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Chemotactic Effect of Common Food Chemicals on the Escherichia Coli Present in the Gastrointestinal Tract of Homo sapiens.

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Abstract— This study was conducted to detect the effect of common food chemicals on the *Escherichia coli* flora present in the large intestine of the gastrointestinal tract. *E.coli* is a symbiotic bacillus, one of the frequently occurring microbes which produce vitamin K_2. Bacterial populations in the large intestine digest carbohydrates, proteins and lipids that escape digestion and absorption in the small intestine. The intensity of fermentation depends on the number of microbes. The composition of the microbial colony varies with age, state of health and diet. The present study was therefore aimed at finding the effect of commonly used food chemicals like Monosodium glutamate (FAC: 621), saccharin(FAC: 954), sodium nitrite (FAC: 250) on the intestinal *E.coli*. *E.coli* isolates were variably susceptible to various concentrations of MSG, saccharin and sodium nitrate. The increase in concentration of these chemicals increasingly inhibits the growth of intestinal *E.coli.* The decrease in E.coli population may scale down the vitamin K_2 production in the body. This may lead to the deficiency of vitamin K_2, which reduces the level of active osteocalcin, which in turn increases the risk of fragile bones. The vitamin K_2 deficiency may also lead to *Calcium Paradox*, which causes Osteoporosis.

Keywords— *E.coli*, Monosodium glutamate, saccharin, Calcium paradox, osteocalcin and osteoporosis

1. Introduction

Escherichia coli (also known as *E.coli*) is a Gram-negative, facultative anaerobic, rod-shaped bacterium of the genus Escherichia that is commonly found in the lower intestine of endotherms (Singleton P et al, 1999). Most *E.coli* strains are harmless, the harmless strains are part of the normal flora of the gut, and can benefit their hosts by producing vitamin K_2 (Bentley R, Meganathan R, 1982) and preventing colonization of the intestine with pathogenic bacteria. New evidence, however, has confirmed that vitamin K_2's role in the body extends far beyond blood clotting to include protecting us from heart disease, ensuring healthy skin, forming strong bones, promoting brain function, supporting growth and development and helping to prevent cancer. *E.coli* and other facultative anaerobes constitute about ~0.1% of gut flora (Paul B. Eckburg et al, 2005) and faecal–oral transmission is the major route through which pathogenic strains of the bacterium cause disease. *E.coli* is the most widely studied prokaryotic model organism, and an important species in the fields of biotechnology and microbiology, where it has served as the host organism for the majority of work with recombinant DNA. Under favourable conditions, it takes only 20 minutes to reproduce. It also has 100% frequency of occurrence in population in the large intestine.

The gastrointestinal tract contains an immensely complex ecology of microorganisms. A typical person harbours more than 500 distinct species of bacteria, representing dozens of different lifestyles and capabilities. The composition and distribution of this menagerie varies with age, state of health and diet. The number and type of bacteria in the gastrointestinal tract vary dramatically by region. In healthy individuals the stomach and proximal small intestine contain few microorganisms, largely a result of the bactericidal activity of gastric acid; those that are present are aerobes and facultative anaerobes. In sharp contrast to the stomach and small intestine, the contents of the colon literally teem with bacteria, predominantly strict anaerobes (bacteria that survive only in environments virtually devoid of oxygen). Between these two extremes is a transitional zone, usually in the ileum, where moderate numbers of both aerobic and anaerobic bacteria are found. The gastrointestinal tract is sterile at birth, but colonization typically begins within a few hours of birth, starting in the small intestine and progressing caudally over a period of

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several days. In most circumstances, a "mature" microbial flora is established by 3 to 4 weeks of age.

The intestinal E.coli plays a very important role in producing vitamin K_2. The deficiency of vitamin K_2 is very rare in human beings since it is produced by the intestinal E.coli. There may be possibilities of vitamin K_2 deficiency if the activity of E.coli present in lower intestine is inhibited due to some harmful chemicals which are consumed. In the present era there has been an enormous increase in consumption of food chemicals like taste enhancers, artificial sweeteners, colouring agents, etc. There is an increase in the consumption of food containing high levels of calories from sugar or fat with little protein, vitamins or minerals. Today's food has little "nutritional value" and contains excessive fat, sugar, salt, and calories. High protein food contains large amounts of meat prepared with, for example, too much unhealthy saturated fat e.g. hamburgers, fried chicken, etc. Processed foods are laden with sweeteners, salts, artificial flavours, factory-created fats, colourings, chemicals that alter texture, and preservatives.

