 9.4 (Chhikara & Rana 2013) whereas Verma and Sarma tested textile waste-water which was brownish-black in color with unpleasant odor at pH 8.3 (Varma & Sharma 2011).

### 3.2. Isolation, screening and identification of dye degrading bacteria

On the basis of the decolorizing capacity and colony characters 3 isola om sludge-2 and the isolates were named as CV–S1, CV–S2 and CV–S3 after 72 hours of incubation CV-S1 yielded up to 81.25% Crystal Violet dye degradation while the rest two CV-S2 and CV-S3, exhibited up to 64.58% and 25% dye degradation respectively. Thus CV-S1 isolate was selected for identification. The Gram's staining indicates that CV-S1 was Gram-negative rod-shaped bacteria.

*reference should be made to appropriate figure*

*stating the result of gram staining is not necessary since its only for preliminary identification.*

*this section is continuation of the above section under isolation and identification. preferably merged together*

### 3.3 Genomic analysis of the isolated bacteria:

The best sequenced portion of 580 bp of 16S rDN bp exhi identity (99%) with *Enterobacter* sp. HSL69 according to isolation source. T corresponding aligned sequences, shown in Table 1, revealed that the phylogenetic relationship between the isolated bacterial strains with other related bacterial strains. During phylogenetic tree construction, strain CV–S1 had formed a new branch and the homology indicated that the strain CV–S1 is under the genus *Enterobacter*. Therefore, the isolate was identified and named as *Enterobacter* sp. CV–S1. The newly formed branch confirms that the identified *Enterobacter* sp. CV–S1 is a novel species of *Enterobacter* genus. Numerous potential dye decolorizing bacteria have been reported by scientists from the textile dye effluents, contaminated soil with dyes, dying waste disposal sites, and wastewater treatment plant (Khadijah et al. 2009; Pokharia & Ahluwalia 2013).

249

250 **Table 1.** Similarity between the isolated bacterial strains CV–S1 with other related bacteria found
251 in the GenBank database.

252

253

254 **Figure 1.** Phylogenetic tree analysis: The evolutionary history was inferred using the Neighbor–
255 Joining method. Highlighted bacterial strains are the isolated bacteria. The phylogenetic tree was
256 reconstructed using the maximum likelihood method implemented in the PhyML program (v3.0
257 aLRT) (Dereeper et al., 2010; Edgar, 2004).

258

259 3.4. Growth characteristics

260 The maximum growth of CV– S1was observed at temperature 35ºC and pH 6.50 while the
261 growth started decreasing within 60-72 hours of incubation. Bacterial growth is a complex
262 process associated with various anabolic and catabolic reactions. Eventually, these biosynthetic
263 reactions result in cell division (Raina MM 2009). As the growth-rate hypothesis (GRH) predicts
264 positive correlations among RNA content, phosphorus (P) content and biomass, such
265 relationships have been used to assume patterns of microbial activity, resource availability, and
266 nutrient recycling in ecosystems (Franklin et al. 2011). Hence, the degradation study required
267 considerable of 72 hours of cultivation time.

268

269 3.5. Influence of environmental parameters on crystal violet dye degradation

270 The results of degradation experiment of crystal violet dye by *Enterobacter* sp. CV–S1 was
271 involved with the effect of pH, temperature, initial dye concentration and inoculum size under
272 aerobic shaking condition at 120 rpm.

273

274 3.5.1. Effect of pH on dye degradation

275 This experiment revealed that the percentage of Crystal Violet dye degradation had improved
276 with the change of pH in the medium. The higher degradation was observed at pH between 6.50
277 to 7.00 while the highest decolorization rate (100%) was observed at pH 6.50 and lowest (12.5%)
278 was at pH 6.00. However, organism showed very low decolorization above pH 7.50 (Figure-2).

