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Decolorization of Azo Dye by Enteropathogenic *Eschericha coli* Strains Isolated from Wastewater

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The presence of enteropathogenic *E. coli* (EPEC) in wastewater represent a public health problem because it is causative agent of outbreaks diarrheal, the interaction between this pathogens and chemical pollutants in wastewater effluent it is poorly understood, nevertheless it is well known that decolorization of dyes resulted in the formation of intermediate metabolites could be more toxic that dyes, the objective of this study was recovered from wastewater enteropathogenic *E. coli* strains able to decolorize azo dye. Samples of wastewater were collected and examined for identification and characterization of EPEC pathotype by PCR, after were quantified percent of decolorization the azo dye Direct Black 22 at 200 and 300 ppm for 10 days, and finally performed partial characterization of reaction products. A total of 106 *E. coli* strains isolated in this study, 66 (62.02%) were characterized into pathotype (EPEC) and were able to decolorize azo dye to 200 and 300 ppm. In this study evidenced a double concern about presence of EPEC, first leads to the risk of acquisition of diarrheal disease and second per possible participation in water chemical pollution toxic metabolites generated from the decolorization of azo dye.

Key words: Decolorization, Azo dye, *E. coli*, Wastewater.

Water pollution is an environmental problem due to direct discharge a lot of urban and domestic waste into rivers and streams, turning them in wastewater with chemical, physical and biological pollutants that affect ecosystem and public health [1].

The presence of biological pollutants by pathogens microorganisms in wastewater represent an important source of infection for acquisition of gastrointestinal disease, enteropathogenic *E. coli* (EPEC) is a causal agent of diarrhea, causing high infant morbidity rates in developing countries, can be transmitted through ingestion of contaminated water and food[2]. The EPEC infection process involves a bundle-forming pili (BFP) that virulence factor characteristic. In Mexico has been isolated pathogen *E. coli* about 63.9% as agent principal producer diarrhea following an overflow of sewage water [3].

Regarding chemical pollutants, the higher affectation are dyes because they are generally recalcitrant to biodegradation and microbial attack[4].

Synthetic dyes most used and discharged into water bodies are azo dyes[5] , they are characterized by the presence of one or more azo groups N=N[6], the biodegradation of azo dyes produces metabolites which are toxic, carcinogenic and mutagenic, affecting hydric ecosystem and public health[7]. Some bacterial genres, have enzyme systems that metabolized azo dyes such as

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Bacillus[8], *Proteus*[9], *Pseudomona*[10] and others. But is little known about decolorization of azo dyes by pathogen microorganism and its implications, therefore, in this study, was determinate percent of decolorization of azo dye Direct Black 22 by EPEC strains, previously isolated and characterized from wastewater.

MATERIALS AND METHODS

Study site : Alseseca river is located in city of Puebla, Pue. Mexico on geography coordinates of 19º 05‘50" N y 98º 05‘24" W, length of 31,491 km. This hydric body has be converted an receptor of industrial and domestic wastewater. The river crosses city from north to south until lead to Manuel Avila Camacho dam.

Samples collection: Overall 40 wastewater samples (250ml) were collected during two months of spring and summer 2011, in agreement with NOM-NMX-AA-003-1997[11], were taken in sterile containers of 250 ml and transported in cold for microbiological isolated.

Isolation and identification of *E. coli* strains: After homogenize the wastewater sample for 5 minutes, 100 µL was deposited on selective mediums Eosin Methylene Blue Agar (EMB) and MacConkey agar plates were incubated for 24 h at 37°C, colonies with similar morphology to *E. coli* were isolated and identified by biochemical tests.

