Journal of Food and Dairy Sciences

Journal homepage: www.jfds.mans.edu.eg
Available online at: www. jfds.journals.ekb.eg

Effect of Heat Treatment on the Activity of the *Enterococcus* spp. and *Escherichia coli* Nissle 1917 in the Presence of Lactulose and Apple and Orange Extracts

Nassib, T. A. and Esraa M. El-Amir*

Dairy Department, Faculty of Agriculture, Mansoura University

ABSTRACT

The present studies are carried out to elucidate the following; Effect of heat on the activity of the *Enterococcus* spp. isolates and *Escherichia coli* Nissle 1917 in the presence of lactulose and apple and orange extracts. During the course of examination the activity of certain specie of bacteria, namely *Enterococcus molodoratus, Enterococcus faecles, Enterococcus faecium and Eschericia coili nissilewere examined* for their activity at 55, 60 and 65°C.durin the incubation for different times. Rsults revealed wide variation according to the tested organism, temperature and the exposure time. For the enumeration of the examined cultures, the Mac Konkey agar No.3 was used for the *Escherichia coli*, and tryptone soy agar was used for the *Entreococcus spp*. Survival of the examined strains were tested at 55, 60, 65°C.

Keywords: Oligosaccharides, probiotic, tryptone ,soy , broth

INTRODUCTION

Prebiotics are oligosaccharides, non-digestible carbohydrates, being not fermentative and used by probiotic bacteria to stimulate their growth. Oligosaccharides are of the most important source of them. Prebiotics are not enzymatically broke-down in the human digestive system. (Hutkins *et al*., 2016 , Ambalam, P. *et al*. 2016 and Bertelsen,, *et al*. 2016). GOS is a group of carbohydrates made up of oligo-galactose along with glucose and lactose

The present studies are carried out to elucidate the following; Effect of heat on the activity of the *Enterococcus* spp. isolates and *Escherichia coli* Nissle 1917 in the presence of lactulose and apple and orange extracts.

A variety of benefits have been attributed to prebiotics intake. Relief from constipation. Prebiotics are mainly carbohydrates, which liberate gases when fermented in the large intestine, and increase the volume and diminish the transition time of the digested food in the intestine. Slow transit time results in constipation. Reducing the transit time thus leads against constipation and carbohydrates that present reaches the large intestine and have a laxative effect on bowel habit (Cuello-Garcia *et al*., 2016 and Broadbent, *et al*; (2018)

MATERIALS AND METHODS

The effect of heat on the survival of probiotic bacteria

Bacterial strains used in the present studies were kindly obtained from the stock cultures of the department of Dairy science, faculty of Agriculture, Mansoura University.

An overnight culture of each probiotic strain used in the current study (*Enterococcus malodoratus* , *Enterococcus feacalis*, *Enterococcus faecium*, and *Escherichia coli* Nissle 1917) was inoculated at 1% (v/v) into tryptone soya broth (TSB) enriched with 1% (v/w) lactulose, oligosaccharides from orange, and apple peels extracts. Inoculated media were heated at 55°C, 60°C, and 65°C./ 60 for 60 min, and samples were taken when fresh at zero time and after 5, 10, and 20 min for assessing the viability of the probiotic tested strains. For the enumeration of the examined cultures, the Mac Konkey agar No.3 was used for the *Escherichia coli*, and tryptone soy agar was used for the *Entreococcus spp*. Survival of the examined strains were tested at 55, 60, 65°C.

RESULTS AND DISCUSSION

Studying the effect of thermal treatment at 55, 60 and 65C° in the presence of lactulose and apple and orange peels extracts on the activity of the isolate *Enterococcus malodoratus* in Table(1) and Figures(1-3) .

Isolate *Enterococcus feacalis* in Table (2) and Figs.(4-6) and isolate *Enterococcus faecium* in Table (3) and Figs.(7-9).Results concerning the isolate *Enterococcus malodoratus* at 55°C. Present in Table (1-3) and Figs.(1-9) revealed that the growth of the isolate decreases over time ,and the growth rate increases over time ,while in the case of using lactulose sugar the growth rate is fixed over time compared to the control .When studying the effect of thermal treatment on the activity of isolate *Enterococcus malodoratus* it was noted the when thermal treatment at 55°C in the presence of apple extract ,the growth of isolation decreases over time ,and then the rate of growth rate is fixed over time compared to the control(Ryu and Beuchat 1998; Ryu *et al*., 1999) Akhter, N., Wu, B., Memon, A. M., & Mohsin, M. (2015).

