Available online at www.sciencedirect.com

ScienceDirect

journal homepage: www.jfda-online.com

Original Article

Microbiological, histological, and biochemical evidence for the adverse effects of food azo dyes on rats

Khaled Elbanna [a,b], *Osama Mohamed Sarhan* [b,c], *Manal Khider* [d], *Mohamed Elmogy* [e], *Hussein Hasan Abulreesh* [b,*], *Mohamed Rabie Shaaban* [b,f]

[a] *Department of Agricultural Microbiology, Faculty of Agriculture, Fayoum University, Egypt*
[b] *Department of Biology, Faculty of Applied Science, Umm Al-Qura University, Saudi Arabia*
[c] *Department of Zoology, Faculty of Science, Fayoum University, Fayoum, Egypt*
[d] *Department of Dairy Science, Faculty of Agriculture, Fayoum University, Fayoum, Egypt*
[e] *Department of Entomology, Faculty of Science, Cairo University, Egypt*
[f] *Department of Chemistry, Faculty of Science, Cairo University, Cairo, Egypt*

ARTICLE INFO

Article history:
Received 3 October 2016
Received in revised form
10 January 2017
Accepted 13 January 2017
Available online 15 February 2017

Keywords:
dairy products
food additives
food azo dyes
lactic acid bacteria
probiotic bacteria

ABSTRACT

In this study, 120 lactic acid bacterial strains from different fermented dairy products as well as 10 bacterial intestinal isolates were evaluated for *in vitro* and *in vivo* degradation of various food azo dyes. Of these isolates, lactic acid bacteria (LAB) strains 13 and 100 and the intestinal isolates Ent2 and Eco5 exhibited 96–98% degradation of the tested food azo dyes within 5–6 hours. High performance liquid chromatography mass spectra of sunset yellow (E110) and carmoisine (E122) anaerobic degradation products by the intestinal isolates showed that they were structurally related to toxic aromatic amines. For an *in vivo* study, eight groups of rats were treated for 90 days with either the food azo dyes or their degradation products. All groups were kept for a further 30 days as recovery period and then dissected at 120 days. Hematological, histopathological, and protein markers were assessed. Rats treated with either E110/E122 or their degradation products exhibited highly significant changes in red blood cell count, hemoglobin, hematocrit, mean corpuscular volume, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration, and white blood cell count. In addition, alanine and aspartate aminotransferases, amylase, total bilirubin, blood urea nitrogen, creatinine, glucose, total protein, and globulins were significantly increased. Furthermore, marked histopathological alterations in the liver, kidney, spleen, and small intestine were observed. Significant decreases in inflammation and a noticeable improvement in the liver, kidney, spleen, and small intestine of rats treated with LAB and food azo dyes simultaneously were observed. Finally, these results provide a reliable basis for not only a better understanding of the histological and

* *Corresponding author*. Department of Biology, Faculty of Applied Science, Umm Al-Qura University, P. O. Box 7388, Makkah 21955, Saudi Arabia. Tel.: +966 555519597.
E-mail address: hhabulreesh@uqu.edu.sa (H.H. Abulreesh).
http://dx.doi.org/10.1016/j.jfda.2017.01.005

biochemical effects of food additives, but also for early diagnostics. In addition, LAB strains 13 and 100 may play an important role as potential probiotics in food and dairy technology as a probiotic lactic acid starter.

1. Introduction

Synthetic colors are man-made compounds that are not found in nature; these are often azo dyes. Certain artificial colors that are used in foods have been linked to negative health issues [1]. In recent years, food containing synthetic colors have been increasingly avoided by consumers as studies of the effect of a combination of certain artificial food colors and sodium benzoate on childhood behavior concluded that there was a possible link between the consumption of these additives and increased hyperactivity in 3-year-old and 8–9-year-old children [2]. Food azo dyes are the largest group of synthetic chemicals containing one or more azo groups (–N=N–) that are widely used in foods to improve their appearance [3]; they are also used in drugs, cosmetics [4], textile dyeing, paper printing, color photography, and leather industries [5,6]. The annual global production of food azo dyes is estimated to be around 1 million tons [7], and more than 2000 structurally different dyes are currently in use [8].

