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Role of Some Enzymes Produced by Egyptian Bacterial Isolate on Decolorization of Blue and Yellow Textile Dyes

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DECOLORIZATION of textile reactive azo dyes, blue and yellow, by 15 bacterial isolates collected from Egyptian arid soil was investigated. Of these, S8 isolate revealed a highest decolorization performance. Here, we identified S8 isolate on a molecular level based on type I chaperonin universal target sequence (cpn60). The most similar DNA sequence to the S8 DNA sequence was for *Klebsiella oxytoca* partial CDS (AB008147.1) coding for GroES and GroEL chaperon homologues (acronyms of cpn60) with 88% DNA sequence similarity. The impact of numerous exterior parameters to improve the decolorization abilities of this isolate was studied. A maximum decolorization activity occurred at a medium containing glucose, soybean husk at a C/N ratio of 12:1 supplemented with dye concentration of 100mg/L and amended with 3% (v/v) inoculum. Incubation for 4 days at 35°C±2 with shaking at 150rpm reached the decolorization activity to 89.35 and 78.23% for blue and yellow dyes, respectively. The ascending levels of bacterial enzymes like azo-reductase, phenol red manganese peroxidase and ascorbate oxidase indicated their prominent roles in dye degradation.

Keywords: Dyes, Decolorization, Degrading enzymes, *Klebsiella oxytoca*, cpn60, Gene sequence.

Introduction

Azo synthetic dyes are used extensively in textile, dying and paper painting due to their easy production, low cost, fastness and greatest variety of colors (Pandey & Dubey, 2012; Jafari et al., 2013 and Lade et al., 2015). The term azo dyes are applied to synthetic organic colorants that are characterized by one or more azo linkage (R1–N=N–R2) and by aromatic structures (Franciscon et al., 2009; Bayoumi et al., 2010 and Jafari et al., 2013).

The discharge of huge amounts of textile effluents contains harmful dyes that deteriorated water quality, affecting plant photosynthesis and decrease dissolved oxygen levels (Franciscon et al., 2009 and Javaid et al., 2016).

Several physiochemical methods are used for decolorization in the therapy of textile effluents, but they have many disadvantages. These methods are sometimes ineffective or very costly and often produce great quantities of toxic waste (Li et al., 2004 and Gregorio et al., 2010). Biological treatments offer alternative method which is not expensive, friendly to the environment and can completely degrade organic pollutants (Anjaneyulu et al., 2005 and Asad et al., 2007).

Many microorganisms belong to the bacterial community have been recently reported for their ability to decolorize azo dyes (Telke et al., 2008; Mendes et al., 2011; Feng et al., 2012 and Pandey & Dubey, 2012). Such oxidoreductase enzymes, like azo-reductase, tyrosinase, peroxidase and laccases, were associated with the decolorization process and reported in different bacterial studies

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(Telke et al., 2008; Mendes et al., 2011 and Javaid et al., 2016).

Type I chaperonin Cpn60 (known as Hsp60 or GroEL/ES) is a heavily preserved protein found in bacteria and some archaea (Wick et al., 2004 and Horwich et al., 2007). The universal target cpn60 is really an optimum molecular barcode which code for a protein coding region, thus it is more variable than 16S ribosomal ribonucleic acid (rRNA) genes among heavily associated taxa (Hill et al., 2006). Cpn60 DNA sequence comparisons are now useful for microbiological research findings for investigating phylogeny, evolution and microbiome (Hill et al., 2006 and Gupta & Sneath, 2007).

In the current study, we screened and isolated local bacterial isolates from arid soil for investigating their capacities to decolorize the commercially reactive azo dyes, reactive blue 19 and reactive yellow 4GL 150%. Most efficient isolate in decolorization was selected and identified. Several exterior parameters that enhance decolorization process by this selected isolate were studied. The activity levels of dye degrading associating enzymes, reductase and oxidase, were also assessed.

Materials and Methods

Sampling

An arid soil was sampled from Aswan Governorate to collect decolorization bacterial isolates. Two reactive textile dyes were used, namely, SIMFIX NAVY SP (C.I. reactive blue 19) and reactive yellow 4GL 150% (C.I. reactive yellow 160), and referred in the text as blue and yellow dyes, respectively. All commercial dyes were obtained from Amoun Company for chemicals, Cairo, Egypt.

Isolation and screening of textile dye decolorizing bacteria

The soil sample was serially diluted (10^{-6}) in sterile distilled water, spread on plates containing nutrient glucose agar and inoculated into 250 ml Erlenmeyer flasks having 200ml broth medium (Difco™ and BBL™ Manual, 2009) supplemented with 100mg/L either blue or yellow dye and incubated for 5 days at 30°C on a shaker with a speed of 150rpm. After incubation period, clear halo-zones surrounded the colonies indicating the decolorization of the dyes were measured (mm). These colonies were further picked, then re-cultivated several times for purity.