These observations indicated that the organism could treat efficiently neutral to weakly acidic dyeing waste. The effect of pH value on decolorization studies had shown a significant role as it regulated the surface charge of the bio-sorbent and the degree of ionization as well as the speciation of the dye solution. In case of red azo dye decoloration by *Aspergillus niger*, it was observed that the removal percent increased with the rise of pH and the maximum removal efficiency was reached (99.69%) at pH 9.0. Thereafter, whenever the pH value increases, the decolorization process appeared to decrease (Mahmoud et al. 2017).

**Figure 2:** The effect of pH on crystal violet dye degradation by *Enterobacter* sp. CV–S1

3.5.2. Effect of temperature on dye degradation

The maximum (100%) degradation was observed at temperature 35°C while at temperature 30°C and 40°C, the much adverse effect on the degradation was found and it was 37.5% in both cases (Figure-3). This might have occurred due to an adverse effect of lower and higher temperature other than 35°C on the enzymatic activities and the rate of chemical reaction directly related to temperature change. In addition, Bacteria need optimum temperature for growth. Since dye decolorization is a metabolic process, the change in temperature causes change from optimum results into a decline dye decolorization. The higher temperature causes thermal inactivation of proteins and probably affects cell structures such as the membrane (Shah 2013). Wanyonyi et al., observed the optimal temperature for decolorization of Malachite Green by using novel enzyme from *Bacillus cereus* strain KM201428 at 40°C (Wanyonyi et al. 2017).

**Figure 3.** The effect of temperature on crystal violet dye degradation by *Enterobacter* sp. CV–S1

3.5.3. Effect of initial dye concentration on dye degradation

It was observed that *Enterobacter* sp. CV–S1 can degrade 150 mg/l Crystal Violet dye within 72 hours. However in higher concentration, dye degradation rate was reduced remarkably (Figure-4). This may be due to the decreasing of nucleic acids content ratio, i.e., RNA/DNA, which

309 results to lowering the protein synthesis that inhibits cell division. The effect of dye concentration
310 on growth plays an important role in the choice of microbes to be used in the bioremediation of
311 dye wastewater, for instance high concentrations can reduce the degradation efficiency due to the
312 toxic effect of the dyes (Khehra et al. 2006). Furthermore, initial dye concentration provides an
313 essential driving force to overcome all mass transfer resistance of the dye between the solid and
314 aqueous phases (Parshetti et al. 2006).

315

316 **Figure 4.** Degradation of different concentration of crystal violet dye by *Enterobacter* sp. CV–S1

317

318 3.5.4. Effect of initial inoculums size on dye degradation

319 It was observed that the dye removal capacity had affected by the inoculums size used. The
320 degradation rate had decreased with the declining of inoculum sizes. The most significant result
321 (100%), was obtained when 10% inoculum was used. A similar pattern was observed and
322 reported by Ayed et al. (Ayed et al. 2009) that the dye removal capacity had increased
323 significantly with the escalation in inoculum size. After optimizing the environmental parameters,
324 100% degradation of 150 mg/l Crystal Violet was observed within 72 hours at 35°C and pH 6.50
325 under aerobic shaking condition by 10% (v/v) *Enterobacter* sp. CV–S1 without supplying extra
326 carbon and nitrogen source as shown in Figure-5 and Figure-6. Ayed et al. (2009) isolated
327 *Bacillus* sp., was able to decolorize 500 ppm crystal violet within 2.5 h under shaking condition
328 at temperature 30 °C and pH 7. In another study, the Brilliant Green dye (10 mg/l) removal by the
329 *Klebsiella* strain Bz4 in static conditions was observed 81.14 % after 24 hours of incubation and
330 100% dye removal was observed after 96 hours of incubation (Zabłocka-Godlewska et al. 2015).

331

332

333

334 **Figure 5.** 10% (v/v) of *Enterobacter* sp. CV–S1 showed 150 mg/l Crystal violet dye degradation
335 at temperature 35ºC and pH 6.50 under shaking condition (C, control; R1, R2, R3, replication 1,
336 2 and 3 respectively).

337

**Figure 6.** Degradation rate of Crystal Violet by *Enterobacter* sp. CV–S1 after optimizing the environmental parameters at different time intervals

## 4. Conclusion

recast based on the results obtained. the first two sentences are sufficient. the rest can be deleted. the last sentence too is ok.

