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Effect of Food Coloring Dyes on Bacterial Growth

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Abstract:- Effect of 11 commercially available food coloring dyes was tested on referent bacterial strains: Staphylococcus aureus ATCC 25923, Pseudomona aeruginosa ATCC 27853, Escherichia coli ATCC 25922 and Enterococcus faecalis ATCC 29212. Mueller-Hinton agar was used as media for bacterial growth to which different concentration of food coloring dyes were added. Two different types of food coloring dyes were used, powder and gel. The results indicated that different food dyes have different effect on bacterial growth due to difference in their ingredients, such as sugars, coloring and emulsifiers. Difference in growth, on media containing food coloring dyes, was also noticed between Gram-negative and Gram-positive bacteria.

Keywords:- *Food Coloring Dyes; Bacteria; Emulsifiers; Sugar; S.Aureus; P.Aeruginosa; E.Coli; E.Faecalis.*

I. INTRODUCTION

The use of food coloring dyes in drinks and food products is often encountered in the industry today to enhance the products visual quality and to make it more attactive to the consumer [1]. Food coloring dyes are classified as natural or synthetic. Natural food coloring dyes are derived from a variety of sources and natural products such as seeds, fruits, vegetables, insects and microorganisms. Natural food coloring dyes do not undergo chemical treatments [2]. Since prehistoric times people obtained colorants. The first natural colorants were usually obtained from the sources like flowers, leaves, berries, blossoms, barks and roots [3,4]. Later on, with advances in technology, different chemical procedures for synthetic production were introduced. In the middle of the nineteenth century, introduction of synthetic color marked the decline in the use of natural colors to color foods, drugs and cosmetics [5]. Synthetic food coloring dyes are usually water-soluble chemical substances made in factory and can be used in foods without any further processing [6,7]. Relative to natural colorants which are costly and less stable, synthetic colors are reliable and economical for restoring the original shade of the foods [8].

All food coloring dyes are mainly sugar based, with sets of different emulsifiers for specific color. The effects of sugars on bacterial growth have been studied for years now [9,10], and it has been reported that different emulsifiers and colorants have effect on bacterial growth [11,12], both found in food coloring dyes as mentioned. Sadar et al., (2017) tested the effect and toxicity of synthetic food coloring dyes on human normal flora and yeast (*Saccharomyces cerevisiae*) [8]. Therefore, the present investigation was planned to elucidate the effect of food coloring dyes on growth of referent bacterial strains: *Staphylococcus aureus* ATCC 25923, *Pseudomona aeruginosa* ATCC 27853, *Escherichia coli* ATCC 25922 and *Enterococcus faecalis* ATCC 29212.

II. MATERIALS AND METHODS

A. Bacteria

Microbial cultures were procured from American Type Culture Collection (ATCC). The Gram-positive bacteria Staphylococcus aureus ATCC 25923 and Enterococcus faecalis ATCC 29212, and the Gram-negative bacteria Pseudomona aeruginosa ATCC 27853 and Escherichia coli ATCC 25922, referent strains were used.

B. Media

We used 11 food coloring dyes commercially available in Europe, of which 4 are gel tubes (Dr. Oetker), and 7 are powders (SPM and ETERIKA) (Table 1). Different concentrations of each food coloring dyes were made. In Mueller-Hinton agar 1g and 0.5g of each food coloring dye were separately diluted. Staphylococcus aureus ATCC 25923, Pseudomona aeruginosa ATCC 27853, Escherichia coli ATCC 25922 and Enterococcus faecalis ATCC 29212 were inoculated on plates of all the tested colors and concentrations.

III. RESULTS

Before The growth of the Gram-positive bacteria Staphylococcus aureus ATCC 25923 and Enterococcus faecalis ATCC 29212, and the Gram-negative bacteria Pseudomona aeruginosa ATCC 27853 and Escherichia coli ATCC 25922, upon adding different food coloring dyes and food coloring dye concentrations, evaluated by Mueller-Hinton Agar plate analysis is presented below.

Upon the addition of different commercially available food coloring dyes, and due to different ingredients of food coloring dyes, growth of bacterium significantly differed (Table 2).

Mueller-Hinton agar was used as the negative control. Staphylococcus aureus ATCC 25923, Enterococcus faecalis ATCC 29212, Pseudomona aeruginosa ATCC 27853 and Escherichia coli ATCC 25922 were inoculated on a plate containing exclusively MH agar, and the growth of these bacteria observed after 24 hours of incubation was high.