For broth culture, 10ml samples were collected, then centrifuged at 10000rpm for 10min at 4°C. Decolorization was measured in free supernatant using UV/Vis spectrophotometer (Unico S2100 series) at wavelength of 595nm for the blue dye (El-Bindary et al., 2016) and at 413nm for the yellow dye (Sabrien, 2016). Dyes removal percentage was calculated by the equation of: (Initial OD - Final OD X 100)/Initial OD) where OD is the optical density (Cheriaa et al., 2012). The most efficient bacterial isolate in decolorizing blue and/or yellow dye(s) in solid and/or liquid media was selected for further study.

Identification of the most decolorizing bacterial isolates

Morphological characteristics of the selected isolate was determined.

Bacterial DNA extraction and PCR amplification of cpn60

Bacterial genomic DNA had been isolated from S8 isolate using the GeneJet DNA purification kit (Thermo) according to the manufacturer's producer. DNA concentration and quality were estimated by the NanoDrop spectrophotometer (Thermo Fisher Scientific Inc., Burlington, ON). PCR amplification of cpn60UT was performed (Hill et al., 2002). A couple of primers were used for PCR amplification and sequencing, H1511 (5'-GACGTCGCCGGTGACGGCACCACCAC-3') and H1261 (5'-CGACGGTCGCCGAAGCCCG GGGCCTT-3'). The amplified DNA fragment migrated on 1% (w/v) agarose gel electrophoresis, then purified using QiaQuick gel extraction kit (Qiagen, Germany) following the manufacturer's procedure (Nimnoi et al., 2010). The purified PCR product was sequenced using the massive-dye terminator package abi 310 genetic analyzer (Applied Biosystems, America). The obtained nucleotide sequences were searched by basic local alignment search tool, BLAST, (http:/blast.ncbi.nlm.gov/Blast.cgi) to identify the closest known sequence related to our query.

Phylogenetic analysis

The evolutionary phylogenetic tree was inferred by using the Maximum Likelihood method and Tamura-Nei model (Tamura & Nei, 1993). Initial tree(s) for the heuristic search were obtained automatically by applying Neighbor-

Join and BioNJ algorithms to a matrix of pairwise distances estimated using the Maximum Composite Likelihood (MCL) approach, and then selecting the topology with superior log likelihood value. The tree is drawn to scale, with branch lengths measured in the number of substitutions per site (above the branches). This analysis involved 7 nucleotide sequences. Evolutionary analyses were conducted in MEGA X (Kumar et al., 2018).

Preparation of bacterial inoculum

Pure culture of selected isolate from the nutrient agar slant was transferred to the pre-sterilized 50 ml nutrient glucose broth medium in a 100ml conical flask and incubated at 30°C for 24hr with shaking at 150rpm. Approximately 2% (v/v) of cell suspension containing $6.8x10^7$ colony forming units (cfu)/ml was used as the inoculum.

Batch culture

Tested isolate was grown on **a** nutrient glucose broth medium under the same growth conditions formerly described (methods, isolation and screening) except that the standard inoculum was added at the rate of 2% (v/v). Periodically, samples were taken as needed for separating pellets and supernatants. Pellets were used to assess cell dry weight and to prepare a mixture of crude enzymes. Supernatants were used to determine the dye decolorization.

Incubation period

Samples (10ml) of a batch culture were drawn every 6hr up to 120hr under aseptic conditions. Collected samples were used to measure cell dry weight (pellets) and dye decolorization (supernatants).

Inoculum size

The impact of the inoculum size in range of 1 to 5% (v/v) on biomass and decolorization of dyes by selected isolate grown on nutrient glucose broth medium was examined.

Improvement of decolorization percentage by selected isolate

Batch culture of selected isolate was inoculated with a recommended inoculum size and incubation period; other growth conditions were constant as formerly described. The classical method of the medium improvement involving changing one variable at a time, while keeping others at fixed levels was followed (Saharan et al., 2011). The impact of nutritional and environmental variables on decolorization percentage was tested.

Nutritional factors

Carbon sources

Glucose (10g/L) of the medium was independently substituted with galactose, fructose, sucrose, lactose and mannose.

Nitrogen sources

The recommended nitrogen source was used. Six organic nitrogen sources (beef extract + peptone (control with 3+5g/L)), beef extract, yeast extract, peptone, tryptone, soybean husk extract and corn steep liquor) and inorganic sources (NH_4NO_3, NH_4Cl, $(NH_4)_3PO_4$ and $(NH_4)_2C_6H_6O_7$) were separately tested.

C/N ratios of 4/1, 7/1, 9/1, 11/1, 12/1 and 14/1, the best sources of carbon and nitrogen previously described were evaluated.

Dye concentrations

The impact of blue or yellow dye on decolorization was tested on a concentration of 50, 100, 200, 300 and 400mg/L.

Environmental factors

Optimum sources of carbon and nitrogen, C/N ratio and dye concentration were used in the subsequent experiments. The recommended level of each factor(s) was used when studying subsequent factors. The following factors were investigated:

a. Initial pH: The initial pH was adjusted at 5.5, 6.0, 6.5, 7.0, 7.5, 8.0 or 8.5.

b. Incubation temperature: The incubation temperature was tested at 20, 25, 30, 35, 40 and 45°C.

c. Aeration: The shaking of cultures was carried out at a speed of 0, 50,100, 150, 200 and 250rpm.

