Enhancement of enteric bacterial growth in culture media supplemented with cyclamate and aspartame.

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الملخص

هدفت هذه الدراسة الى معرفة تأثير السايكلوميت والاسبارتام على نمو بكتريا *E.oli, Klebsiella pneumoniae, Enterobacter* المعزولة سريريا . أظهرت النتائج أن عزلات الكليبسيلا كانت غير قادرة على النمو في التراكيز القليلة من مركب الاسبارتام (0.9 mg/ml ، 1.8) غير أنها أظهرت نموا" جيدا في التراكيز المرتفعة نسبيا" من هذا المركب. أظهرت النتائج أن عزلات *Enterobacter* كان الأكثر نموا"(10^9 cells/ml) في التراكيز العالية من هذا المركب (9.0 mg/ml). أظهرت النتائج أن مركب السايكلوميت يعزز نمو هذه الأنواع البكتيرية الثلاث في مختلف التراكيز وان عزلات *E.oli* كانت هي الأكثر نموا"(10^9-10^{10} cells/ml) في التراكيز المختلفة من هذا المركب.

Abstract

This study aimed to show the effect of sweeteners Sodium cyclamate and aspartame on culture media and its role in the enhancement of the growth of some clinical isolates of *Escherichia coli, Klebsiella pneumoniae* and *Enterobacter.* Results showed that *Klebsiella pneumoniae* isolates were unable to grow in low concentration of aspartame (0.9, 1.8 mg/ml) but they showed well growth at relatively high concentrations of this compound. Results also showed that *Enterobacter* spp was the best growers (10^9 cells/ml) at high concentration of aspartame (9.0 mg/ml) P> 0.05 . Sodium cyclamate enhanced the growth of *E.coli, Klebsiella pneumoniae* ,and *Enterobacter* spp. at different concentrations P< 0.001. *Escherichia coli* showed heavy growth and was the best growers (10^9-10^{10} cells/ml) at different concentrations of Sodium cyclamate.

Introduction

Sodium cyclamate and aspartame were used as a non nutritive sweetener. Cyclamate is odourless white crystalline powder with about 30 times the sweeteing power of sucrose [1].

Sodium cyclamate and aspartame have no caloric value and are poorly obsorbed from the gastrointestinal tract. However , a variable amount is hydrolyzed by bacteria in the intestinal tract to form cyclohexylamine , which is a potential carcinogen [2,3]. Two scientific studies prior to the production of cancerous tumors in the bladder, of rates. This lead to an immediate ban on use of the compound, in many countries subsequent research has failed to demonstrate the carcinogenic properties of cyclamate. This has led to reapprove of their use in many countries, though they remain banned in the united state, its use during pregnancy and by children is one of the greatest aspartame dangers of all [4,5] . The gut bacteria in man and other

species acquire the ability to convert cyclamate is administered ability[6,7]. The number of bacteria of the various genera occurring in human feces were not altered by daily cyclamate administration , but in the rat the number of clostridia in the faces were significantly increased (from 10-10^3/g to 10-10^5/g) by cyclamate feeding [8]. The structure of aspartame , seem simple . Two isolated amino acid, in aspartame are fused together by its third component , deadly methanol. It's the root of disease such as aspartame allergy , and central nervous system, aspartame and infertility …… etc. the chemical structure of aspartame causes the body to mimic these disease symptoms[9,16].

This study aimed to show the effect of sweeteners Sodium cyclamate and aspartame on bacterial growth and its role in the enhancement of the growth of some bacterial isolated belonging to enteric group.

Materials and Methods

1- **Sodium cyclamate and aspartame, tablets. 5 mg (Oxoid. UK) were obtained from local markets.**
2- **Bacterial isolates include: *E.coli* (15 isolates) *Klebsiella pneumoniae* (20 isolates) , and *Enterobacter spp* (15 isolates) were obtained from department of Microbiology, College of Medicine , Babylon University . All isolates were diagnosed according to morphological and biochemical reactions as described in [10].**
3- **Pouring technique was carried out for each isolates by inoculating of 10^7 bacterial cell/ ml growing on nutrient broth at 37C° on the following media:**

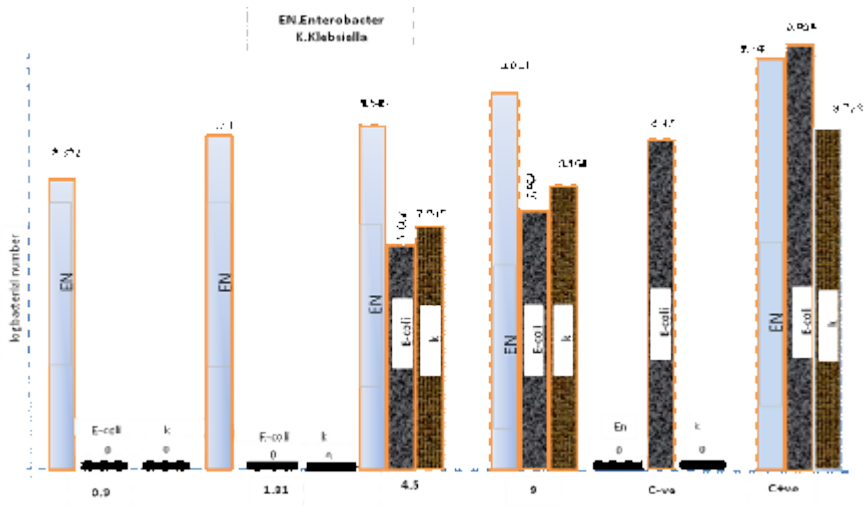
Freshly prepared nutrient agar without any type of sugar, and it composed of peptone meat, sodium chloride ,and agar .The previous media mixed with one of the sweeteners (sodium cyclamate and aspartame) at different concentrations (0.90 , 1.80 , 4.50 , 9.00 mg/ ml) (1.56, 3.12, 7.80, 15.60 , mg/ml) respectively. The plates were incubated aerobically at 37C° for 24hr. Nutrient agar medium without the sweeteners as (control + ve), and nutrient agar without any sugar as (control – ve) [1,3].