Regarding the activity of the strain *Enterococcus feacalis*, results in Table (2) and Figs.(4-6) in the presence of apple extract revealed an increase of the growth of the isolate *Enterococcus feacalis*, followed by decrease by prolonging time, compared with the control, while in the presence of orange extract or lactulose the growth rate increases, followed by decrease by prolonging the incubation period (Ryu and Beuchat 1998; Ryu*et al*., 1999 and Wotzka, *et al* ,2018, Rodrigues, 2016 and Nooshka and Madadlou 2016))

* **Corresponding author.**
E-mail address: esraaalamir314@gmail.com
DOI: 10.21608/jfds.2020.78885

Table 1. Activity of *Enterococcus malodoratus* during incubation at different temperarure

Temp.	Time	R (71)				F	P	LSD
		Control (n = 3)	Isolate L (n = 3)	Isolate A (n = 3)	Isolate O (n = 3)			
55	0	44.50 ± 3.18	41.50 ± 4.91	49.50 ± 1.44	89.50^{abc} ± 6.64	25.008*	<0.001*	14.640
	5	46.50 ± 0.87	35.0^{a} ± 0.58	5.0^{ab} ± 1.15	43.0^{c} ± 6.35	33.331*	<0.001*	10.680
	10	52.0 ± 9.81	53.50 ± 1.44	6.0^{ab} ± 0.58	17.0^{ab} ± 2.31	22.587*	<0.001*	16.664
	20	45.0 ± 0.58	22.50 ± 0.29	3.50 ± 0.87	72.67 ± 44.05	1.830	0.220	71.968
60	0	86.0 ± 4.62	328.0^{a} ± 16.17	52.0^{ab} ± 4.62	47.50^{ab} ± 3.75	226.438*	<0.001*	29.132
	5	12.0 ± 0.0	50.0^{a} ± 9.81	3.50^{b} ± 0.29	2.50^{b} ± 0.29	20.815*	<0.001*	16.046
	10	27.50 ± 0.87	15.0^{a} ± 1.15	4.50^{ab} ± 1.44	7.50^{ab} ± 1.44	67.240*	<0.001*	4.084
	20	2.0 ± 0.58	20.0 ± 8.66	14.0 ± 9.29	4.50 ± 1.44	1.711	0.241	20.902
65	0	111.0 ± 63.51	89.0 ± 50.81	117.0 ± 6.93	61.0 ± 4.04	0.385	0.767	133.491
	5	23.50 ± 12.99	3.50 ± 0.87	5.0 ± 1.53	1.0 ± 0.0	2.469	0.136	21.412
	10	1.0 ± 0.0	7.50 ± 3.75	1.50 ± 0.29	1.0 ± 0.0	2.847	0.105	6.148
	20	5.33 ± 4.33	2.0 ± 1.0	1.0 ± 0.0	1.0 ± 0.0	0.854	0.503	7.264

Data expressed by using mean ± SEM. F: F for ANOVA test, Pairwise comparison bet. each 2 groups was done using Post Hoc Test (LSD)
p: p value for comparing between the studied groups a: Statistically significant with control
b: Statistically significant with Isolate L c: Statistically significant with Isolate A *: Statistically significant at p ≤ 0.05
L = lactulose A = Apple O = Orange 71=*E.malodoratus*

Table 2. Activity of *Enterococcus feacalis* during incubation at different temperarure

