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Biodegradation of Food Dyes by *Lactobacillus Paracasei*

KEYWORDS: Azo dyes Biodegradation and Lactobacillus paracasei

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ABSTRACT *Azo dyes belong to the most important group of synthetic colorants and are used extensively in the textile industries and food coloring. the present study the ability of Lactobacillus paracasei. isolate to modify the dyes; Tartrazin, Allura red and Amaranth was investigated. lactic acid bacteria (LAB) isolates were isolated from dried milk and identified by API 50 CHL Kit. All isolates screened for their capability to modify the food coloring Tartrazin, Amaranth, Allura red. The isolate modified dyes under anaerobic conditions, in MRS Broth. The product had a different color or colorless according differences in incubation time (0, 4, 24,72) hr. and different PH (5,6,8). It absorbed light at 427 and 520 nm,504 nm for Tar., Ama., All. Respectively that degrade dyes were characterized as . Were able to decolorize azo dyes (0.OO6 g/l00ml) within 24hr at optimized conditions pH 5- 6- 8, temperature 37°C.*

Introduction
Azo dyes are widely used as colorants in foods such as soft drinks, candy, hot dogs, ice cream, and cereals and in drugs, cosmetics, etc. The Food and Drug Administration (FDA) has approved the use of some azo dyes in the food, drug and cosmetic industries. The following azo dyes are used as colorants in the food industry: Citrus Red ,Allura Red (Allura Red; Food Red 17; E129: FD&C Red 40 Color Index No.: CI 16035), Tartrazine : (Hydrazine yellow; E102; FD&C yellow #5) Color Index No.: CI 19140 , Sunset yellow, Orange Band and Amarath (FD&C Red No. 2, E123, C.I. Food Red 9, Acid Red 27, Azorubin S, or C.I. 16185)(Krishna and Gunnu, 2011) . The chemical structure of these compounds features substituted aromatic rings that are joined by one or more azo groups (–C=C-, -N=N-, -C=O, -C=N-)(.Chen et al, 2004). The annual world production of azo dyes is estimated to be around one million tons (Chung, 2000, Stolz, 2001).It has been known that the reductionof the azo bonds is important for toxicity, mutagenicity and carcinogenicity of the azo dyes (Parkinson and Brown,1981) .Azo dyes are degraded by intestinal microorganisms in vivo and it is possible that the toxic and/or carcinogenic effects of these dyes in the gut may be due to their degradation products(Chung et al,1978). Many microorganisms belonging to the different taxonomic groups of bacteria, fungi, actionmycets and algae have been reported for their ability to decolorize azo dyes .However, maintaining the purity of single cultures in the large scale application at field level. The syntrophic interactions present in the mixed communities lead to complete mineralization of azo dyes.(Chen et al,2003). In the present study an attempt has been made for the degradation and decolonization of different azo dyes using of Lactic acid bacterial culture and effect of various process parameters like PH, incubation time.

Material and Methods
Azo dyes
The commercial food azo dyes, Allura red ,Amaranth and Tartrazin were purchased from the local market of Baghdad capital. These dyes were selected on the basis of their structural diversity and frequent use in local food industries. The stock solution of dyes (0.006 g/100 ml) was prepared by dissolving in distilled water and filtration through Millipore filter 0.45µm.

Isolation of bacteria
Lactobacillus isolate (LAB) isolated from dried milk was grown in deMan Rogosa and Sharpe agar (MRS) .Agar plate were incubated anaerobic conditions at 37˚C for 48 hr. (Hungate , 1969).

Identification of bacteria
Isolates capable of degrading azo dyes were further characterized by using the API 50 CHL System identification Kit (Bio Me'rieux, France) and the species were preliminarily identified from the API database.

Screening for de colorization activity
Screening of isolate was done in two steps. Primary screening was done only on visibility basis i.e. change in color of media containing respective dye. In secondary screening, de colorization was measured as decrease in optical density using spectrophotometer). as describe bellow:

4ml of MRS broth for isolate inoculated with 100µl of overnight bacterial culture, 1ml of azo dye solution were added then incubated at 37 ˚C for 48 hr. For measurement of de colorization of the three tested dyes cultures were centrifuged at 12,000 rpm for 15 min. The absorbance of centrifuged supernatant samples was red at 427nm Tar., 504 nm All. and 520 nm Ama., respectively using a JASCO V-530 UV-VIS spectrophotometer. All assays were performed in triplicate and compared with an un inoculated control (Perez and McFeeters,2009).

Effects of different conditions on de colorization:
De colorization under different conditions was done by changing one at time, the factors with the basic conditions of temperature 37 ˚C. the effect of incubation time on the de colorization was studied by incubating the medium containing dyes under a range of time (4, 24 and 72 hr).