The chronic effects of dyestuffs, especially food azo dyes, have been studied for several decades. The reduction of azo dyes, i.e., the cleavage of the azo linkage(s), leads to the formation of aromatic amines that are known mutagens and carcinogens. In mammals, metabolic reduction of food azo dyes is mainly attributable to bacterial activity in the anaerobic parts of the lower gastrointestinal tract [5]. Various other organs, especially the liver and the kidneys, can, however, also reduce food azo dyes. After degradation in the intestinal tract, the released aromatic amines are absorbed by the intestine and are then excreted via the kidneys [4].

Azo dyes are generally recalcitrant to biodegradation because of their complex structures, but some microbial consortia or combinations of anaerobic and aerobic systems achieve complete degradation [8–12]. Lactic acid bacteria (LAB) as promising probiotic isolates could completely metabolize some azo dyes under anaerobic/aerobic regimes [13]. Probiotics are health-promoting live microorganisms that improve the intestinal microbial balance and produce various compounds that inhibit the growth of various bacterial pathogens [14].

Tartrazine (E102), sunset yellow (E110), carmoisine (E122), and ponceau 4R (E124) are mostly used in ice cream, yoghurt, soft drinks, instant puddings, flavored chips, cake mixes, custard, candy, and fermented dairy products. They are also used in some pharmaceutical products, cosmetics, moisturizers, and crayons [15,16]. The European Parliament and Council of the European Union have made a political decision that food or drinks containing some artificial colorings must be labeled with the text "May have an adverse effect on activity and attention in children" [17]. So, this work aims to (1) study the potential degradation of food azo dyes by bacterial consortia, (2) identify the degradation products by high performance liquid chromatography (HPLC), and (3) evaluate the *in vivo* toxicity of both food azo dyes and their metabolites in rats as a mammalian model.

2. Materials and methods

2.1. Food azo dyes

The commercial food azo dyes tartrazine (E102), sunset yellow (E110), carmoisine (E122), and ponceau 4R (E124) were purchased from Sigma (Cairo, Egypt). These dyes were selected on the basis of their frequent use in the local food, dairy, drug, and cosmetics industries. For determination of the *in vitro* degradation, a stock solution of each dye (10 g/L) was prepared by dissolving in distilled water and filtering through a 0.45-μm filter.

2.2. Screening and identification of food azo dye degrading bacteria

A total of 120 LAB strains isolated from different fermented dairy products were screened for anaerobic degradation of different food azo dyes as described by Elbanna et al [13]. Furthermore, screening and isolation of intestinal food azo dye degrading bacteria were conducted using an enrichment technique. For this, Hungate tubes (16 mL) containing 14 mL of nutrient broth medium were prepared and sterilized at 121°C for 15 minutes before a final concentration of 100 mg/L of the stock solution of each dye was added. All tubes were inoculated with different fresh child feces, and then incubated at 37°C until complete decolorization was achieved within 48 hours. After that, a loop-full of each enrichment culture from each dye was streaked onto Eosin Methylene Blue agar plates and incubated at 37°C for 24 hours. After incubation, different colonies were picked and purified, then tested for degradation of the different food azo dyes. The most efficient intestinal strains capable of decolorizing all tested azo dyes were selected for further studies. Ten different intestinal isolates were characterized and identified by API 20 E kit (BioMérieux, Marcy l'Etoile, France) using the API database and the protocol given in *Bergey's Manual of Determinative Bacteriology* [18].

2.3. Preparation of food azo dye degrading LAB

For preparation of the active fresh lactic acid bacteria used in this study, 100 μL of both LAB13 and LAB100 were propagated in sterilized and homogenized skim milk (3%) at 37°C for 48 hours, then subcultured in MRS broth several times. The