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According to our obtained results using commercially available food coloring dyes affected bacterial growth differently, depending on ingredients of food coloring dyes, texture and type on bacteria (Gram-positive and Gram-negative). All Gram-positive bacterial grew noticeably more than all Gram-negative bacteria.

Food Coloring dye	**Manufacturers and Ingredients**						
	Texture	***Manufacturer***	***Ingredients***	***Dye***	***Gelling agent***	***Acidity regulators***	***Preservative***
Yellow	gel	Dr. Oetker	Glucose syrup, Sugar, Water	Curcumin	Carrageenan	Lactic acid, Acetic acid, Sodium lactate	Potassium sorbate
Red	gel	Dr. Oetker	Glucose syrup, Sugar, Water	E-102, E-161b	E-407	E-330, E-270, E-260, E-325	E-202
Black	gel	Dr. Oetker	Glucose syrup, Sugar, Water	Vegetable Charcoal	Carrageenan	Lactic acid, Acetic acid, Sodium lactate	Potassium sorbate
Green	gel	Dr. Oetker	Glucose syrup, Sugar, Water	Safflower and lemon concentrate, E-133	E-407	E-325, E-270, E-260	E-202
Extra Red Strawberry	powder	SPM (Stamenković)	Dextrose	E-124			
Extra Yellow	powder	SPM (Stamenković)	Dextrose	E-102			
Extra Purple	powder	SPM (Stamenković)	Dextrose	E-122, E-151			
Extra Green	powder	SPM (Stamenković)	Dextrose	E-104, E-131			
Extra Orange	powder	SPM (Stamenković)	Dextrose	E-110			
Silver Powder	powder	SPM (Stamenković)	No sugars	Potassium aluminium silicate E-555 (pearlescent pigments), Titanium dioxide E-171, E-131			
Blue Powder	powder	ETERIKA	Dextrose	E-102, E-121, E-13, E-151			

Table 1:- List of Used Commercially Available Colors, their Manufacturers and Ingredients

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Food Coloring Dye name	Sugar type	Amount of food coloring dye (g)	*Bacteria*			
			***E.faecalis* (gram +)**	***S.aureus* (gram +)**	***P.aeruginosa* (gram -)**	***E. coli* (gram -)**
Negative Control (MH Agar)	none	0g	yes ++++	yes ++++	yes ++++	yes ++++
Yellow Tube	glucose	1g	yes ++++	yes ++	No	yes +
Yellow Tube	glucose	0.5g	yes +++	yes+	No	yes -/+
Red Tube	glucose	1g	yes ++++	yes +++	yes -/+	yes +
Red Tube	glucose	0.5g	yes +++	yes++	No	yes -/+
Green Tube	glucose	1g	yes ++++	yes +++	No	yes +
Green Tube	glucose	0.5g	yes +++	yes++	No	yes -/+
Black Tube	glucose	1g	yes ++++	yes +++	No	yes +
Black Tube	glucose	0.5g	yes +++	yes++	No	yes -/+
Blue Powder	dextrose	1g	yes ++++	yes +++	No	yes -/+
Blue Powder	dextrose	0.5g	yes +++	yes++	No	yes +
Purple Powder	dextrose	1g	yes ++++	yes +++	No	yes -/+
Purple Powder	dextrose	0.5g	yes +++	yes++	No	yes +
Silver Powder	none	1g	yes ++++	yes +++	No	yes -/+
Silver Powder	none	0.5g	yes +++	yes++	No	yes +
Orange Powder	dextrose	1g	yes ++++	yes +++	No	yes +
Orange Powder	dextrose	0.5g	yes +++	yes++	No	yes +++
Yellow Powder	dextrose	1g	yes ++++	yes +++	yes -/+	yes -/+
Yellow Powder	dextrose	0.5g	yes +++	yes++	yes -/+	yes +
Red Powder	dextrose	1g	yes ++++	yes +++	No	yes -/+
Red Powder	dextrose	0.5g	yes +++	yes++	No	yes +
Green Powder	dextrose	1g	yes ++++	yes +++	No	yes -/+
Green Powder	dextrose	0.5g	yes +++	yes++	No	yes +

Table 2:- Effect of Food Coloring Dyes on Bacterial Growth; Yes ++++ (High Bacterial Growth); Yes +++ (Moderate to High Bacterial Growth); Yes ++ (Moderate Bacterial Growth); Yes + (Low Bacterial Growth); Yes +/- (Very Low Bacterial Growth); No (No Bacterial Growth)

Using commercially available gel tube food coloring dyes, showed increased growth of all bacterial strains used, when compared to bacterial growth on plates containing the powder food coloring dye, for both concentrations of food coloring dye.