Temp.	Time	R (41)				F	p	LSD
		Control (n = 3)	Isolate L (n = 3)	Isolate A (n = 3)	Isolate O (n = 3)			
55	0	34.0 ± 13.28	82.50^{a} ± 14.72	71.0^{a} ± 8.66	146.5^{abc} ± 3.75	18.186*	0.001*	35.867
	5	63.0 ± 10.39	21.50^{a} ± 5.48	45.50^{b} ± 2.60	147.5^{abc} ± 0.29	82.863*	<0.001*	19.663
	10	32.50 ± 0.29	51.0 ± 2.31	49.50 ± 2.60	145.5^{abc} ± 19.92	25.717*	<0.001*	33.030
	20	139.5 ± 22.81	63.0^{a} ± 8.08	17.50^{ab} ± 2.02	2.50^{ab} ± 0.87	25.673*	<0.001*	39.684
60	0	67.50 ± 0.87	44.0^{a} ± 10.97	59.50 ± 4.91	337.5^{abc} ± 7.22	400.816*	<0.001*	22.941
	5	12.0 ± 2.89	9.0 ± 2.31	40.50 ± 0.29	50.0 ± 28.29	2.059	0.184	46.605
	10	10.67 ± 6.49	1.50 ± 0.29	5.0 ± 0.58	107.0^{abc} ± 24.83	15.655*	0.001*	41.927
	20	5.0 ± 0.0	3.0 ± 1.15	5.33 ± 1.45	11.33 ± 5.55	1.511	0.284	9.555
65	0	182.0 ± 21.94	7.50^{a} ± 0.87	73.0^{ab} ± 13.86	64.0^{ab} ± 2.31	31.311*	<0.001*	42.576
	5	6.67 ± 4.70	7.50 ± 0.29	7.0 ± 2.89	54.67 ± 33.95	1.917	0.205	56.180
	10	3.0 ± 1.15	1.0^{a} ± 0.0	1.0^{a} ± 0.0	2.33 ± 0.33	2.976*	0.049*	1.963
	20	1.0 ± 0.0	2.67 ± 1.67	1.0 ± 0.0	1.0 ± 0.0	1.000	0.441	2.722

Data expressed by using mean ± SEM.
F: F for ANOVA test, Pairwise comparison bet. each 2 groups was done using Post Hoc Test (LSD)
p: p value for comparing between the studied groups a: Statistically significant with control b: Statistically significant with Isolate L
c: Statistically significant with Isolate A *: Statistically significant at p ≤ 0.05
L = lactulose A = Apple O = Orange 41=*Enterococcus feacalis*

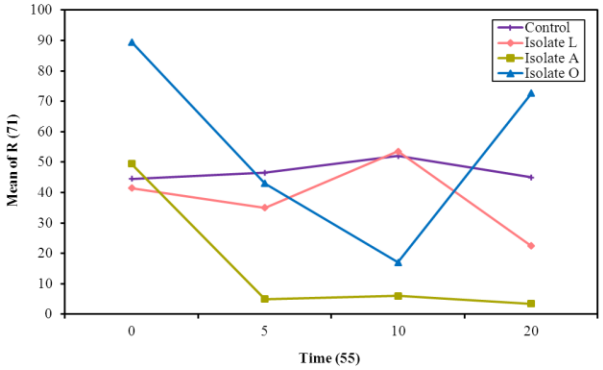

Figure 1. Activity of *Enterococcus malodoratus* during incubation at different temperarure

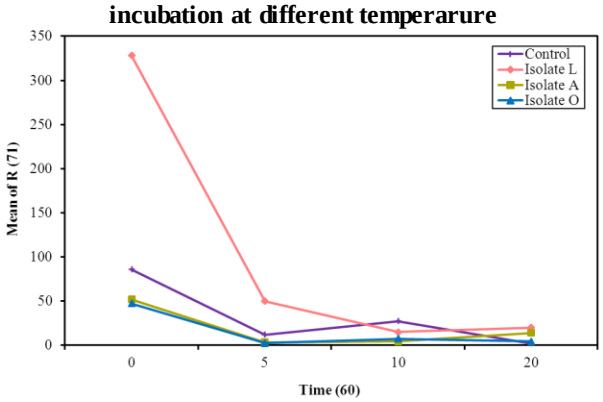

Figure 2. Activity of *Enterococcus malodoratus* during incubation at different temperarure