In this study, the newly isolated bacteria *Ent* had demonstrated potentiality for its Crystal Violet dye degradation. The c g parameters of the study were concentration of dye (150 mg/l), inoculums s rature (35ºC), pH (6.50), with a rotation of 120 rpm. Under these aerobic *acter* sp. CV-S1 was able to decolorize Crystal Violet dye completely within 72 hours of incubation. The efficient degradation of Crystal Violet dye by this newly isolated bacterium highlights the significance of large-scale treatment of textile effluent. This experiment might meet the industrial demand as dye degradation occurs at the mesophilic range and near to neutral pH. It can be concluded from the overall findings that the isolated bacteria *Enterobacter* sp. CV–S1 could effectively be used as an alternative to the physical and chemical process of textile effluents as they are able to decolorize or degrade highly potential Crystal Violet dye.

## Funding

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## Acknowledgement

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## Table 1(on next page)

Similarity between the isolated bacterial strain CV–S1 and other related bacteria found in the GenBank database

Table : Similarity between the isolated bacterial strain CV–S1 and other related bacteria found in the GenBank database.

| Isolated strain | Closed bacteria | Accession no. | Identity (%) |
|---|---|---|---|
| CV–S1 | *Enterobacter cloacae* RU14 | KJ607595.1 | 99 |
| | *Enterobacter cloacae* RJ04 | KC990807.1 | 99 |
| | *Enterobacter cloacae*BM8 | JX514423.1 | 99 |
| | *Enterobacter* sp. HSL69 | HM461195.1 | 99 |
| | *Enterobactersacchari* SP1 | NR_118333.1 | 98 |

## Figure 1(on next page)

Phylogenetic tree analysis: The evolutionary history was inferred using the Neighbor–Joining method. Highlighted bacterial strains are the isolated bacteria. The phylogenetic tree was reconstructed using the maximum likelihood method implemented in the Ph

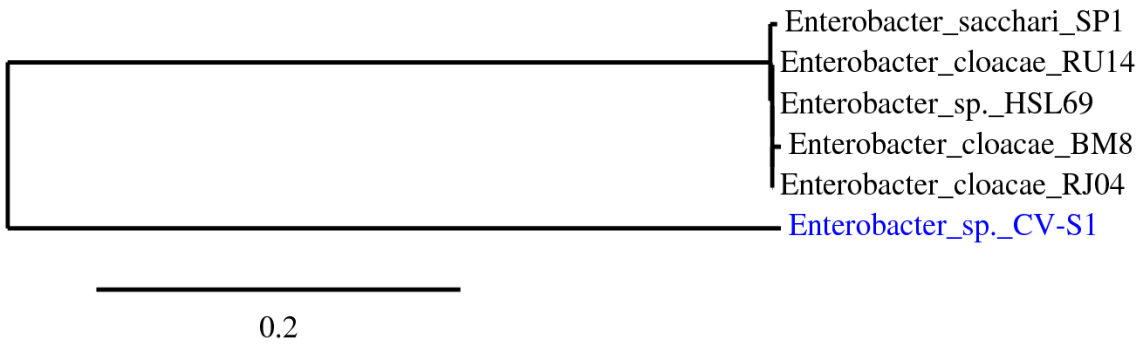


**Figure 1.** Phylogenetic tree analysis: The evolutionary history was inferred using the Neighbor–Joining method. Highlighted bacterial strains are the isolated bacteria. The phylogenetic tree was reconstructed using the maximum likelihood method implemented in the PhyML program (v3.0 aLRT) (Dereeper et al., 2010; Edgar, 2004).