All bacteria grew more on MH agar plates containing 1g of gel tube food coloring dyes, then on plates containing 0.5g of gel tube food coloring dyes. In the case of the powder food coloring dyes, all bacteria, except E.coli grew more on MH agar plates containing 1g of powder food coloring dyes, than on plates containing 0.5g of powder food coloring dyes. E. coli was the only bacteria which grew more on plates containing 0.5g of powder, than 1g of powder. Growth of S.aureus was slightly inhibited (moderate to low) on MH agar plates containing both 1g and 0.5g of gel food coloring dye.

P.aeruginosa did not grow on any media containing food coloring dyes except negative control which contains only Mueller-Hinton agar alone, and plates containing MH agar with yellow powder and MH agar plate with red tube food coloring dye. P.aeruginosa grew equally on both MH agar plates containing 1g and 0.5g of yellow powder, and only on MH agar plate containing 1g of red gel tube food coloring dye, not on the MH agar plate of 0.5g red gel tube food coloring dye. Plates containing MH agar with yellow powder and red tube food coloring dye, differ from other food coloring dyes used, due to one particular ingredient, E-102. This is also know as Tartrazine, used as yellow pigment in food industries.

It has been observed that growth of E.coli was significantly increased on the plate containing MH agar with orange powder (1g and 0.5g) in comparison to MH agar plates containing other food coloring dyes.

E.faecalis and S.aureus had stable, high to moderate growth on all plates, containing different food coloring dyes and food coloring dye concentrations.

IV. DISCUSSION AND CONCLUSION

All Gram-positive bacteria grew noticeably more than all Gram-negative bacteria. Similar behaviour have been noticed in research related to sugar metabolism of Gram-positive and Gram-negative bacteria [13,14].

Gel tube and powder food coloring dyes were used. Gel tube food coloring dyes contain glucose as main sugar, while powder food coloring dyes contain dextrose. Using commercially available gel tube food coloring dyes showed increased growth of all bacterial strains used, when compared to bacterial growth on plates containing powder food coloring dye, for both concentrations of food coloring dye. This is likely to be due to gel tube colors containing glucose (L-glucose) as one of their main ingredients, which is known to promote bacterial growth in some cases [15]. On contrary, powder food coloring dyes contain dextrose, also known as D-glucose, as one of their main ingredients, are known to inhibit bacterial growth in certain cases [16]. Both sugars are glucose isomers, but as shown, have noticeable differences in the way they affect growth of bacteria. It has been also reported that all bacteria grew more on plates containing 1g of gel tube food coloring dyes, then on plates containing 0.5g of gel tube food coloring dyes. In the case of powder food coloring dyes, all bacteria, except E.coli grew more on plates containing 1g of powder food coloring dyes, than on plates containing 0.5g of powder food coloring dyes. E. coli was the only bacteria which grew more on plates containing 0.5g of powder, than 1g of powder, probably due to dextrose having inhibitory effect on E.coli. Growth of S.aureus was slightly inhibited (moderate to low) on MH agar plates containing both 1g and 0.5g of gel food coloring dye, probably due to curcumin being used as colorant for this dye, showed to have inhibitory effect on S.aureus strains [11]. Interestingly P.aeruginosa did not grow on any media containing food coloring dyes except negative control which contains Mueller-Hinton agar alone, and plates containing MH agar with yellow powder and MH agar plate with red tube food coloring dye. P.aeruginosa grew equally on both MH agar plates containing 1g and 0.5g of yellow powder, and only on MH agar plate containing 1g of red gel tube. This is most likely due to the fact that P.aeruginosa is non-fermenter bacteria [12]. Plates containing MH agar with yellow powder and red tube food coloring dye, differ from other food coloring dyes used, due to one particular ingredient, E-102. This is also know as Tartrazine, which according to some studies have an effect on bacterial growth, and are proven to increase growth of P.aeruginosa [17].

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