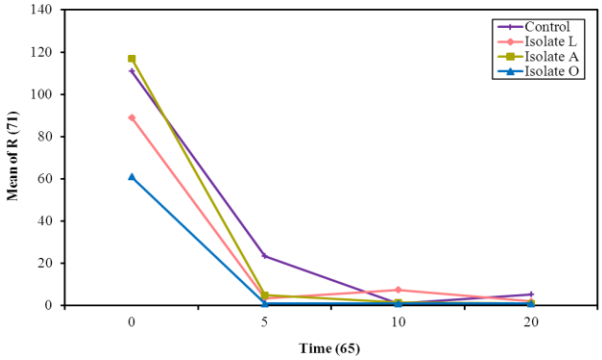

Figure 3. Activity of *Enterococcus malodoratus* during incubation at different temperarure

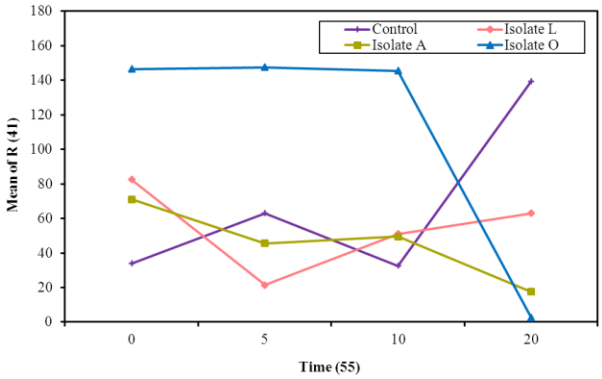

Figure 4. Activity of *Enterococcus feacalis* during incubation at different temperarure

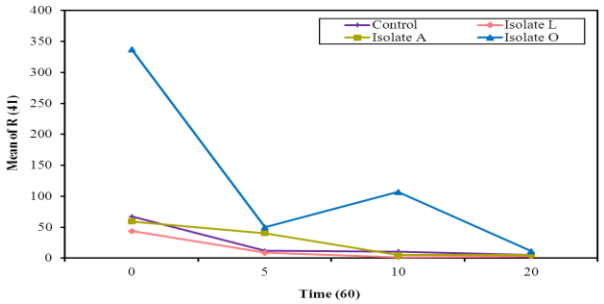

Figure 5. Activity of *Enterococcus feacalis* during incubation at different temperarure

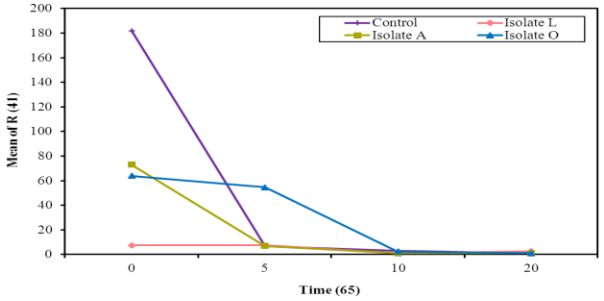

Figure 6. Activity of *Enterococcus feacalis* during incubation at different temperarure

Meanwhile, the presence of orange extract resulted in decrease in the growth rate and activity of the isolate *Enterococcus feacalis* observed throughout the incubation period. It could also be observed that the growth rate and activity of this isolate increased in the presence of lactulose. It was also observed that when thermal treatment at 55°C in the presence of apple extract ,the growth rate of the isolate *Enterococcus feacalis* during the incubation period ,and when using orange extract, growth stability was observed within 10 min, followed by decrease . (Ryu and Beuchat 1998; Ryu *et al*., 1999). Raising the temperature to 60°C in the presence of apple extract and lactulose, growth rate of the tested isolate *Enterococcus feacalis* decreased by proceeding the incubation period .However, reduction in the growth rate of this isolate in the presence of orange extract, followed by an increase then after time (Ryu and Beuchat 1998; Ryu *et al*., 1999). The presence of lactulose resulted in more active growth of the examined isolate *Enterococcus feacalis* at 55°C. (Ngono *et al*., 2019 , Broadbent, 2018 and Broadbent, 2018**)**

By examining the activity of the isolate *Enterococcus faecium* at 55°C in the presence of apple extract, growth stability could also be detected in the Table (3) and Figs.(7-9) after 5 min, followed by the activity in the presence of orange extract, growth stability after that decreased by proceeding the incubation period. (Ryu and Beuchat 1998; Ryu *et al*., 1999). Treating the isolate *Enterococcus feacium* at 65°C in the presence of apple extract, on the other hand, resulted decreasing the growth rate of isolate during the incubation period, and in case of using orange extract and lactulose, an increase in the incubation (Ryu and Beuchat 1998; Ryu *et al*., 1999).The best growth temperature for this isolate in the presence of orange extract is 65°C, which is considered the optimum for its growth. However, in the presence of lactulose sugar, the optimum temperature for its activity is 55°C, and65°C.