## Figure 2(on next page)

The effect of pH on crystal violet dye degradation by *Enterobacter* sp. CV–S1

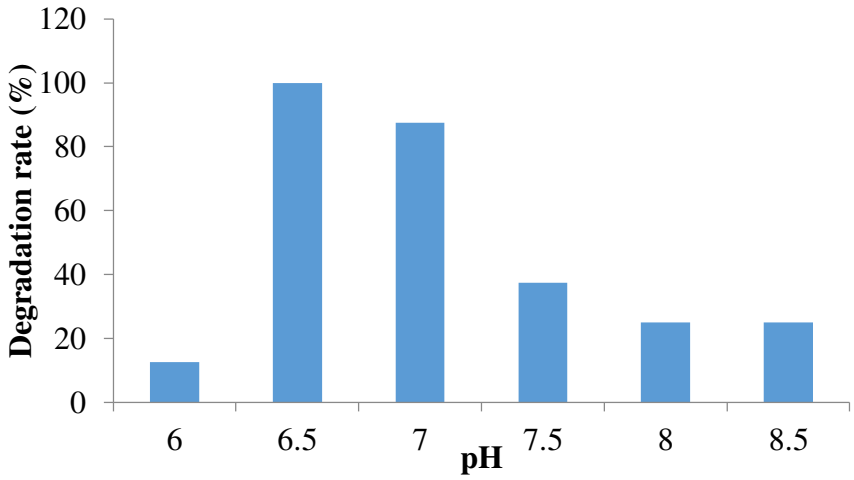


**Figure 2:** The effect of pH on crystal violet dye degradation by *Enterobacter* sp. CV–S1

## Figure 3(on next page)

The effect of temperature on crystal violet dye degradation by *Enterobacter* sp. CV–S1

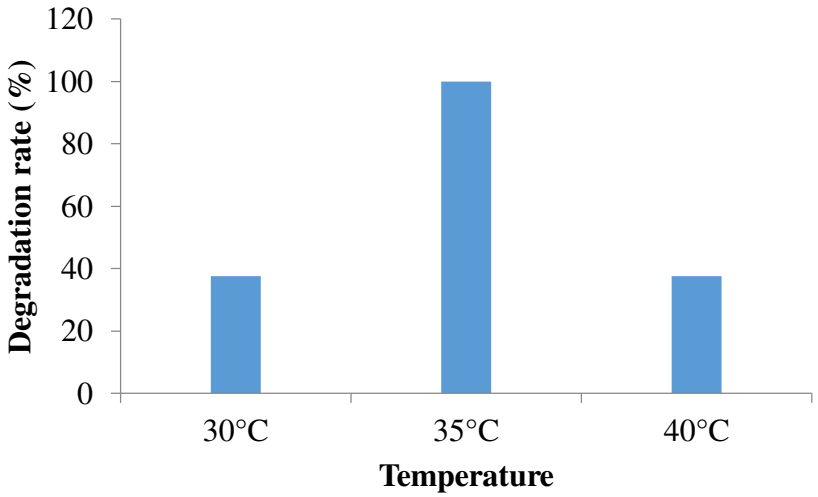


**Figure 3.** The effect of temperature on crystal violet dye degradation by *Enterobacter* sp. CV–S1

## Figure 4(on next page)

Degradation of different concentration of crystal violet dye by *Enterobacter* sp. CV–S1

