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Virulence factors among *Enterococcus* species isolated from local sources

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SUMMARY

Our study indicated that all tested isolates showed haemolytic activity as 52 out of the tested 80 isolates showed β haemolytic activity, while obtained results also declared that 20 and 8 isolates out of the 80 tested isolates showed γ and α haemolysis, respectively. Most isolates of Ras and Domiati cheese (15 and 14 out of 20 isolates, respectively) showed β-haemolytic activity, while 11 isolates of Raw milk and 12 of Karish cheese were considered to be β-haemolytic isolates.

Results also showed that none of studied ***Enterococcus species***, had the ability to produce gelatinase. Moreover, most isolated enterococci (76 out of 80) showed low and moderate aggregation percentages being < 17- 30%. Ras cheese (5) Domiati cheese (5) and Raw milk (3) isolates gave lower aggregation phenotype values, being < 17. The higher aggregation percentage was only noticed with Ras cheese isolates (4 out of 20), being > 30%. All Karish cheese isolates showed only moderate aggregation activity.

Minimum and maximum hydrophobicity percentages values were observed with Karish cheese and Domiati cheese isolates, being 55.90% and 67.82% respectively. In addition 13 out of the studied 80 enterococci isolates showed high adherence towards xylene (63% < 65%), while the best strains (10) exhibited hydrophobicity values (≥ 65%). In conclusion all tested isolates showed good affinity for xylene, which indicates hydrophobic cell surface.

INTRODUCTION

Virulence factors enable the bacteria to act as "opportunistic pathogens". Enterococci have been recognized in recent years as major nosocomial pathogens, one should carefully consider the potential virulence factors of this group of microorganisms before use (**Foulquie et al., 2006**).

Haemolycin production can increase severity of enterococcal infections and presence of genes involved in haemolysin/cytolycin production is also considered a risk factor (**Thacker et al., 1992 and Jett et al., 1994**). Haemolycin plays an important role in enterococcal virulence as it may increase the chance of infection (**Morandi et al., 2006**).

The main role of both gelatinase and serine protease in enterococcal pathogenesis is thought to be in producing nutrients to the bacteria by degrading host tissue, although they also have some function in biofilm formation (**Gilmore et al., 2002 and Mohamed and Hung., 2007**).

The hydrophobic nature of outermost surface of microorganisms has been implicated in the attachment of bacteria to host tissue (**Liungh and Wads tom 1982; and Kiely and Olson., 2000**) and hence is an essential feature in order to import beneficial effect to the host. The information regarding the hydrophobic interactions as well as adherence ability of the enterococcal isolates is very sparse.

In vivo, aggregation substance (**Mundy et al., 2000 and Foulquie et al., 2006**) may contribute to the pathogenesis of enterococcal infection through a number of

mechanisms. Aggregation substance is among the virulence factors able to influence host / parasite relationships that were described in *E. faecalis* (**Cariolato et al., 2008**). In addition to haemolysin / cytolysin, ESP and aggregation substance were clustered together on the large pathogenecity island (**Hallgren et al., 2009**). Aggregation substances increase the hydrophobicity of the enterococcal surface, which may induce localization of cholesterol to phagosomes and prevent or delay fusion with lysosomal vesicles (**Mundy et al., 2000**).

MATERIALS AND METHODS

Haemolytic activity of enterococci:

Haemolytic activity of enterococcal strains was evaluated on nutrient agar supplemented with 5 % of sheep erythrocytes. The plates were incubated at 37 °C for 24h and the haemolytic reaction was recorded by observation of a clear zone of hydrolysis around the colonies (β haemolysis),a partial hydrolysis and greening zone (α haemolysis) or no reaction (γ haemolysis) according to **Jurkovic et al. (2006)**.

Hydrophobicity studies:

The cell surface hydrophobicity of examined isolates was determined according to **Fortina et al., 2008**. Cells were harvested (late log phase from M17 medium), washed twice in PBS buffer and resuspended in 0.1M KNO_3 (pH 6.2) to give a cell suspension with an $OD_{600\ nm}$ of 0.5–0.6 (A0). Three ml of cell suspension were mixed with 1 ml of xylene. After a 10 min preincubation at room temperature, the two-phase system was mixed by vortexing for 2 min. The aqueous phase was removed after 20 min of incubation at room temperature, and its absorbance at 600 nm (A1) was measured. The percentage of bacterial adhesion to solvent was calculated as (1−A1 /A0) ×100.