Table 3. Activity of *Enterococcus faecium* during incubation at different temperarure

Temp.	Time	R (16)				F	p	LSD
		Control (n = 3)	Isolate L (n = 3)	Isolate A (n = 3)	Isolate O (n = 3)			
55	0	50.0 ±24.52	84.67±25.51	156.0^{ab}±1.73	146.5^{ab}±3.75	8.079*	0.008*	58.184
	5	68.33±28.98	7.67^{a}±1.67	157.0^{ab}±12.12	147.5^{ab}± 0.29	20.120*	<0.001*	51.394
	10	43.33±36.33	81.33±37.05	72.0 ±20.78	109.5 ± 0.87	0.952	0.460	91.315
	20	3.0 ± 0.58	1.0 ± 0.0	5.50^{b}±1.44	2.50^{bc}±0.87	4.421*	0.041*	2.907
60	0	50.0 ±24.52	84.67±25.51	168.0^{ab}±2.89	337.5^{abc}±7.22	50.140*	<0.001*	59.169
	5	54.33±32.35	81.33±40.13	88.0 ±9.81	86.0 ±7.51	0.348	0.792	86.580
	10	42.33±33.12	44.0 ±29.37	40.50± 0.29	63.0 ± 0.58	0.223	0.878	72.306
	20	1.0 ± 0.0	4.33±2.40	11.67±5.61	11.33±5.55	1.640	0.256	13.469
65	0	89.0 ± 0.58	219.5^{a}±7.22	146.5^{ab}±18.19	157.5^{ab}± 0.29	29.852*	<0.001*	31.977
	5	4.67±1.76	1.0 ± 0.0	30.0^{ab}±5.20	2.0^{c}± 0.58	25.055*	<0.001*	9.013
	10	2.33 ± 0.88	53.0^{a}± 29.44	1.0^{b}± 0.0	21.0 ± 7.02	4.166*	0.045*	49.466
	20	17.67±16.67	2.0 ± 0.0	2.0 ± 0.58	2.0 ± 0.58	0.881	0.490	27.256

Data expressed by using mean ± SEM. F: F for ANOVA test, Pairwise comparison bet. Each 2 groups was done using Post Hoc Test (LSD)
p: p value for comparing between the studied groups a: Statistically significant with control b: Statistically significant with Isolate L
c: Statistically significant with Isolate A
***: Statistically significant at p ≤ 0.05**
L = lactulose A = Apple O = Orange 16=*Enterococcus faecium*

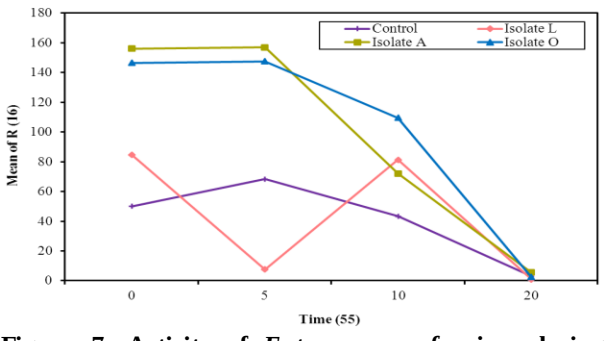

Figure 7. Activity of *Enterococcus faecium* during incubation at different temperarure

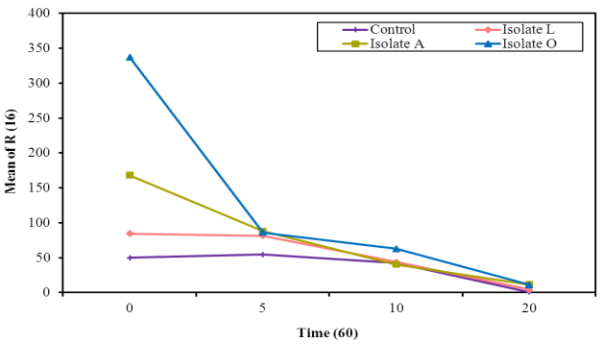

Figure 8. Activity of *Enterococcus faecium* during incubation at different temperarure