Molecular characterization of EPEC strainsReference strains used for characterization: E2348/69 (enteropathogenic *E. coli*) as positive control and *Shigella flexneri as* negative control, from Microbial Pathogenicity Laboratory, Center for Research in Microbiological Sciences Institute of Science, Autonomous University of Puebla (CICM-ICUAP). PCR reported by Vidal et, al (2007)[12] with modifications, was used to amplify *bfp*A gene characteristic of EPEC, for this genomic DNA of controls and *E. coli* isolates was extracted by heat shock, after were centrifuged at 12000 rpm during 5 minutes and the supernatant was used as the template DNA. The following specific primers were used: EP1 (5´CAA TGG TGC TTG CGC TTG CT-3´) y EP2 (5´-GCC GCT TTA TCC AAC CTG GT-3´) reported by Gomez et al, (2010)[13]. The 25 µL PCR reaction mixture contained 10 µL of *Taq* polymerase, 2 µL of EP1, 2 µL of EP2, 3 µL of water and 8 µL of template DNA. Amplified products were analyzed by 1% agarose gel electrophoresis and visualized under UV transillumination.

Azo dye decolorization assay. Were used control strains: *P. aeruginosa*, DH5á (*E. coli* non-pathogenic) y E2348/69 (from CICM-ICUAP) for compared percentage decolorization of dye with EPEC strains. The control and EPEC strains were grown for 24 h at 37°C in LB medium, after were performed suspension of each bacterial strain with optical density of McFarland 0.5 ($1X10^8$ UFC/ml), that was used as inoculum. Direct Black 22 was carried out in 30 ml nutrient broth medium at 200 and 300 ppm with inoculum concentration 10 % v/v incubated at 37°C under static conditions for 10 days, assays were done in duplicate. After incubation samples were drawn at 24 hours intervals with aliquot (3ml) centrifuged of the culture media was centrifuged at 6000 rpm for 6 minutes to clear supernatant and analyzed wavelength at ë=480 nm. Control used was uninoculated sterile medium supplemented with dye. The percent of decolorization was calculated from de following equation used by Ponraj et, al (2011)[14].

$$\%\text{Decolorization} = \frac{\text{Initial OD} - \text{Final OD}}{\text{Initial OD}} \times 100$$

Reaction products partial characterization. 100 µL of each assays and control were diluted 10^1 and was used to partial characterize reaction products by UV-Vis absorption spectra of 200 nm to 800 nm.

Statistical Analysis

Data of the experimental design were analyzed with ANOVA using R program.

RESULTS AND DISCUSSION

A total of 106 *E. coli* strains were recovered and isolated from 40 water samples of Alseseca river city of Puebla, 66(62.26%) strains were detected as EPEC for presence of *bfp*A gene, because generated amplification product of 326 pb in similarity with control strain (Fig. 1) and 40 (37.74 %) *E. coli* strains did not hybridize with probe but, presumably, other diarrheagenic categories of *E. coli* (ETEC, EAEC, EHEC, EIEC)

could be found. We encounter a high prevalence of EPEC in wastewater, the interpretation can be complex, it does not reduce the importance in finding, as with other pathogroups, is an important cause of morbidity in developing countries [15], causing gastroenteritis and diarrheal outbreaks[16]. Obi et al, (2004)[17], isolated *E. coli* pathogen strains an 67.5% in rivers located in Limpopo Province, the prevalence of EPEC was 34.1%, in the river Tocantis, Brasil, analyzed water samples founding the presence of *E. coli* an 91.1% (149/163) only two strains were EPEC[18]. Other aspect important to considered with encounter of EPEC, is the preoccupation by previously treatment to wastewater used in agricultural zones, constitutes a risk for consumers, Castro et. al, (2012)[19] reported a contamination by faecal coliforms in salad samples with 85% and 7% detected diarrheagenic patogroups *E. coli*, the salads were prepared with vegetables irrigating with wastewater.

Dye decolorization

EPEC strains recovered from effluent (here in after called "wild strains") and control strains were able to decolorize azo dye Direct Black 22 at 200 and 300 ppm. Due different percent of decolorization at 200 ppm of wild strains, were grouped into three groups with different ranges of percent: A from 27 to 41% (21/66), B from 42 to 56% (31/66) and C from 57 to 70% (14/66) and for percentages obtained at 300 ppm we formed three groups were as follows: A' from 5 to 23% (17 strains), B' from 24 to 42% (38 strains) and C' from 43 to 60% (11 strains) (Table 1). Manivannan et al, (2011)[20] determined percent decolorization of *E. coli* isolated from textile effluent, dyes against: Orange 3R (79%), Blue T (66%), Antroquinone Blue T (56%), Black R. L(25%), Yellow GR (15%), that results propose the ability of microorganisms for decolorize pigments with different dye concentration, chemical structure and incubation conditions.