Gelatinase activity:

Production of gelatinase was tested on BHI agar plates containing 10g/l peptone and 30g/l gelatin. After over night incubation at 37°C, the plates were placed at 4°C for 5 h before examination for zones of turbidity around the colonies indicating hydrolysis of gelatin. This assay was performed according to **Cariolato et al. (2008)**.

Aggregation assay:

This assay was performed according to **Fortina et al. (2008)**. Isolated enterococci were grown at 37°C for 18 h in M17 broth and the cells were harvested by centrifugation at 5000 g for 15 min, washed twice and resuspended in phosphate buffered saline (PBS) to give an $OD_{600\ nm}$ of 0.580 corresponding to viable counts of approximately 3.5×10^7 CFU/ml. Aggregation was evaluated at room temperature on 4 ml of cell suspension mixed by vortexing for 10 s. Every hour 0.1 ml of the upper suspension was transferred to another tube with 0.9 ml of PBS and the absorbance (A) was measured at 600 nm. The aggregation percentage was expressed as 1− (At/A0) ×100, where At, represents the absorbance at time (t =1, 2, 3, 4 or 5 h) and A0 the absorbance at t = 0.

RESULTS AND DISCUSSION

Results of Table (1) indicate that most tested isolates showed haemolytic activity as 52 out of the 80 tested isolates showed β haemolytic activity. Distribution of α, β and γ haemolytic of studied isolates from Raw milk, Ras, Domiati and Karish cheeses could be extracted from Table (1). Data obtained showed that most isolates of Ras cheese and Domiati cheese (15 and 14 out of 20 isolates of each, respectively) were considered to be β-haemolytic, while lower values actually, 11 and 12 isolates from. Raw milk and Karish cheese respectively, showed β-haemolytic reaction.

Frequency distribution of haemolytic activity (cytolytic) among *Enterococcus* species isolated from different sources presented in Table (1). Most isolated enterococci from all tested samples were haemolytic strains with different rates. Where, β-haemolytic pattern possessed the highest value, actually 52 isolates. while the lowest figure, being 8 isolates was recorded in α haemolysis pattern. Considering that genetic determinants encoding for β-haemolytic may be easily transferred by means of conjugative plasmid **(Ike et al., 1987)**, β-haemolytic isolated enterococci from studies milk and dairy products (52 out of 80 isolates) are considered undesirable and not recommended to be used in dairy industry.

Ras cheese samples possessed the highest value of β haemolytic battern strains being 15 and 14 out of 20 of each, respectively. While 11 and 12 haemolytic isolates of Raw milk and Karish cheese were detected.

The most haemolytic ***Enterococcus*** species isolates (Table 2) were *E. faecalis*. being 21 isolates followed by ***E. durans***, 18 and ***E. faecium***. 13 strains. It worthy to say that most haemolytic ***E. faecalis*** were isolated from Ras cheese samples (12 out of 21) followed by isolates from Domiati cheese (6 out of 21). Similarly most haemolytic ***Enterococcus*** species belonged to ***E. faecium*** were isolates from Domiati cheese (5) and Raw milk (4), while Karish cheese posses more than 30% of haemolytic ***E. durans*** isolates (7 out of 18) followed by 6 isolates in Raw milk samples.

Untabulated results showed that none of studied ***Enterococcus species***. *E. faecalis*. ***E. faecium*** and ***E. durans***, had the ability to produce gelatinase. Similar results by Yoon **et al. (2008)** were obtained by studying 7 strains of ***E. faecium***. On the other hand, the presence of gelatinase production among food ***E. faecalis*** strains is high **(Eaton** and **Gasson., 2001 and Franz et al., 2001)**. However, the first author demonstrated that even the Gel gene is present; a negative phenotype can be found. In addition none of the ***E. faecium*** strains involved in both studies produce gelatinase. Only one of VREFs (vancomycin resistant ***E. faecalis***) was found to be gelatinase producer **(Campargo et al., 2008)**. In contrast to ***E. faecalis*** where 48 out of the 80 strains showed gelatinase activity, none of studied ***E. faecium*** produced gelatinase **(Gomes et al., 2008)**.