Commonly used food chemicals include Aspartame, Monosodium glutamate (MSG), Saccharin, Trans fats, Sodium Nitrite, etc.MSG is a flavour enhancer, most often associated with Chinese food, but it's actually in countless processed food products ranging from frozen dinners and salad dressing to snack chips and meats. MSG is an excitotoxin, which means it overexcites your cells to the point of damage or death, causing brain dysfunction and damage to varying degrees -- and potentially even triggering or worsening learning disabilities, Alzheimer's disease, Parkinson's disease, Lou Gehrig's disease and more. Saccharin is a no-calorie sweetener 300 times sweeter than table sugar. Today saccharin is used in a wide range of low- and no-calorie and sugar-free foods and beverages, including table top sweeteners, baked goods, jams, chewing gum, canned fruit, candy, dessert toppings and salad dressings as well as cosmetic products, vitamins and pharmaceuticals. It is also used in table-top sweeteners under the brand names Sweet n' Low®, Sugar Twin® and Necta Sweet.

The present study was therefore aimed at finding the effect of commonly used food chemicals like MSG, saccharin, sodium nitrite on the intestinal *E.coli.*

2. Method

i) Isolation of E.coli

E.coli was isolated from human faecal matter. The human stool was taken and streaked over the EMB and Nutrient Agar. The petri plates were incubated at 37°C for 24 hours.

ii) Gram staining

Gram staining was done to confirm the *E.coli* culture. A small portion of the culture was taken and heat fixed on a clean grease free slide. Crystal violet stain was added and kept for few minutes. Later it was washed off. Iodine was added to the culture, which act as a mordant. Decolourization was done using 95% ethanol. Gram negative cells can be rendered visible with a suitable counter stain, which is usually positively charged safranin, which stains them pink. Gram negative rod shaped *E.coli* was observed under microscope. Various other biochemical tests were done to confirm the presence of *E.coli.*

iii) Serial dilution (Hyunwoo Bang et al, 2004)

Saccharin and MSG were serially diluted using distilled water. 1g of MSG was taken and dissolved in 10 ml of distilled water in a test tube. From the first test tube 1ml was taken and was transferred to the next test tube with 9 ml distilled water. This was repeated till the concentration of $10^{-5.}$ The same procedure was done for saccharin and sodium nitrite. This was done in aseptic condition.

iv) Lawn culture (Dinesh Kumar et al, 2013)

The lawn culture was done on MHA agar. Sterile cotton swab was used to spread the culture over the medium. The culture was sterilized in flame. A loop full of culture was mixed in normal saline. The cotton bud after sterilization was dipped in the saline containing *E.coli* culture. The spreader was moved in top to bottom or side to side motion to spread the inoculum over the surface of the agar.

v) Paper disc diffusion method (Somayed Razmavar et al, 2014

Paper discs of equal diameter were placed over the culture using sterilized forceps. Paper disc diffusion method is mostly preferred as it is easier when compared to the gel puncture method. The petri plates were incubated at 37°C for 24 hours.

3. Observation

Different inhibitory zones with various diameters were observed. The zone of inhibition was formed depending on the effect of these chemicals with various concentrations on the E.coli culture. The zone of inhibition indicates that the E.coli in that region was seriously affected by the toxicity of these chemicals. Graphs were plotted after the

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observation and calculation. The following results were observed:

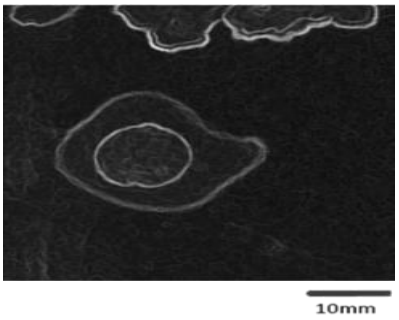

Fig 1: The zone of inhibition of the *E.coli* caused due to various concentrations of MSG

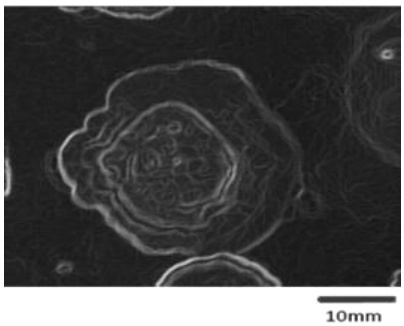

Fig 2: The zone of inhibition of the *E.coli* caused due to various concentrations of Saccharin..

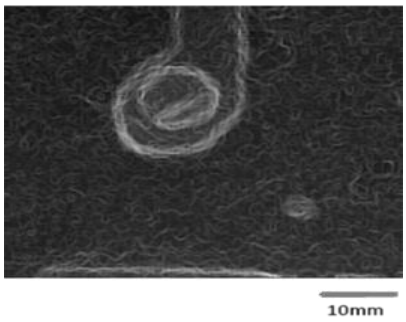

Fig 3: The zone of inhibition of the *E.coli* caused due to various concentrations of Sodium Nitrite.