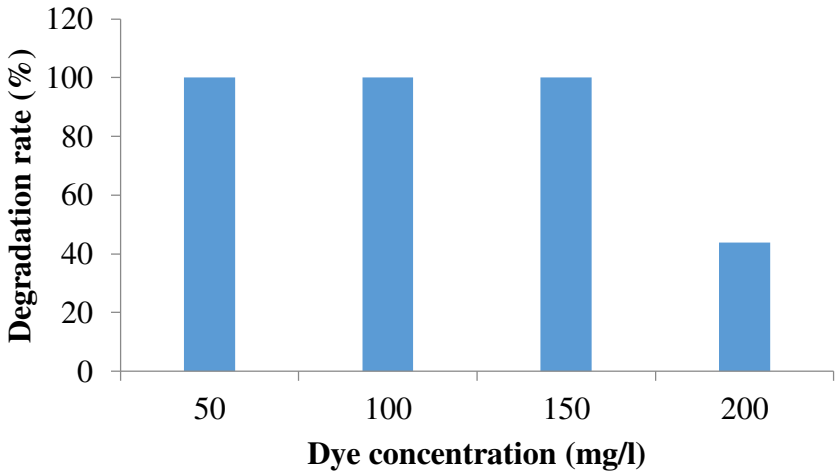


**Figure 4.** Degradation of different concentration of crystal violet dye by *Enterobacter* sp. CV–S1

## Figure 5(on next page)

10% (v/v) of *Enterobacter* sp. CV–S1 showed 150 mg/l Crystal violet dye degradation at pH 6.50 and temperature 35ºC under shaking condition (C, control; R1, R2, R3, replication 1, 2 and 3 respectively).

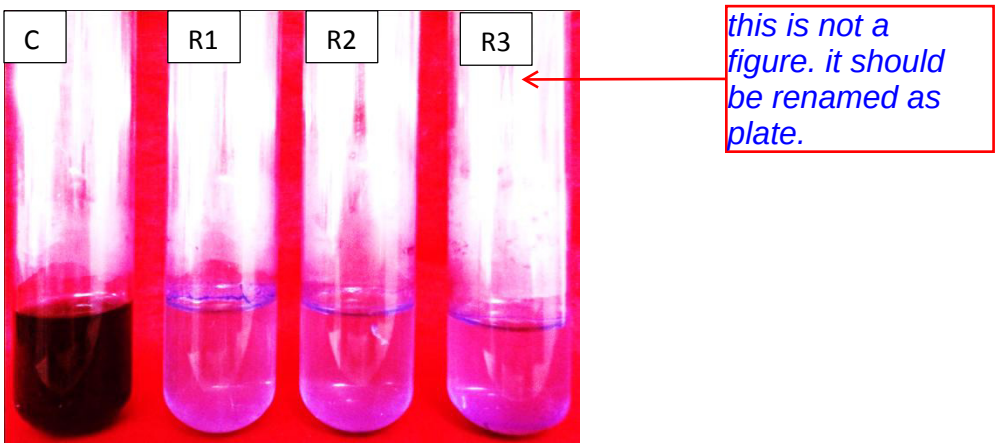


**Figure 5.** 10% (v/v) of *Enterobacter* sp. CV–S1 showed 150 mg/l Crystal violet dye degradation at pH 6.50 and temperature 35ºC under shaking condition (C, control; R1, R2, R3, replication 1, 2 and 3 respectively).

## Figure 6(on next page)

Degradation rate of Crystal Violet by *Enterobacter* sp. CV–S1 after optimizing the environmental parameters at different time intervals

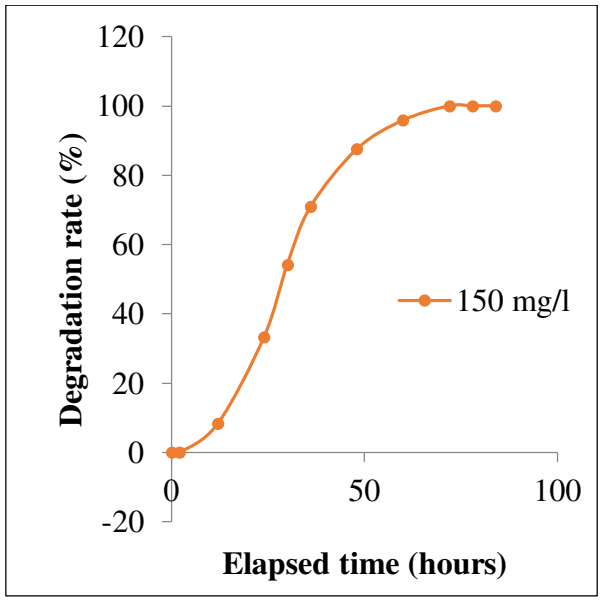


**Figure 6.** Degradation rate of Crystal Violet by *Enterobacter* sp. CV–S1 after optimizing the environmental parameters at different time intervals