The association between an enterococcal gelatinase and virulence was stated by many authors **(Campargo et al., 2008 and Gomes et al., 2008)**. Accordingly, all our studied ***Enterococcus*** strains may be considered as safe and should be studied for other different virulence factors.

In general, most isolated enterococci (76 out of 80) showed low and moderate aggregation percentages, being < 17- 30%. Results of Table (3) clearly indicate that Ras cheese, Domiati cheese and Raw milk isolates gave lower aggregation phenotype value. being < 17% with only 5, 5 and 3 out of 20 tested enterococci isolates, respectively

while none of Karish cheese isolates showed this level of aggregation. On the other hand, all Karish cheese isolates exhibited moderate aggregation level, being 17 - 30 %. Similar moderate aggregation levels were also observed with most of Raw milk and Domiati cheese isolates, being 17 and 15, respectivcly and this figure was 11 in case of Ras cheese isolates. High aggregation percentage (> 30%) was only noticed with Ras cheese isolates actually 4 strains.

The biotechnological and safety properties of the novel enterococcal species of dairy origin ***E. italicus*** were investigated by **(Fortina et al., 2008)**. They found that all strains tested exhibited a moderate autoaggregation phenotype with values ranging from 17 to 30%. Consequently, most studied enterococci from local sources are considered to be safe, (Table 4) expect four isolates from Ras cheese samples and these strains belonged to ***E. faecalis*** (Rc1-1 and Rc4-2) and ***E. faecium*** (Rc2- 1 and Rc3-1). **Eaton and Gasson (2001)** and **Franz et al. (2001)** stated that aggregation substance is exclusively found in ***E. faecalis*** strains. However **Valenzuela et al. (2008)** found that aggregation substance showed a very low incidence among ***E. faecalis***, and its incidence among food isolates seems to be high and this was supported by **Yousif et al. (2005) and Valenzuela et al. (2008)** who stated that none of ***E. faecium*** strains in their study produce aggregation substance.

On the other hand, and in agreement with our results **Munoz et al. (2008) and Hallgren et al. (2009)** mentioned that number of ***E. faecium*** and ***E.fecalis*** isolates carried the asal gene was detected in 74 out of 94 isolates. In this respect, as far as we were, there is no any information concerning the aggregation properties of ***Enterococcus species*** isolates from Egyptian milk and cheeses.

Minimum and maximum of aggregation percentages of isolated enterococci are shown in Table (5). Aggregation percentages were increased by time and reached maximum values after 5 hours with different increasing values. Data of this table also indicate that the minimum rate of aggregation after 5 hour was noticed with Raw milk and Ras cheese isolates (11.53% and 11.11%) and higher minimum percentage showed by Karish cheese isolates (17.02%). On the other hand, maximum rate of aggregation after 5 hours was observed with Ras cheese (32.61%), followed by Domiati cheese isolates (28.94%), and approximately similar rate of aggregation was observed with Raw milk and Karish cheese isolates being 26,82 % and 26.31 %, respectively. It seems to be true , that Ras cheese enterococci isolates reached higher maximum aggregation percentages after 1, 2, 3, 4 and 5 hours than all tested enterococci isolated from different sources, being 14.63%, 17.07%, 23.4%, 29.78% and 32.61%, respectively.

In conclusion Ras cheese isolates showed high increasing rate of aggregation than these isolates from different sources studied; which clearly proved the high tendency of Ras cheese isolates towards the aggregation activity. **Foulquie et al. (2006)** came to conclusion that, these important features of virulence factors are strain – dependant and for these reasons, the selection of ***Enterococcus*** strains of interest in food industry should be based on the absence of any possible pathogenic properties.

Results of Table (7) indicate that percentages of hydrophobicity (xylene adhesion) ranged from 55.9% Karish cheese (minimum) to 67.82% Domiati cheese (maximum). Similar conclusion was recorded by **Fortina et al. (2008) and Bhardwaj et al. (2011)** with their strains which exhibited xylene adhesion ranged from 57 to 99 and 65.5 to 91± 0.7%, respectively. Generally all tested ***Enterococcus spp.*** from different sources gave

approximately similar minimum and maximum values. However, different ranges of hydrophobicity with different values were found by **Fortina et al. (2008) and Bhardwaj et al. (2011)** i.e 61- 99% and 65.5 - 91%, respectively. **Morata et al. (1998)** stated that adhesion depends upon the origin of strains as well as surface properties. The information regarding the hydrophobic interactions as well as adherence ability of the enterococcal isolates is very sparse **(Bhardwaj et al., 2011)**.