Crude enzyme preparation

After incubation, cultures were centrifuged at 10,000rpm for 5min at 4°C, and the pellets were washed three times with 5ml of a 50mM phosphate buffer solution (pH 7), then frozen at -20°C. The frozen pellets were resuspended in a 50 mM phosphate buffer solution (pH 7) with lysozyme (0.1g/ml), and incubated for 20min at 37°C, then centrifuged at 10,000rpm for 5min at 4°C. The collected supernatant was used as crude enzymes (Leelakriangsak & Borisut, 2012).

Enzyme assays

Azo-reductase (AR) activity was assayed by the reduction of the color density at 595 nm for the blue dye and at 413 nm for the yellow dye following the method of Pandey & Dubey (2012). About 200µl of crude extract was mixed with a 200µl of 100mg/L dye (as substrate) and 400µl of a sodium phosphate buffer (50mM, pH 7.0). The reaction was started by the addition of 200µl of a 2mM nicotinamide adenine dinucleotide hydrate (NADH) (7.09mg/ml) for 5min. In blank, the crude extract was replaced by a 0.1g/ml lysozyme dissolved in a phosphate buffer. One unit (U) of the enzyme activity was characterized as the amount of the enzyme deemed necessary to reduce 1µ mole of reactive dye/min.

The phenol red manganese peroxidase (MnP) activity was measured as recommended by Mercer et al. (1996). The reaction mixture containing 0.1mM $MnSO_4$, 0.1mM phenol red, 100mM potassium phosphate buffer (pH 7.0) and 1.0ml crude extract. The reaction was started by the addition of 50mM H_2O_2 for 1min, then stopped by the addition of 5M NaOH. Absorption was measured at 610nm against a blank without manganese. The oxidized phenol red molar extinction coefficient is $22mM^{-1}$ cm^{-1}. The enzyme activity (U) was described as the quantity of the enzyme demanded to oxidize 1 µ mole of substrate/ml/min.

The ascorbate oxidase activity was evaluated as reported by Nakano & Asada (1981). A reaction mixture containing 0.5ml of 10mM ascorbic acid solution (176mg L-ascorbic acid/100ml of HCl solution (1.0mM) and 1.0mM ethylenediaminetetraacetic acid (EDTA)), 0.5ml Na_2HPO_4 solution (10mM) and 1.0ml crude extract were mixed, then incubated at 30°C for 5min. About 3.0ml of HCl (0.2N) was added to end the reaction. The absorbance shift was measured every 30sec at 245nm for 5min against water (ε=$2.8mM^{-1}$ cm^{-1}). One unit (U) of the enzyme activity was represented as the amount of enzyme to oxidize 1µmol of ascorbate/ml/min.

Protein concentration

The concentration of protein was determined by Bradford (1976) using bovine serum albumin as standard.

Statistical analysis

All data was statistically analyzed using IBM ® SPSS® Statistics (2011) version 19 based on Duncan's Multiple Range Test (Duncan, 1955) at the level of 5%.

Results and Discussion

Screening and isolating dye decolorizing bacteria

An arid soil sample was collected from Aswan governorate for screening dye decolorizing bacteria. Fifteen isolates exhibited abilities to decolorize blue and/or yellow reactive dyes were isolated and purified. The decolorizing ability of each isolate was estimated by measuring the decolorization zone on solid medium and decolorizing percentage by spectrophotometer in liquid medium. These isolates varied greatly in their capabilities to decolorize blue and/ or yellow dyes. Out of these isolates, S8 isolate exhibited a maximum decolorizing ability for both dyes. The decolorization zone reached by this isolate to 33 and 21mm, and the decolorization percentage was 69.1 and 58.5% for the blue and yellow dyes, respectively. S8 isolate was therefore selected for further studies.

Molecular identification of S8 isolate

From the genomic DNA of bacterial genomic DNA, a PCR reaction for amplifying cpn60 was performed, then the PCR product was sequenced using Sanger method (methods). The DNA sequence from S8 isolate was identified by BLASTN search through the GenBank NR database. The most similar DNA sequence was for *Klebsiella oxytoca* partial CDS (AB008147.1) coding for GroES and GroEL chaperon homologues (acronyms of cpn60) with 88% DNA sequence similarity. The multiple sequence alignment by ClustalW and the evolutionary phylogenetic tree was generated using the Maximum Likelihood method (Fig. 1). These analysis involved seven nucleotide sequences including S8 tested isolate sequence and the most similar *Klebsiella oxytoca* partial GroES or GroEL CDS (AB008147.1, AY301254.1, AY301253.1, AY301252.1, AY301251.1 and AY301255.1). A Jalview multiple sequence alignment using ClustalW revealed conserved regions across the aligned DNA sequences. Of the tree, two clades (I and II) are indicated presenting the distance between our tested isolate and most similar sequences in the NR database from the Genbank. This may refer to identifying a new isolate. Further molecular studies are need for a complete identification.