For the study of effect PH on de colorization of dyes. Colonies of an overnight grown culture was used to inoculate the medium containing dye. The PH of the medium was adjusted to (5, 6 and 8).

Results and Discussion:
Based on their superior de colorization potential, Lactobacil-

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lus paracasei. was identified on the basis of biochemical test using API 50CHL test, isolate was identified as Lactobacillus paracasei (Figure 1).

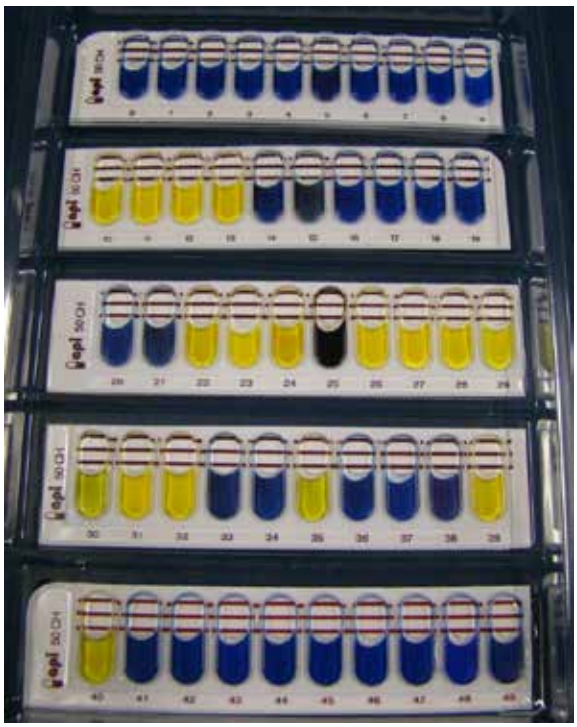

Figure 1. Identification of Lactobacillus paracasei with API 50CHL Kit

The biodegradation of dyes is illustrated by spectrophotometric measurement at 520 nm,427 nm,504nm for Amaranth , Tartrazin , Allura red respectively. The decrease in dye absorbance for all three dyes was observed for all isolates. There was a significant decrease in absorbance at 48 hours for the (LAB) isolate. The data suggests that after a period of48 hours there was complete reduction of all three dyes (Figure 2) this result is agreement with (Jessica et al, 2012). The reduction observed occurs an aerobically, and thus the gastrointestinal tract, particularly the colon, which is the most anaerobic environment in the body, is probably the sutable site for the reduction of azo dyes.

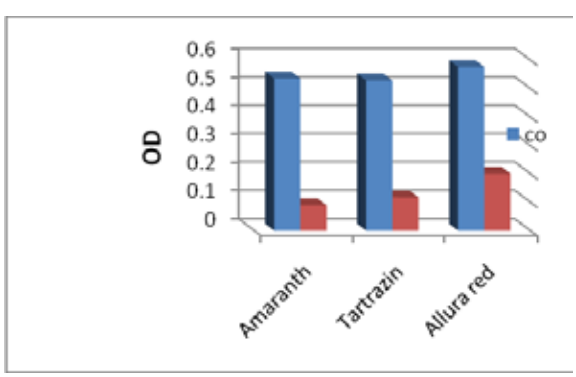

Figure 2. decrease of Azo dyes by Lactobacillus paracasei. For three different OD

Effect of incubation time on decrease azo dyes

To test the affect time on de colorization activity. The first experiment used different time (4, 24 and 72 h.). Using 0.006 g/100 ml of Allura red, Amaranth and Tartrazin. Gradually reduction of all three dyes was observed (Figure 3,4 and 5). After 4hr. Amaranth was reduced (higher) when compared to Tartrazin and Allura red after 24 hr. respectively. Interestingly, the different dyes may caused some change in the reduction of Tartrazine and Allura red, suggesting some conditions were not optimal.