The Average percent of decolorization of dye at 200 ppm by wild strains were as follows: A (35.82%), B (48.91%) and C (62.66 %), and control strains were *P. aeruginosa* (46.91%), *E. coli* non-pathogenic DH5á (39.66%), *E. coli* pathogenic

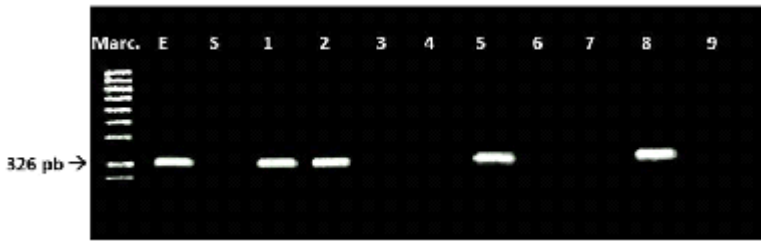

Fig .1. Agarose gel 1% shows the amplification of 326pb corresponding to *bfpA gene*. E= *E.coli* control (+), S = S. flexneri (S) control (-), wells of 1 to 18 contain DNA of recorvered strains

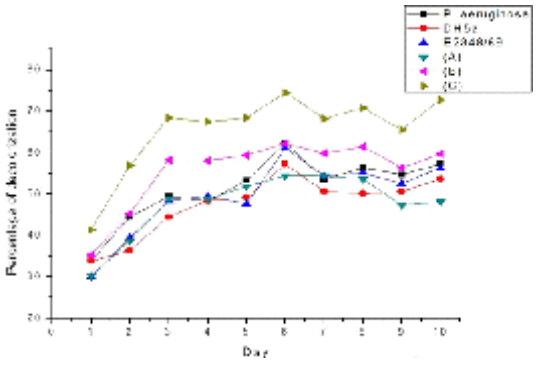

Fig .2. Percentage of decolorization of dye at 200ppm for 10 days of study by strain: P aeruginosa (), E234/69 (), DH5a (), E. coli silvestres: A(), B (), yC().

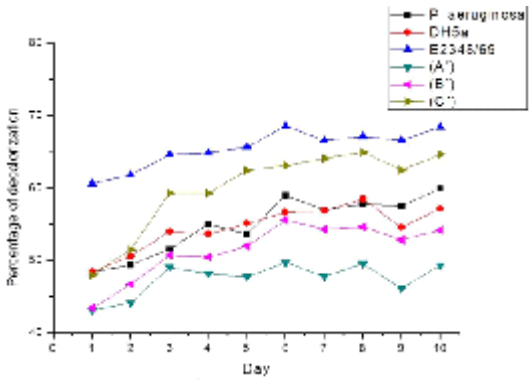

Fig .3. Percentage of decolorization of dye at 300ppm for 10 days of study by strain: P aeruginosa (), E234/69 (), DH5a (), E. coli silvestres: A(), B (), yC().

E2348/69 (52.87 %), no significant difference was found between strains (p= 0.640). At 300 ppm wild strains showed the following percentages: A' (18.72 %), B' (32.04 %) and C (50.01%), latter significantly exceeded percent of decolorization of *P. aeruginosa* (34.56%) this strain has been reported as capable of decolorizing dyes in high percentages, such as Sivakalai and Ramanathan, (2013) [21] mention that *P. aeruginosa* isolated from wastewater of treatment plant was able to decolorize dye Congo Red by 97.32%. In this study, the wild strains and type strain (E2348/69) presented a percent of decolorization similar of Direct Black 22, that did not successes with non-pathogenic *E. coli*, that could be due have more capacity to decolorize azo dyes, independent of concentration, used different azoreductase enzymes, with the ending of utilize the dye compound as the carbon source or only decolorize for survival in wastewater [22].

In figure 2 and 3 can be seen the lectures of absorbance for 10 days that decolorization of dye by wild and control bacteria at 200 and 300 ppm, starts from the first day of evaluation, continues to rise until sixth day, where a decrease in the percentage which may result from the toxicity of dyes to bacteria through the inhibition of metabolic activities as mentioned Chen et al, (2003)[23].