4. Result and Discussion

4.1 Size of zone of Inhibition

The zone of inhibition obtained due to the bacteriocidal effect of the food chemicals on *E.coli*were calculated. Table 1 shows the Size of the zone of inhibition, by various concentrations of MSG and Saccharin.

Size of zone of inhibition	Concentration of food chemicals				
	10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}
Size of zone of inhibition caused by MSG (diameter in mm)	24	20	17	16	12
Size of zone of inhibition caused by Saccharin (diameter in mm)	35	21	15	12	9
Size of zone of inhibition caused by Sodium Nitrite (diameter in s)	15	12	7	6	4

Table 1: Size of the zone of inhibition, by various concentrations of MSG and Saccharin.

4.2 Graphical Analysis:

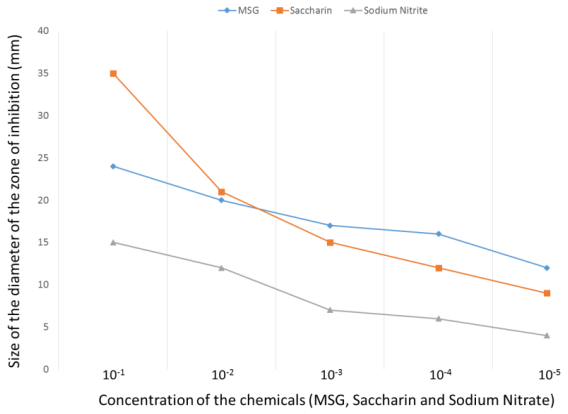

Fig 4: Graphical representation of the bactericidal effect of common food chemicals on the Normal resident *E.coli* flora of the large intestine

4.2 The vitamin K_2 deficiency

Thus the graphical analysis clearly shows that if there is increase in concentration of the chemical there is increase in the size of Inhibitory zone. The food chemicals MSG, saccharin and sodium nitrite thus proved to be harmful to human health as they inhibit the growth of the intestinal E.coli.

In comparison to MSG and Saccharin, Sodium Nitrite proved to relatively less harmful. MSG proves to be more harmful as it shows comparatively more zone of inhibition i.e. 12mm even in 10^{-5} concentration. Though Saccharin

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proves to be more bactericidal in its higher concentration, its activity gradually decreases more with respect to concentration when compared to the other two chemicals.
The *E.coli* in the large intestine digest the undigested food by fermenting them and thus produce Vitamin K_2. The increase in inhibition zone indicates the decrease in the population number of the *E.coli*. Reduction in the number of microbial flora causes reduction in the intensity of fermentation. This may hinder the production of vitamin K_2 and cause vitamin deficiency. The following are the health risks caused due to inhibition of intestinal vitamin K_2 producing *E.coli* by such interactions. The ties over which any given social unit connects denote the convergence of the various social contacts of that unit. This theoretical approach is, essentially, relational.

- **Osteoporosis**

Vitamin K_2 deficiency results in a decreased level of active osteocalcin, which in turn increases the risk of fragile bones (Booth et al, 2013). Chronic pain and a decreased ability to carry out normal activities may occur following a broken bone.

- **Heart calcification**

Patients suffering from osteoporosis were shown to have extensive calcium plaques, which impaired blood flow in the arteries. This simultaneous excess of calcium in one part of the body (arteries), and lack in another (bones) is known as Calcium Paradox(Gast et al, 2009). The underlying reason is the deficiency of vitamin K_2. The strongly protective effect of K_2 on cardiovascular health was confirmed by Geleijnse et al in the Rotterdam study (2004) performed on a group of 4800 subjects. The long chain types of K_2 are the most important for efficiently preventing excessive calcium accumulation in the arteries.

- **Reproduction**

The consumption of these chemicals increases the chance of infertility. They can raise the level of blood pressure in pregnant women due to which the infant may suffer from muscular dysfunction and a chance of organ failure also occurs.

5. Conclusion

It can be concluded that the common food chemicals like MSG, Saccharin and Sodium nitrite are harmful to human health as they reduce the *E.coli* population in the large intestine and thus causes vitamin K_2 deficiency. These chemicals are commonly present in junk foods and in the present era it is also been used in homes. This should be avoided and people need to be made aware about the ill-effects of consumption of these chemicals.

List of Symbols and Abbreviations

1) MSG: Monosodium glutamate
2) FAC: Food Additive Code
3) *E.coli: Escherichia coli*

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