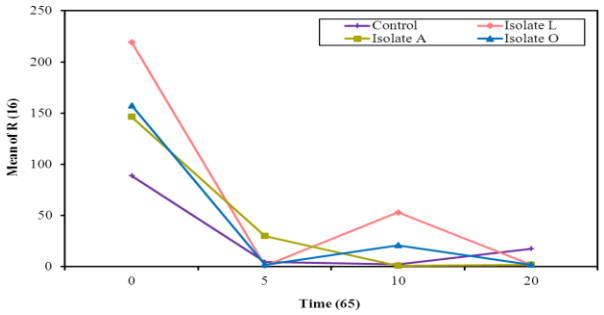

Figure 9. Activity of *Enterococcus faecium* during incubation at different temperarure

Regarding the activity of the isolate *Escherichia coli* Nissle 1917 in the presence of the examined two extracts from apple and orange and lactulose at 55, 60and 65^0 C is shown in Table (4) and Figs.(10-12).

The obtained data indicated that treating the isolated strain of *E. coli* Nissle 1917 at 55πC in the presence of an apple extract resulted in decrease in its growth rate during the incubation time, while growth stability was first observed by using lactulose, followed by decrease after 10 min. It could also be detected that raising the temperature to 60^0 C in the presence of an apple, orange extracts and lactulose, *E. coli* Nissle 1917 characterized with lower growth rate during the incubation period (Ryu and Beuchat 1998; Ryu *et al*., 1999 Sharma, *et al*. (2017).

Meanwhile, at 65°C in the presence of apple ,orange and lactulose extract,the growth rate decreased (Ryu and Beuchat 1998; Ryu *et al*., 1999) Uyeno, Y., Shigemori, S., & Shimosato, T. (2015).

The best effect of orange extract on the activity of the examined isolate was observed at 65°C. Growth stability of these bacteria was observed after 10 min. incubation at 65°C,. Markowiak, P., & Śliżewska, K. (2017).

Table 4. Activity of *Escherichia coli nissile* during incubation at different temperarure

Temp.	Time	R(E.C.N)				F	p	LSD
		Control (n = 3)	Isolate L (n = 3)	Isolate A (n = 3)	Isolate O (n = 3)			
55	0	38.0 ±17.90	43.50±9.53	58.50±8.37	96.50^{ab}±15.30	3.899*	0.045*	43.684
	5	31.50±8.37	18.50±2.02	2.67^{ab}±1.20	13.0^a± 0.58	7.595*	0.010*	14.235
	10	5.0 ±1.15	5.0 ±1.53	5.0 ±1.15	1.0^{abc}± 0.0	3.200*	0.048*	3.652
	20	1.0 ± 0.0	1.0 ± 0.0	2.0 ± 1.0	1.0 ± 0.0	1.000	0.441	1.633
60	0	142.0 ±15.59	232.5^a±7.22	82.0^b±35.22	38.0^{ab}±8.66	17.598*	0.001*	65.549
	5	2.0 ± 0.58	2.0 ± 0.58	13.67^{ab}±3.93	5.33±3.84	3.297*	0.045*	9.078
	10	1.0 ± 0.0	1.50± 0.29	6.0^{ab}±2.31	1.0^c± 0.0	4.354*	0.043*	3.802
	20	1.0 ± 0.0	2.0 ± 0.58	3.67±1.33	2.50± 0.29	2.241	0.161	2.420
65	0	78.50±22.23	59.50±9.53	108.5 ±23.38	54.50±1.44	2.111	0.177	54.997
	5	1.0 ± 0.0	2.0^a± 0.58	1.0^b± 0.0	2.0^{ac}± 0.0	4.000*	0.042*	0.943
	10	1.0 ± 0.0	1.0 ± 0.0	1.0 ± 0.0	1.0 ± 0.0	–	–	–
	20	1.0 ± 0.0	1.50^a± 0.29	1.0^b± 0.0	1.0^b± 0.0	3.050*	0.049*	0.472