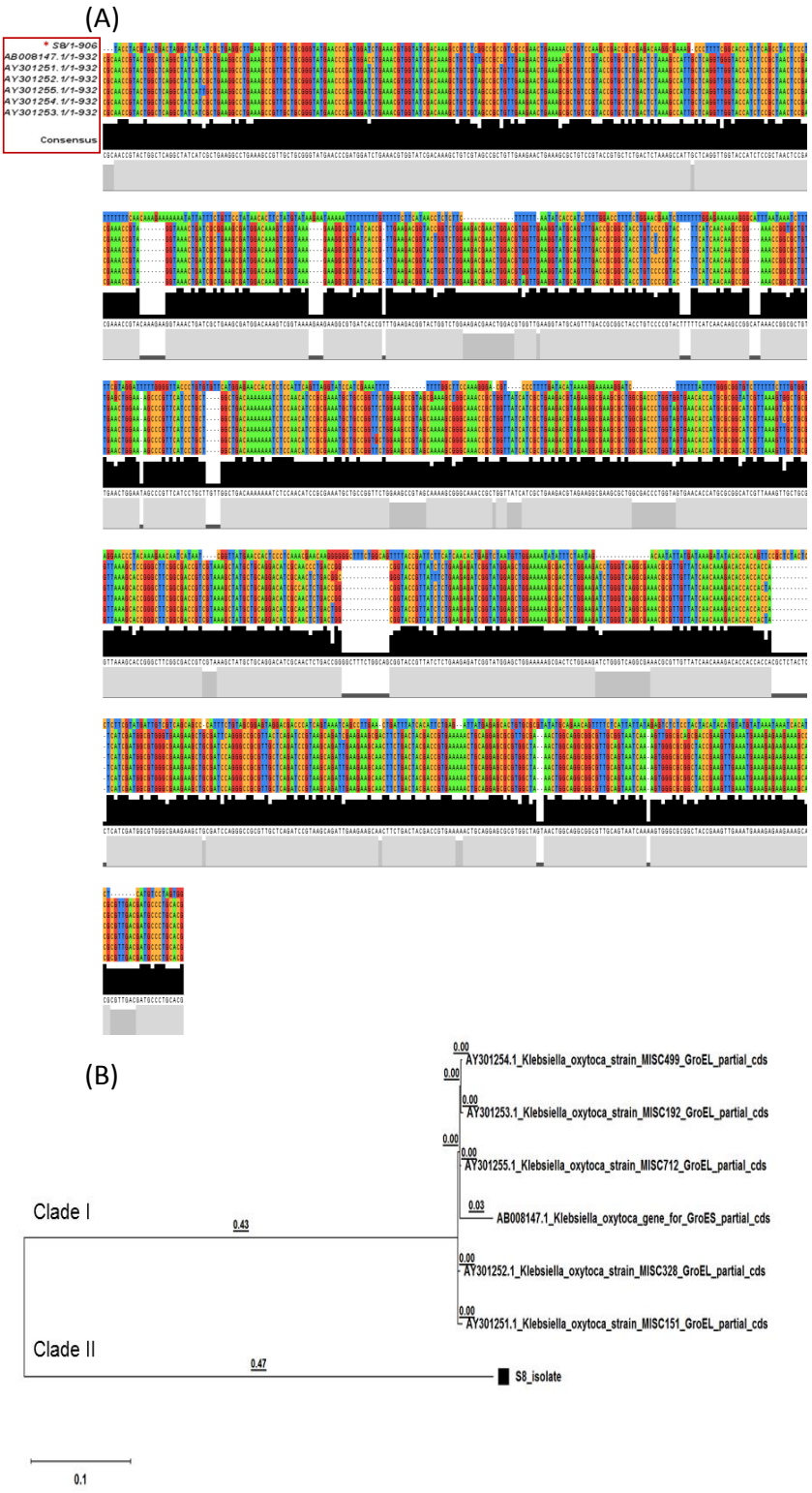

Fig. 1. A multiple sequence alignment and rooted phylogenetic tree of S8 and most similar six *Klebsiella oxytoca* GroEl and GroES CDS; (A) A Jalview multiple sequence alignment using ClustalW revealed conserved regions across the aligned DNA sequences. Red square represent S8 tested isolate along with the most similar sequences tagged with their accesition IDs from the GenBank, (B) Evolutionary analysis by Maximum Likelihood method [Two clades (I and II) are indicated. Numbers indicate the branch length, the scale bar for the branch length is 0.1].

Incubation period

On a batch culture of *S8 isolate* supplemented with blue or yellow dye, the bacterial growth showed successive increase up to 96 h and reached to 1.52 and 1.10g/L for the blue and yellow dyes, respectively (Fig. 2). Thereafter, the same bacterial growth ceased until the minimum was reached after 120hr. However, the growth was always higher in the blue than the yellow treatment.

The higher growth was related to decolorization percentage when it peaked after at 70.26 or 60.00% for blue and yellow dyes treatments, respectively, after 96hr.