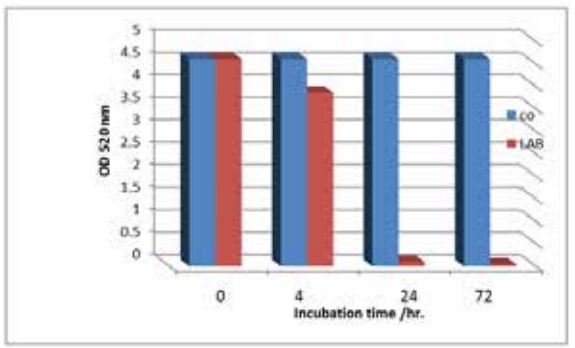

Figure 3. Changes in decolourisation during incubation time of Amaranth

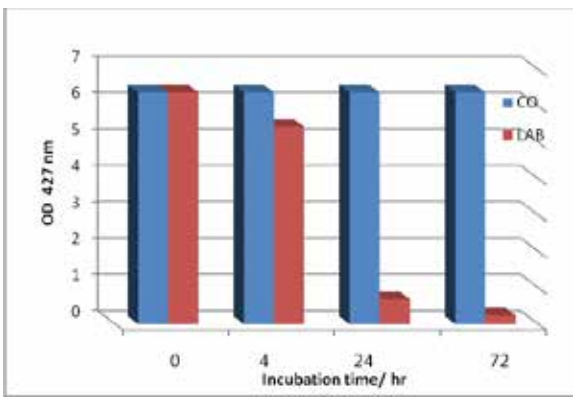

Figure 4. Changes in decolourisation during incubation time of Tartarazine

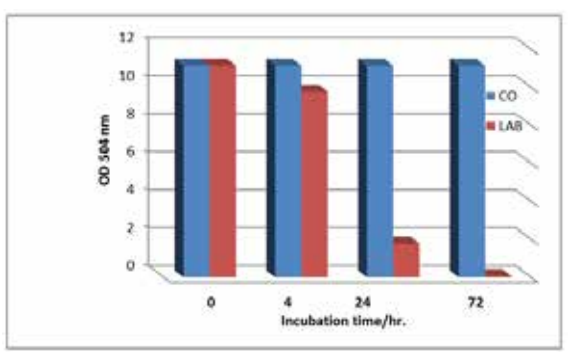

Figure 5. Changes in de colorization during incubation time of Allura red

Effect of PH on decrease azo dyes

Based on the time course experiments, a time point measurement of the complete reduction of dyes. Maximum de colorization was obtained at PH 5.0, 6.0 and 8.0 respectively for all three used dyes. De colorization was faster with Amaranth respect to others dyes as the PH increased to acidic or alkaline range. Reductive cleavage of azo bond (-N=N-) in azo days generates colorless aromatic amines (Mohammed , 2009). The increase in PH towards the alkaline range may be attribute to the accumulation of these basic aromatic amines and / or other metabolites (Figure 6, 7 and 8).

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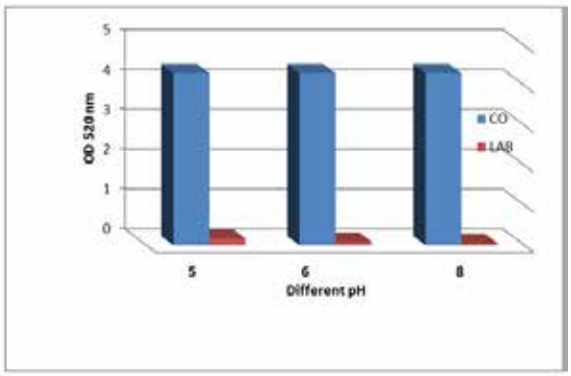

Figure 6. Effect of PH on decrease Amaranth by Lactobacillus paracasei

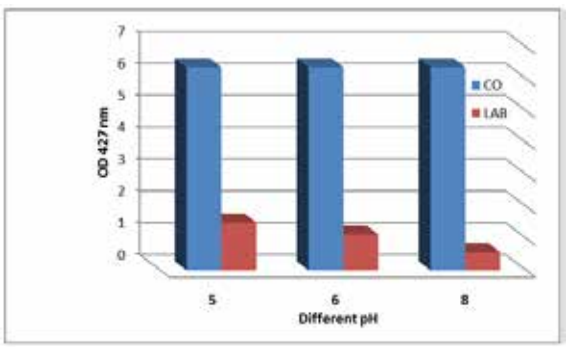

Figure 7. Effect of PH on decrease Tartrazin by Lactobacillus paracasei

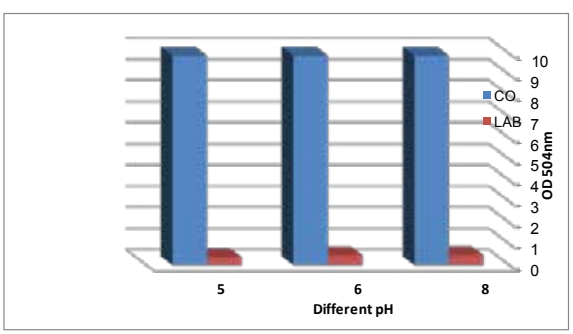

Figure 8. Effect of PH on decrease Allura red by Lactobacillus paracasei

In conclusion:

The results obtained in this study are very promising for the study of lactobacillus reductase gene which convolging in dye reduction and could help us to achieve complete mineralization of azo dyes for safe products in rapid and short time. However, further work is need to identify the gene(s) responsible.

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