Data expressed by using mean ± SEM. F: F for ANOVA test, Pairwise comparison bet. each 2 groups was done using Post Hoc Test (LSD)
p: p value for comparing between the studied groups a: Statistically significant with control
b: Statistically significant with Isolate L c: Statistically significant with Isolate A
***: Statistically significant at p ≤ 0.05**
L = lactulose A = Apple O = Orange *E.C.N= Escherichia coli Nissile*

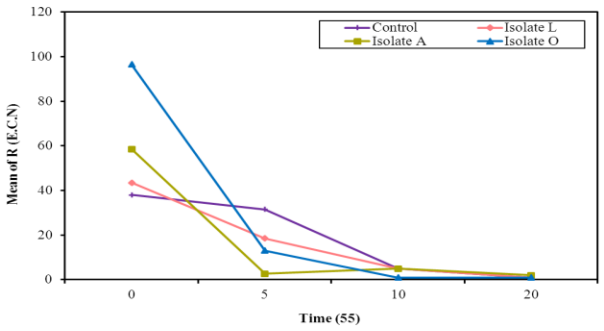

Figure 10. Activity of *Escherichia coli nissile* during incubation at different temperarure

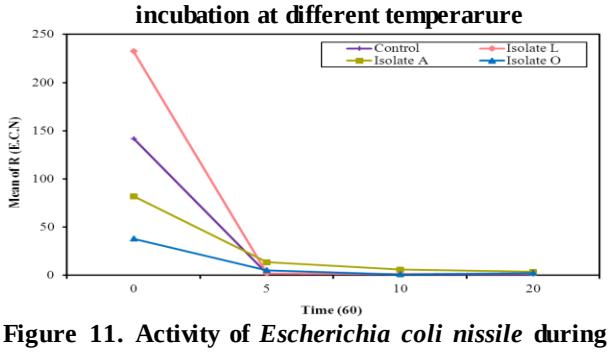

Figure 11. Activity of *Escherichia coli nissile* during incubation at different temperarure

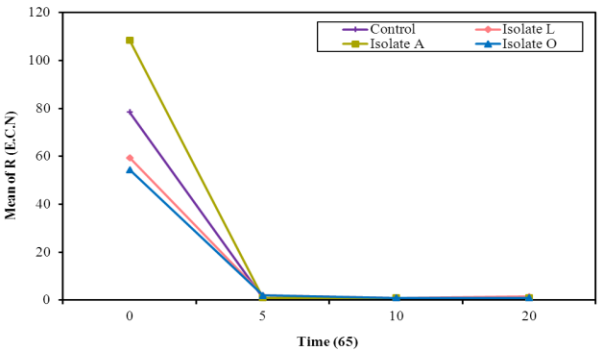

Figure 12. Activity of *Escherichia coli nissile* during incubation at different temperarure

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تأثير المعاملة الحرارية على نشاط عزلات ال .*Enterococcus* spp و عزلة ال *E. coli Nissle* 1917 في وجود مستخلص التفاح والبرتقال و اللاكتيلوز

طه عبد الحليم نصيب وإسراء محمد الأمير

قسم الألبان -كلية الزراعة - جامعة المنصورة.