Higher cell surface hydrophobicity (≥ 65%) was noticed with Ras cheese and Domiati cheese isolates, being 5 and 3 out of 20 isolates , and only one isolate of each Raw milk and Karish cheese showed similar degree of hydrophobicity (Table 6 and 7). Second higher degree of hydrophobicity was observed (63 - < 65%) within 5, 3, 2 and 3, while (60 - < 63%) by 8 , 9 , 7 , and 7 isolates from Raw milk , Ras cheese , Domiati cheese and Karish cheese, respectively. Results of the same table also show that 26 of enterococci out of the tested 80 isolates had relatively good hydrophobic property (< 60%).

It could be extracted from the results of Table 6, that 13 isolates (4 ***E. durans,*** 4 ***E. faecium*** and 5 ***E. faecalis***) from raw milk out of 80 showed high adherence towards xylene (63 - < 65%). However the best strains, (10) were exhibited hydrophobicity values (≥ 65%). In conclusion all tested isolates showed good affinity for xylene, which indicates hydrophobic cell surface.

Result of Tables (6 and 7) also showed that all isolated ***E. faecalis*** from different sources were not safe expect four isolates (Rm9-2, Rm10-2, Rc9-2, Rc10-2 and k2-1) which not posses any of the four studied virulence factors. However, less than half (12 out of 25) of ***E. faecium*** isolates were safe and did not harbored any of virulence factors. These results are in agreement with many authors. **Eaton and Jasson. (2001)** and **Faranz et al. 2001** and **Yousif et al. (2005).** The present results (Table 6 and 7) clearly indicate that the incidence of safe ***Enterococcus species*** were 8 of Raw milk and 6 of Domiati cheese isolates, besides 3 from Ras cheese and 8 from Karish cheese.

Enterococcus faecium isolates were considered to be not virulence carriers, for humans since only one virulence determinant was found and overall they were different to the type of virulence determinant isolated in patients according to **Mannu et al., (2003)**. Consequently, out of the 28 isolates which may considered to be safe. as they did not harbored any of studied virulence factors (Table 4, 6 and 7), only 2 strains of ***E. faecium*** (Rc2-1 and Rc3-1) showed high aggregation percentage (>30%).

In conclusion, results of the present study of 80 ***Enterococcus species*** isolated from Egyptian dairy sources suggested that these isolates should be regarded with caution since most of these isolates may contribute a reservoir of virulence factors. On the other hand, these 25 isolates with no incidence of such traits studied (except 2 strains of ***E. faecium***) should be studied as possible alternatives for use as starters. adjuncts or probiotics to displace the populations of higher risk in dairy products. It is worthy to state that these 25 isolates one or null incidence of such traits and usefulness of these strains should be studied, and similar conclusion was noticed by **Valenzuela et al. (2009)**. In addition, **Valenzuela et al. (2008)** stated that the risk of enterococci has to be interpreted as some of several factors rather than individual traits. Finally, as far as we aware, there is no any information concerning the virulence factors among enterococci isolated from Egyptian raw milk or cheeses.

Table (1): Frequency distribution of haemolytic ***Enterococcus species*** isolated from different sources

Samples	α- Haemolysis	β- Haemolysis	γ- Haemolysis
Raw milk	2	11	7
Ras cheese	1	15	4
Domiati cheese	1	14	5
Karish cheese	4	12	4
Total	8	52	20

α- Haemolysis = a partial hydrolysis and greening zone.
β- Haemolysis = clear zone of hydrolysis around the colonies.
γ- Haemolysis = no reaction.

Table (2): Frequency distribution of Hemolytic (β- Haemolysis) ***Enterococcus*** strains isolated from different sources.

Samples	***Enterococcus species***		
	E.faecalis	***E.faecium***	***E.durans***
Raw milk	1	4	6
Ras cheese	12	1	2
Domiati cheese	6	5	3
Karish cheese	2	3	7
Total	21	13	18

Table (3): Pattern variations of isolated enterococci aggregation assay.