Results

Results showed that *Klebsiella pneumoniae* isolated were unable to grow in low concentration of aspartame (0.9 , 1.8 mg/ ml) as well as grow well at relatively high concentrations [4,5,9] (figure 1). Results also showed that *Enterobacter* spp was the best growers (10^9 cells/ml) at high concentration of aspartame (9.0 mg/ml).

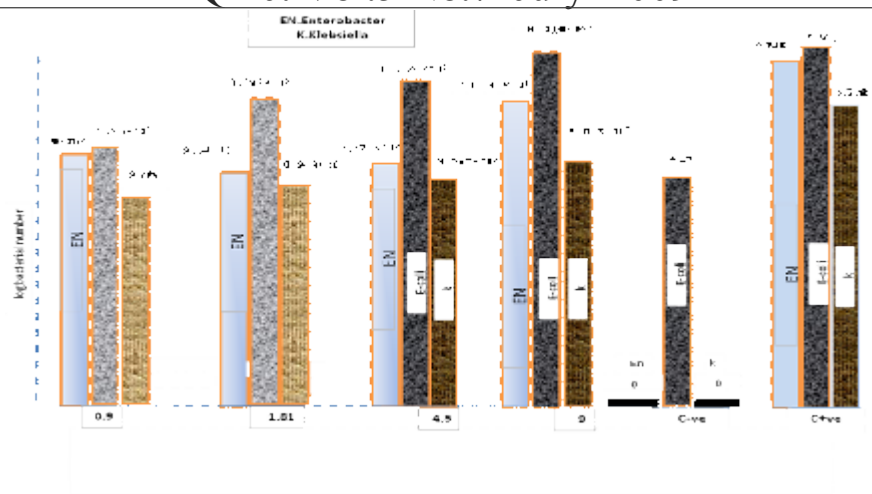
Sodium cyclamate enhanced the growth of *E.coli, Klebsiella pneumoniae* ,and *Enterobacter* spp. at different concentrations. *Escherichia coli* showed heavy growth and was the best growers (10^9-10^{10} cells/ml) at different concentrations of Sodium cyclamate. The results of statistical analysis by using Ki square test show significant realationship between the type of bacteria and concentrations of cyclamate (P< o.oo1) .

The same results were recorded for *E.coli* isolates. Regarding *Enterobacter* isolates, these bacteria were not influenced by various concentration of aspartame and were able to grow at all concentration of aspartame the number increased dramatically with high concentration of aspartame (figure 1) . Regarding bacterial growth in sodium cyclamate, all bacteria isolates were able to grow at various concentration of this sweeter, and the numbers increased dramatically with the increased at all concentration (figure 2) . *E.coli* isolates showed high numbers colony forming unit (CFU) at all concentrations when compared to that of other bacteria. Regarding the growth of bacterial isolates in medium (control _ ve) , all isolates were able to grow well by , when , we compare the growth on medium (control + ve) the growth of *E.coli* was relatively affected at low rate on this medium .



Concentration :(mg/ml)

Figure(1):The growth of E.coli,Klebsiella and Enterobacter on nutrient agar supplenented with Aspartame at differnt concentration .



Concentration :(mg/ml)

figure(2):The growth of E.coli,Klebsiella and Enterobacter on nutrient agar supplenented with sodium cyclamate at differnt concentration .

Discussion

This study revealed that sodium cyclamate enhanced the growth of bacterial isolates *E.coli* , *Enterobacter and Klebsiella pneumoniae* at different concentration, this may due to the ability of these isolates to use the sodium cyclamate as an alternative source for sugar. On the same manner when studying the growth of bacterial isolates on aspartame the growth of *E.coli* and *Klebsiella pneumoniae* at low concentration were highly diminished by comparing with the growth of *Enterobacter* on the same medium or in comparison to the growth of *E.coli* and *Klebsiella pneumoniae* on medium supplemented with sodium cyclamate . This may attributed to the fact that these two isolates were unable to efficiently use the aspartame as an alternative source of sugar as they use sodium cyclamate [15] . Therefore , growth of *E.coli* and *Klebsiella pneumoniae* . On the medium supplemented with aspartame was approximately similar to that of growth of medium (control _ve) (Figure 1 and 2) .

Sodium cyclamate may play a direct role in the enhancement of bacterial growth as a carbon source. The conversion and utilization of cyclamate into cyclohexylamine in man was carried out in the gut [11,13] .

This conversion is carried out by certain gut bacteria [6,12] . The sweeter sugars which plays a direct role in the growth of bacteria by acting as a source for carbon used structurally as well as a source for energy requirement for the bacterial activities [14] .

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