دراسة تأثير المعاملة الحرارية (٦٥،٦٠،٥٥م) في وجود سكر اللاكتيلوز ومستخلص قشور التفاح والبرتقال على نشاط عزلة ال *Enterococcus molodoratus* في الجداول (١) والأشكال (١-٣) وعزلة ال *Enterococcus feacalis* في الجدول(٢) والأشكال (٤-٦) , عزلة ال *Enterococcus faecium* جدول(٣)و الأشكال (٧-٩). النتائج المتعلقة بعزلة ال*Enterococcus molodoratus* على ٥٥م في الجدول (١-٣) والأشكال (١-٩) أظهرت أن معدل نمو العزلة يتناقص بمرور الوقت , ويزيد معدل النمو بمرور الوقت , بينما في حالة استخدام سكر اللاكتيلوز يكون معدل النمو ثابت بمرور الوقت مقارنة بالكنترول , عنددراسة تأثير المعاملة الحرارية على نشاط ال*Enterococcus molodoratus* , لوحظ أنه عند معاملة العزله على ٥٥م في وجود مستخلص التفاح , فإن نمو العزلة يتناقص بمرور الوقت ,ثم يتم تحديد معدل النمو مقارنة بالكنترول . فيما يتعلق بسلالة ال *Enterococcus feacalis* أظهرت النتائج في الجدول (٢) والأشكال (٤-٦) أنه في حالة وجود مستخلص التفاح يزيد معدل نمو ال *Enterococcus feacalis* ثم ينخفض بمرور الوقت مقارنة بالكنترول , بينما في حالة وجود مستخلص البرتقال والتفاح يزيد معدل النمو, يليه انخفاض مستوى النمو بطول فترة التحضين , وفي الوقت نفسه , أدى وجود مستخلص البرتقال إلى انخفاض معدل النمو وزيادة نشاط ال *Enterococcus feacalis* طوال فترة الحضانة , كما يمكن ملاحظة أن معدل نمو ونشاط هذه العزلة زاد في وجود اللاكتيلوز .لوحظ أيضا عند المعاملة الحرارية لعزلة ال *Enterococcus feacalis* على ٥٥م, في حالة وجود مستخلص التفاح انخفاض معدل النمو بمرور الوقت , وفي حالة استخدام مستخلص البرتقال لوحظ ثبات معدل النمو أول ١٠ دقائق ثم الإنخفاض المفاجئ في النمو . وعند رفع درجة الحرارة إلى ٦٠م في وجود مستخلص التفاح واللاكتيلوز , انخفض معدل نمو العزلة المعوية خلال فترة الحضانة , ومع ذلك ,انخفض معدل نمو العزلة في وجود مستخلص البرتقال , يليه زيادة النمو بمرور الوقت . أدى وجود سكر اللاكتيلوز إلى نمو أكثر نشاطا للعزلة *Enterococcus feacalis* عند المعاملة الحرارية على ٥٥م .من خلال فحص نشاط عزلة ال *Enterococcus faecium* عند ٥٥م في وجود مستخلص التفاح لوحظ استقرار النمو في الجدول (٣) والأشكال (٧-٩) بعد ٥ دقائق , يليه نشاط العزلة في وجود مستخلص البرتقال ثم انخفاض معدل النمو خلال فترة التحضين ,من ناحية أخرى , نتج عن معاملة عزلة ال*Enterococcus faecium* عند ٦٥م في وجود مستخلص التفاح , انخفاض معدل النمو خلال فترة الحضانة .أفضل درجة حرارة لنمو هذة العزلة في وجود مستخلص الربتقال هى ٦٥م , والتي تعتبر الأمثل لنموها . ومع ذلك في وجود سكر اللاكتيلوز فإن درجة الحرارة المثلى لنشاط العزلة هي ٦٥،٥٥م .فيما يتعلق بنشاط عزلة ال *Escherichia coli* Nissle 1917 في وجود مستخلص التفاح والبرتقال واللاكتيلوز عند ٦٥،٦٠،٥٥م ,وذلك في الجدول (٤) والأشكال (١٠-١٢) . أشارت النتائج المتحصل عليها أن معاملة السلالة المعزولة من *Escherichia coli* Nissle 1917 عند ٥٥م في وجود مستخلص التفاح أدى إلى انخفاض معدل نموها خلال فترة الحضانة , في حين لوحظ استقرار النمو عندد استخدام سكر اللاكتيلوز أول ١٠ دقائق ثم انخفاض معدل النمو بمرور الوقت , يمكن ايضا ملاحظة أن رفع درجة الحرارة إلى ٦٠م في وجود مستخلص التفاح والبرتقال واللاكتيلوز أدى إلى انخفاض معدل النمو خلال فترة التحضين . وفي نفس الوقت عند رفع درجة الحرارة إلى ٦٥ م في وجود مستخلص التفاح والبرتقال واللاكتيلوز , انخفض معدل النمو .لوحظ أن أفض تأثير لمستخلص البرتقال على نشاط العزلة عند المعاملة الحرارية على ٥٥م , وقد لوحظ استقرار نمو العزلة بعد ١٠ دقائق من الحضانة على ٦٥م .