Samples	No. of isolates	Aggregation Percentages		
		Low <17 %	Moderate 17 – 30 %	High > 30 %
Raw milk	20	3	17	0
Ras cheese	20	5	11	4
Domiati cheese	20	5	15	0
Karish cheese	20	0	20	0
Total	80	13	63	4

Table (4): Aggregation levels of isolated enterococci strains from different sources.

Samples	Low	. Moderate	High
	<17 %	17 – 30 %	> 30 %
Raw milk	*E.faecium* Rm5-1, *E.faecium* Rm7-1, *E.faecalis* Rm9-2	17 out of 20 isolates	None
Ras cheese	*E.durans* Rc3-2, *E.durans* Rc4-1, *E.faecalis* Rc5-2, *E.faecalis* Rc6-2, *E.faecalis* Rc8-1	11 out of 20 isolates	*E.faecalis* Rc1-1, *E.faecium* Rc2-1, *E.faecium* Rc3-1, *E.faecalis* Rc4-2
Domiati cheese	*E.faecalis* Dc2-2, *E.faecium* Dc4-2, *E.faecium* Dc8-1, *E.durans* Dc9-2, *E.faecium* Dc10-2	15 out of 20 isolates	None
Karish cheese	None	All	None

Table (5): Minimum and maximum aggregation percentages of tested enterococci.

Samples	*A1		A2		A3		A4		A5	
	Min	Max	Min	Max	Min	Max	Min	Max	Min	Max
Raw milk	3.27	6.66	7.14	11.76	9.61	15.68	11.53	19.51	11.53	26.82
Ras cheese	1.67	14.63	3.85	17.07	7.69	23.40	9.62	29.78	11.11	32.61
Domiati cheese	2.38	8.51	5.66	14.58	5.66	20.83	9.43	23.07	13.20	28.94
Karish cheese	1.96	6.52	5.17	10.86	8.62	18.42	11.53	23.68	17.02	26.31

*= Time by hours

Table (6): Virulence factors among ***Enterococcus species*** isolated from Raw milk and Ras cheese.

Enterococcus species	Virulence factors		***Enterococcus species***	Virulence factors	
	Hydrophobicity (%)	Haemolysis		Hydrophobicity (%)	Haemolysis
E.durans*Rm1-1	64.10	+	***E.faecalis***•Rc1-1	58.47	+
E.durans Rm1-2	60.81	-	***E.faecalis*** Rc1-2	60.48	+
E.faecium Rm2-1	63.12	+	***E.faecium*** Rc2-1	62.56	-
E.durans Rm2-2	64.53	+	***E.faecalis*** Rc2-2	62.01	+
E.durans Rm3-1	63.10	-	***E.faecium*** Rc3-1	65.56	-
E.durans Rm3-2	57.89	-	***E.durans*** Rc3-2	66.26	-
E.faecium Rm4-1	57.40	-	***E.durans*** Rc4-1	61.00	+
E.faecium Rm4-2	58.11	-	***E.faecalis*** Rc4-2	62.83	+
E.faecium Rm5-1	59.47	-	***E.durans*** Rc5-1	57.95	+
E.faecium Rm5-2	65.09	+	***E.faecalis*** Rc5-2	56.75	+
E.faecium Rm6-1	64.23	-	***E.faecalis*** Rc6-1	60.23	+
E.faecium Rm6-2	60.51	+	***E.faecalis*** Rc6-2	61.66	+
E.faecium Rm7-1	62.90	+	***E.faecium*** Rc7-1	65.01	+
E.durans Rm7-2	59.76	+	***E.faecalis*** Rc7-2	64.57	+
E.durans Rm8-1	61.18	+	***E.faecalis*** Rc8-1	61.34	+
E.durans Rm8-2	58.43	+	***E.faecalis*** Rc8-2	63.71	+
E.durans Rm9-1	62.80	+	***E.faecalis*** Rc9-1	60.21	+
E.faecalis Rm9-2	60.16	-	***E.faecalis*** Rc9-2	65.03	-
E.faecalis Rm10-1	60.77	+	***E.faecalis*** Rc10-1	65.99	-
E.faecalis Rm10-2	62.86	-	***E.faecalis*** Rc10-2	64.00	+

*Rm =Raw milk •Rc =Ras cheese

Table (7): Virulence factors among *Enterococcus species* isolated from Domiati and Karish cheese.