Inoculum size

The bacterial growth medium was inoculated with increasing inoculum size of S8 isolate ranging from 1 to 5% (v/v). Data in Fig. 3 reveal the significant increase in cell mass and decolorization percentage by increasing the volume of the inoculum up to 3% (v/v). At that level, the cell dry weight reached 1.78 and 1.15g/L, while the percentage (%) of the decolorization were 77.42 and 64.47% for blue and yellow dye-treatments, respectively. This bacterial level may accelerate degradation and consumption of dye due to increasing nutrient uptakes, and diffusing dissolved oxygen (Rahman et al., 2005 and Abusham et al., 2009).

Improvement of decolorization percentage of blue and yellow dyes by S8 isolate

For improving decolorization percentage by tested isolate, some nutritional and environmental variables controlling the decolorization process of blue and yellow dyes were investigated. A medium supplied with 100 mg/l dye that amended with 3% (v/v) inoculum was used for this process. A method of medium improvement involved changing one factor at a time while keeping, others at fixed levels was formerly reported by Saharan et al. (2011).

Nutritional factors

Carbon source

Data in Table 1 reveal variation in decolorization percentage of the blue and yellow dyes by S8 isolate in response to carbon sources examined. The maximum percentage of decolorization was recorded in the present of glucose reaching to 77.42 and 64.47 % for the blue and yellow dyes, respectively. Glucose was previously found to increase decolorization percentage (Kapdan et al., 2000 and Velmurugan et al., 2015).

Nitrogen source

Batch cultures containing glucose was supplemented separately with sources of organic and inorganic nitrogen. The organic sources, in general, resulted a higher percentage of decolorization compared to the inorganic sources (Table 1). However, the highest percentage of decolorization was reported in response to soybean husk peaked at 77.48 and 65.01% for the blue and yellow dyes, respectively. The superiority of the organic over inorganic nitrogen sources in increasing decolorization percentage was reported in different organisms (Bhatt et al., 2005; Derle et al., 2012 and Lade et al., 2012).

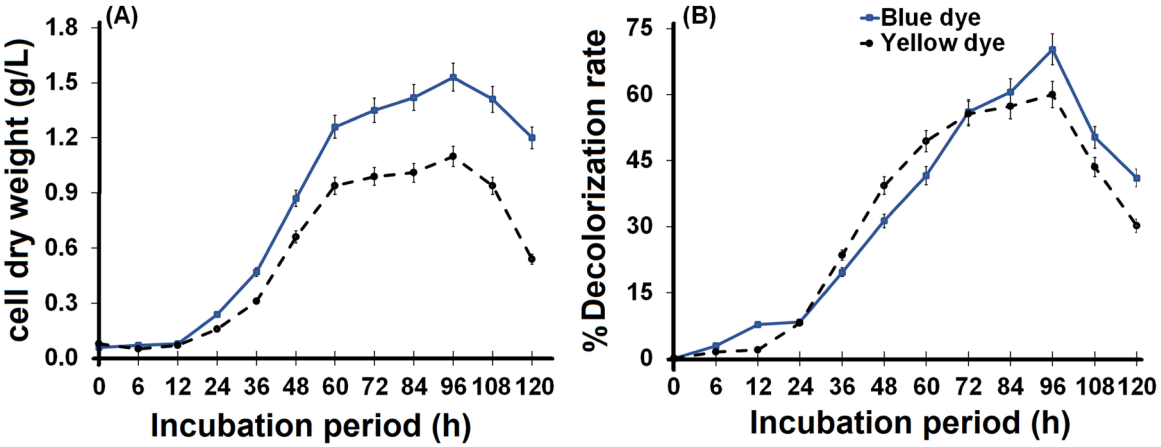

Fig. 2. Effect of incubation period on (A) S8 isolate biomass and (B) decolorization of blue and yellow dyes by S8 isolate [Error bar presented± standard error].

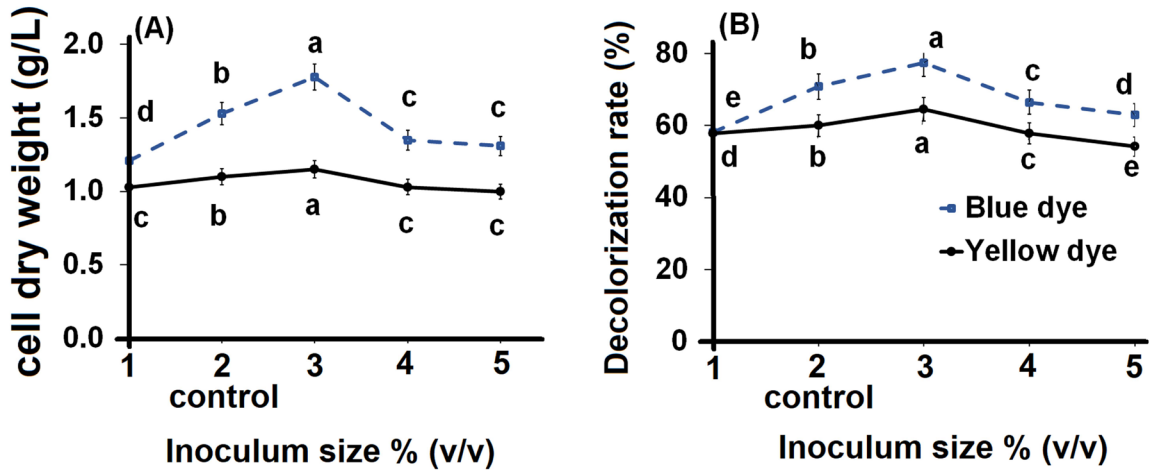

Fig. 3. Influence of inoculum size on (A) S8 isolate biomass and (B) decolorization of blue and yellow dyes by S8 isolate after 96hr of incubation period [Mean values are significantly different with distinct letters on top of points in the same line at P<0.05, error bar presented± standard error].