Characterization partial of reaction products by spectroscopy UV-VIS.

In absorption spectrum UV-VIS can be observed a manifestation of chemical reaction of dye direct black 22 at 200 and 300 ppm, as it presented a interaction to 392 and 398 nm respectively in the spectrums of sonadant before and after reaction. After of reaction presented an increase in the absorbance to wavelengths higher energy, appearing absorption bands to 310 nm and other to 252 nm (Fig. 4). Mohanna et al.(2008)[9] mentioned that the products formed after of decolorization of dye Direct Black 22 by a bacterial consortium were: 1-napthol and diphenylamine absorbing light in wavelengths of 274-340 nm [24] and 247-334nm [25] respectively, indicating that probably these are the products corresponding bands formed our absorption spectrums. EFSA[26] reports diphenylamine is an ecotoxicology pollutant mainly affecting: aquatic microorganisms, bacteria and mammals, not presenting genotoxic or mutagenic affectations to human, is usually degraded in conditions anaerobic and aerobic. Janardhan et al. (2009)[27] report 1-napthol concentration at 0.5 g/L affect growth of *E. coli* strains and can be genotoxic. About effect toxics to humans these will depend of genre and organism

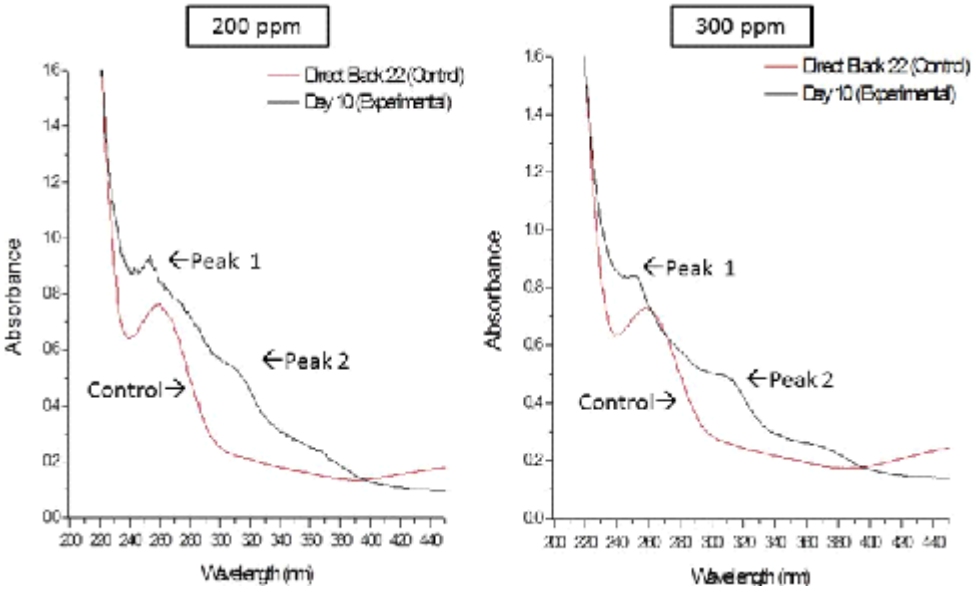

Fig . 4. Comparision of absorption spectrum UV-VIS of dye Direct Black 22 at 200 ppm and 300 ppm with spectrum of product of bacterial metabolism on the tenth day

metabolism, however may affect fetal development by crossing the placenta[28].

The results of this study showed, a latent risk that exists in the wastewater from the Alseseca river in city Puebla, Pue. Mexico, because contain potential pathogenic bacteria for human with the danger to outbreaks infection, nerveless pathogenic strains isolated (EPEC) were able to decolorize azo dye direct Black 22 at 200 and 300 ppm, suggest their double contribution to chemical pollution due formation of products during anaerobic decolorization. A propose for solve the problematic, is a sanitization of wastewater and utilize previously treatment for industrial wastewater.

CONCLUSION

EPEC strains isolated from the effluent decolorize azo dye, suggesting your contribution to pollution of wastewater due intermediate products formed.

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