Enterococcus species	Virulence factors		*Enterococcus species*	Virulence factors	
	Hydrophobicity (%)	Haemolysis		Hydrophobicity (%)	Haemolysis
E.faecium♦Dc1-1	58.31	-	*E.durans*♥K1-1	62.73	-
E.faecalis Dc1-2	58.18	+	*E.faecium* K1-2	59.44	-
E.faecalis Dc2-1	59.88	+	*E.faecalis* K2-1	62.20	-
E.faecalis Dc2-2	60.03	+	*E.durans* K2-2	57.77	+
E.faecalis Dc3-1	60.46	+	*E.faecium* K3-1	58.08	+
E.faecalis Dc3-2	57.43	+	*E.faecium* K3-2	64.19	-
E.faecium Dc4-1	63.68	-	*E.durans* K4-1	61.19	+
E.faecium Dc4-2	58.39	+	*E.durans* K4-2	57.75	-
E.faecium Dc5-1	59.31	-	*E.durans* K5-1	58.76	-
E.faecalis Dc5-2	67.82	+	*E.durans* K5-2	60.10	-
E.durans Dc6-1	63.82	-	*E.durans* K6-1	55.95	+
E.durans Dc6-2	66.20	+	*E.faecium* K6-2	60.43	+
E.faecium Dc7-1	62.32	+	*E.faecalis* K7-1	64.06	+
E.faecium Dc7-2	62.03	+	*E.durans* K7-2	65.74	+
E.faecium Dc8-1	60.94	+	*E.durans* K8-1	59.13	+
E.faecium Dc8-2	59.93	+	*E.faecium* K8-2	62.25	+
E.durans Dc9-1	65.04	+	*E.durans* K9-1	55.90	+
E.durans Dc9-2	60.47	-	*E.durans* K9-2	58.96	+
E.durans Dc10-1	60.66	+	*E.durans* K10-1	62.65	-
E.faecium Dc10-2	57.44	-	*E.faecalis* K10-2	63.90	+

♦Dc =Domiati cheese ♥K =Karish cheese

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الملخص العربى

العوامل المرضية للبكتيريا المعوية (*Enterococcus*) المعزولة من المصادر المحلية

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أظهرت النتائج أن معظم العزلات المختبرة قادرة على التحلل الدموى حيث أظهرت ٥٢ عزلة من اجمالى ٨٠ عزلة نشاطا واضحا من النوع β haemolysis و٢٠ عزلة من النوع γ haemolysis و٨ عزلات من النوع α haemolysis . كانت معظم عزلات الجبن الراس والجبن الدمياطى (١٥ ، ١٤ عزله من ٢٠ عزله لكلا منهم) β haemolysis ، ١١ عزلة من اللبن الخام ، ١٢ عزلة من الجبن القريش β haemolysis .

أظهرت النتائج أن كل أنواع ***Enterococcus*** المعزولة (***E. durans*** ، ***E. faecium*** ، ***E. faecalis***) ليس لديها القدرة على إنتاج انزيم الجيلاتينيز.

كما أظهرت أيضا معظم عزلات Enterococci (٧٦ عزلة من إجمالي ٨٠ عزلة) خاصية التجمع (Aggregation) بنسب منخفضة ومتوسطة أقل من ١٧ ٪ إلى ٣٠ ٪. بالإضافة إلى أن عزلات الجبن الدمياطى والجبن الراس لديها قيم تجمع منخفضة (أقل من ١٧ ٪) بالإضافة إلى أن كل عزلات الجبن القريش كان لها قيم متوسطة وعلى الجانب الآخر أظهرت ٤ عزلات من الجبن الراس قيم تجمع عالية أكبر من ٣٠٪.

أوضحت النتائج أن الحد الأدنى لخاصية (Hydrophobicity) كان بين عزلات الجبن القريش هو ٥٥.٩٠ ٪ بينما كان الحد الأقصى بين عزلات الجبن الدمياطى هو ٦٧.٨٢ ٪. وخلاصة القول أن كل العزلات المختبرة كان لديها قدرة جيدة لهذه الخاصية. أخيرا أمكن التوصل إلى أن هناك ٢٨ من مجموع ٨٠ عزلة تحوى لواحدة فقط أو لا تحوى أى من العوامل الضارة والتى تسمح باستخدامها كبادئات (Starters) أو كمضافات (Adjunct) أو كعوامل مدعمة للحياة (Probiotics).