TABLE 1. Impact of nutritional factors on decolorization percentage of blue and yellow dyes by S8 isolate after 96hr of incubation period.

Factors			**% of decolorization**						
			Source/level						
Carbon			**Glucose***	**Galactose**	**Fructose**	**Sucrose**	**Lactose**	**Mannose**	
		Blu	77.42^{a}	66.73^{d}	71.50^{b}	68.85^{c}	15.07f	30.56^{e}	
		Yell	64.47^{a}	48.23^{d}	63.16^{b}	59.22^{c}	7.29^{f}	45.87^{e}	
Nitrogen	**Organic**		**BE+P***	**P**	**BE**	**T**	**YE**	**CSL**	**SBHE**
		Blu	77.42^{a}	68.85^{c}	60.44^{d}	40.43^{e}	75.53^{b}	75.00^{b}	77.48^{a}
		Yell	64.47^{b}	62.31^{c}	62.46^{c}	56.02^{de}	57.68^{d}	57.29^{d}	65.01^{a}
	Inorganic		**NH_4NO_3**	**NH_4Cl**	**$(NH_4)_3PO_4$**		**$(NH_4)_2C_6H_6O_7$**		
		Blu	10.82^{i}	13.18^{h}	24.00^{g}		35.00^{f}		
		Yell	7.72^{i}	9.10^{h}	15.28^{g}		26.49^{f}		
C/N ratio			**4/1**	**7/1***	**9/1**	**11/1**	**12/1**	**14/1**	
		Blu	49.09^{e}	77.48^{d}	79.54^{bc}	80.98^{b}	84.75^{a}	81.54^{b}	
		Yell	37.38^{e}	65.01^{cd}	65.70^{c}	66.78^{c}	72.20^{a}	69.44^{b}	
Dye concentration (mg/L)			**50**	**100***	**200**	**300**	**400**		
		Blu	84.12^{a}	84.75^{a}	56.06^{b}	36.10^{c}	16.45^{d}		
		Yell	72.16^{a}	72.20^{a}	62.96^{b}	35.15^{c}	20.95^{d}		

*= Control.
- Mean values are significantly different with varying characters in that same row at P<0.05.
- Blu= Blue, Yell= Yellow,
- BE+P= Bbeef extract + peptone, P= Peptone, BE= Beef extract, T= Tryptone, YE= Yeast extract, CSL= Corn steep liquor, SBHE= Soybean husk extract.

C/N ratio

Decolorization percentage was improved by increasing the C/N ratio reaching to 12:1 where the maximum decolorization was peaked at 84.75 and 72.20% for the blue and yellow dye treatments, respectively (Table 1).

Dye concentration

Decolorization percentage of the blue and yellow dyes was at a maximum level at a concentration of 100 mg/L reaching 84.75 and 72.20%, respectively (Table 1). The decolorization percentage continuously decreased during ascending the concentration of dyes which reached 400mg/L for both dyes. Such considerable decrease may be related to the toxic impact of dyes, changing of metabolic enzymes and accumulation of some by-products (Sponza & Işık, 2004 and Barakat, 2013).

Environmental factors

In general, decolorization efficiency of bacteria was known to be significantly affected by numerous of exterior conditions (Lade et al., 2015). A medium containing glucose and soybean husk with a C/N ratio of 12:1 supplemented with 100mg/L of the blue or yellow dye was found to be the best condition for our tested bacterial isolate.

Initial pH

pH study showed that the bacterial isolate (S8) was able to decolorize blue or yellow dye at a broad range of pH. However, the optimum pH was recorded to be 7.5 (Fig. 4 A). At that level, decolorization percentage increased from 84.75 to 86.41% for blue and from 72.20 to 77.58% for yellow treatments. A slight decrease in the decolorization performance was observed at a high pH (8.5). It is suggested that pH may be more clearly related to the transport of dyes across the cell membrane affecting decolorization efficiency (Lourenco et al., 2000).

Temperature

Azo-dyes decolorization collected data suggested that 35°C±2 was the best temperature for improving the decolorization of the blue and yellow dyes that reached to 89.35 and 78.23%, respectively (Fig. 4 B). Further increase or decrease in the temperature caused reduction of the dye decolorization performance. The reduction of decolorization percentage at a higher temperature (40-45°C±2) may be decrease the cell viability or suppress essential enzymes required for dye decolorization process (Kumar et al., 2009 and Jafari et al., 2013).

Aeration

Decolorization percentage inducted by shaking at a rate of 150rpm (Fig. 4 C). It seems likely that the shaking at a speed of 150 rpm is a best condition for higher oxygen transfer and nutrient distribution (Rahman et al., 2005 and Abusham et al., 2009). Lower or higher shaking rates resulted in considerable decrease in a decolorization percentage.

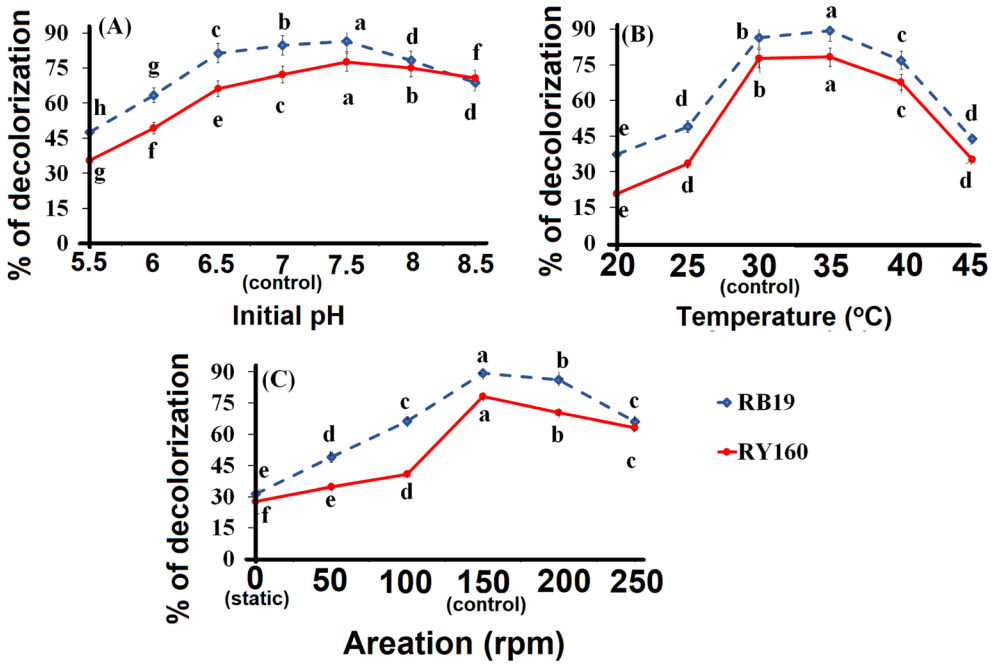

Fig. 4. Influence of initial pH (A), temperature (B) and aeration (C) on decolorization of blue and yellow dyes by S8 isolate after 96hr of incubation period [Mean values are significantly different with distinct letters on top of points in the same line at P<0.05, error bar presented± standard error].

The comparison of two culture conditions aerobic (at 150rpm) and static (at 0rpm) revealed the importance of shaking. It was clear that shaking greatly favored high percentage of decolorization that was 89.35 and 78.23% when compared to static culture 31.36 and 27.73% for the blue and yellow dye, respectively.

Studying different exterior parameters indicated that the mixture of the highest dye decolorization process for the blue and yellow dyes by S8 bacterial isolate as follows: A medium containing glucose and soybean husk at C/N a ratio of 12:1 supplemented with dye concentration of 100mg/L and amended with 3% (v/v) bacterial inoculum supported by incubation for 4 days at 35°C with shaking at 150rpm. The change in one factor at a time for improving growth conditions showed a successful increase in the decolorization percentage (Table 2). At the beginning decolorization percentage was 77.42 and 64.47% for blue and yellow dyes, respectively, then reached to 84.75 and 72.20% after optimizing nutritional factors. An additional increase was observed with optimizing environmental factors reached 89.35 and 78.23% for the blue and yellow dye treatments.

TABLE 2. Summary of the best sources and/or levels factors controlling decolorization of blue and yellow dyes by S8 isolate for 96hr of the incubation period.

Factor	Best	% of decolorization	
	Source/level	Blue dye	Yellow dye
Inoculum size	3% (v/v)	77.42	64.47
Nutritional factors			
Carbon	glucose (10g/L)	77.42	64.47
Nitrogen	soybean husk (8g/L)	77.48	65.01
C/N	12/1	84.75	72.2
Dye concentration	100mg/L	84.75	72.2
Environmental factors			
Initial pH	7.5	86.41	77.58
Temperature	35°C	89.35	78.23
Aeration	150 rpm	89.35	78.23

Enzymatic activities

The presence of the blue or yellow dye in the optimized growth culture of *S8 isolate* significantly stimulated the induction of azo-reductase (5.36 and 3.84U/mg protein), phenol red manganese peroxidase (2.74 and 2.44U/mg protein) and considerably lower levels of ascorbate oxidase (1.55 and 1.63U/mg protein), respectively (Table 3). Stimulating the activity of these oxidoreductase enzymes increased the ability of S8 isolate to decolorize the tested dyes. This indicates their role in the decolorization process. However, azo-reductase, laccases and peroxidases purified from several bacterial strains were reported as important azo dyes degrading enzymes (Kandelbauer & Guebitz, 2005; Telke et al., 2008; Pereira et al., 2009 and Mendes et al., 2015). Azo-reductases are kwon to break dawn azo bonds of azo dyes as initial step in the degradation process producing aromatic amines which are toxic, whereas oxidative enzymes phenol red manganese peroxidase and ascorbate oxidase confirmed the subsequent oxidation of the formed amines (Durán & Esposito, 2000; Kandelbauer & Guebitz, 2005; Franciscon et al., 2009; Rodríguez Couto, 2009 and Jafari et al., 2013).

The presence of oxygen normally inhibits the azo bond reduction by azo reductase (Franciscon et al., 2009; Jafari et al., 2013 and Mendes et al., 2015). In our study, decolorization of the two azo dyes by S8 bacterial isolate was evaluated. Higher decolorization of the blue or yellow dye (89.35 or 78.23%) was observed under aerobic condition, while were considerably low under static condition (31.36 or 27.73%). However, several trials revealed that the azo dye decolorization and degradation was under aerobic conditions (Blumel et al., 1998; Kumar et al., 2009 and Jafari et al., 2013).

TABLE 3. Role of azo-reductase, phenol red manganese peroxidase and ascorbate oxidase produced from S8 isolate on decolorization of blue and yellow dyes after 96hr of the incubation period.

Parameters	Specific enzyme activity (U/mg protein)		
	Control	Blue	Yellow
Azo-reductase	0.85^{c}	5.36^{a}	3.84^{b}
Phenol red manganese peroxidase	1.46^{c}	2.74^{a}	2.44^{b}
Ascorbate oxidase	1.15^{c}	1.55^{b}	1.63^{a}
Decolorization (%)		89.35^{a}	78.23^{b}

Mean values with different letters in the same row are significantly different ($P < 0.05$).

In light of our results, it seems likely that the sequential functions of reductive azo-reductase and oxidative manganese peroxidase and ascorbate oxidase participated effectively in decolorization, degradation and detoxification of blue and yellow dyes.

Conclusions

Azo dyes reactive blue and yellow dyes were mostly biodegraded by S8 bacterial isolate that is most similar to *K. oxytoca* (based on known GenBank NR database) under aerobic conditions. For improving the growth medium for a maximum decolorization percentage, we optimized a medium contained glucose, soybean husk, C/N a ratio of 12/1 with dye concentration of 100 mg/L at pH 7.5 supported by incubation for 4 days at 35°C±2 and shaking at 150rpm. Enzymatic studies stated the role of reductive azo-reductase and oxidative phenol red manganese peroxidase and ascorbate oxidase in the decolorization, degradation and detoxification of both tested dyes. Further initiatives should be provided to estimate the capacity of S8 isolate in a laboratory and pilot-scale for textile effluent treatments. In addition, decolorization ability of this isolate against a variety of azo dyes in textile effluents is needed. The initial molecular identification of our isolate referred to the similarity to *K. oxytoca,* but the DNA sequence similarity (88%) was not a strong support to identify our tested isolate to *K. oxytoca.* Further genomic studies are need; this could help for deciphering the properly new species bacteria.

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دور بعض الانزيمات المنتجة بواسطة عزله بكتيرية مصرية على إزالة اللون من أصباغ النسيج الأزرق والأصفر

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تم دراسة إزالة لون أصباغ الآزو التفاعلية ذات اللون الأزرق والأصفر بواسطة 15 عزلة بكتيرية تم جمعها من التربة القاحلة المصرية. من بين هؤلاء العزلات، حققت العزلة S8 أعلى مستويات إزالة اللون. تم تعريف العزلة S8 على المستوى الجزيئي بناءً على تسلسل الهدف العالمي الأول من النوع chaperonin I (cpn60). كان تسلسل الحمض النووي الأكثر تشابهًا لتسلسل الحمض النووي S8 هو *Klebsiella oxytoca* (AB008147.1) partial CDS لترميزات متجانسة GroES و GroEL بنسبة تشابه تسلسل الحمض النووي. تصل إلى 88%. تمت دراسة تأثير العديد من المعلمات الخارجية لتحسين قدرات إزالة اللون من هذه العزلة. سجل أقصى نشاط إزالة للون على بيئة غذائية تحتوي على جلوكوز وقشور الفول الصويا ذات C/N بمعدل 12: 1 والبيئة مدعمة بصبغة النسيج بتركيز 100 ملجم / لتر وملقحة بـــ 3% (v/v) من اللقاح. وصلت الحضانة لمدة 4 أيام عند 35 درجة مئوية مع اهتزاز عند 150 دورة في الدقيقة إلى زيادة نشاط إزالة اللون إلى 89.35 و 78.23% للأصباغ الزرقاء والصفراء، على التوالي. أشارت المستويات الصاعدة للأنزيمات البكتيرية مثل إنزيم آزو-اختزالية، والفينول الأحمر المنجنيز بيروكسيديز وأكسيداز أسكوربات إلى دورهم البارز في تحلل الصبغة.