PREVALENCE OF Enterococcus IN EGYPTIAN DAIRY PRODUCTS
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ABSTRACT

This study examined the prevalence and diversity of Enterococcus species in traditional Egyptian dairy products. A total of 24 samples of traditional Egyptian dairy products including pickled Domiati cheese (7 samples), fresh Domiati cheese (5 samples), Kariesh cheese (6 samples), and Mish cheese (6 samples) were randomly collected from local markets in Mansoura city and examined for the presence of Enterococcus by plating on Kanamycin Asculin Azid agar. Out of 77 potential Enterococcus isolates recovered from those samples, 61 cultures were confirmed as belonging to the Enterococcus genus. These confirmed cultures were isolated from pickled Domiati cheese (19 isolates), fresh Domiati cheese (28 isolates), Kariesh cheese (7 isolates) and Mish cheese (7 isolates). These cultures were further identified to the species level by examining their ability to ferment glucose, sucrose, lactose, sorbitol, and mannitol, produce pigment and hydrogen sulphide (H₂S), show motility, and utilize pyruvate. Based on these physiological examinations, 80% of the isolates were identified as E. faecium. Other Enterococcus species including E. faecalis, E. gallinarum, and E. malodoratus also existed in the examined samples, but at minor incidence rates of 8%, 2%, and 10%, respectively. E. faecium isolates could be recovered from all examined traditional Egyptian dairy products. E. faecalis, and E. gallinarum were isolated from Domiati cheese, but could not be detected in Kariesh cheese or Mish cheese. Whereas, E. malodoratus could be isolated from both Kariesh cheese and Mish cheese, but could not be cultured from pickled or fresh Domiati cheese. These results highlighted Enterococcus as prevalent lactic acid bacteria that existed as diverse species in traditional Egyptian dairy products. Keywords: Enterococcus, dairy products, Enterococcus faecium, diversity, Lactic acid bacteria

INTRODUCTION

The Enterococcus genus involves Gram-positive, catalase-negative cocci that ferment lactose into lactic acid. Members of the Enterococcus genus are ubiquitous in diverse niches, but more frequently occur in vegetables, plant material, and foods of animal origin including dairy products (Giraffa et al. 1997; Franz et al. 1999). Enterococcus is considered the third-largest genus of lactic acid bacteria (LAB) after the genera Lactobacillus and Streptococcus. It currently involves 37 species, which fall into 7 species groups based on the 16S rRNA sequence similarities (Deviere et al. 2003; Franz and Holzapfel 2006). However, E. faecium and E. faecalis are the most important enterococcal species. Enterococci were found to play an important role in the ripening of various traditional cheese varieties (Giraffa 2003). They were shown to be involved in the proteolysis, lipolysis, and citrate breakdown occurring during ripening, and thus contributing to the development of the typical taste and flavor of ripened cheese. Certain Enterococcus strains have been also reported to produce bacteriocins and others have been employed...
as probiotics (Giraffa 2003). In contrast, enterococci have been reported among the most common nosocomial pathogens that have been incriminated in several cases of endocarditis, bacteremia, and infections of the urinary tract, central nervous system, intra-abdominal and pelvic infections (Endtz et al. 1999; Franz et al. 1999).

The association of Enterococcus with traditional European cheese varieties prepared in Mediterranean countries, such as Greece, Italy, Spain and Portugal, using raw or pasteurized goats', ewes', buffalos' or bovine milk has been extensively studied (Pérez Elortondo et al. 1999; Xanthopoulos et al. 2000; Manolopoulou et al. 2003). These studies showed Enterococcus as predominant LAB in the fully ripened product. However, little is known on the prevalence and diversity of Enterococcus in traditional Egyptian dairy products. The present study was therefore designed to assess the presence and diversity of Enterococcus in Egyptian dairy products including pickled Domiati cheese, fresh Domiati cheese, Kariesh cheese, and Mish cheese.

**MATERIALS AND METHODS**

**Collection of dairy product samples**

A total of 24 samples of traditional Egyptian dairy products were randomly collected from local markets in Mansoura city. These samples included 7 samples of pickled Domiati cheese, 5 samples of fresh Domiati cheese, 6 samples of Kariesh cheese and 6 samples of Mish cheese.

**Isolation of Enterococcus from dairy product samples**

Twenty-five grams of each examined dairy product sample were ground in 225 ml of sterile saline solution (0.85% NaCl), followed by preparing serial dilutions of the resultant sample suspension using the same sterile solution. Suitable dilutions were selected and plated onto kanamycin aesculin azide (KAA) agar (Oxoid, Basingstoke, UK), followed by incubation at 37°C for 24 h. Round, white or grey colonies surrounded by black zone were picked up and maintained for the identification tests.

**Identification of Enterococcus isolates**

Suspected isolates of Enterococcus were subjected to the following morphological and physiological examinations:

**Gram staining**

A 24 h culture of each suspected Enterococcus isolate grown on MRS agar (Oxoid) was Gram-stained and microscopically examined as described by Pollack et al. (2005).

**Catalase activity**

A loopful from one discrete colony of each suspected Enterococcus isolate grown for 24 h on MRS agar was transferred onto a clean glass slide to be mixed with a drop of H₂O₂ (30%). A positive result was indicated by immediate bubbling due to gas formation (Macfaddin 1977).

**Growth at 45°C and 10°C**

A 24 h culture of each suspected Enterococcus isolate grown in the MRS broth (Oxoid) was inoculated at 1% (v/v) into MRS broth, followed by
incubation at 45°C for 48 h and 10°C for 2 weeks. Bacterial growth was observed within these incubation times (Sharpe 1979).

**Growth in 6.5% NaCl**

A 24 h culture of each suspected *Enterococcus* isolate grown in MRS broth was inoculated at 1% (v/v) into MRS broth containing 6.5% NaCl followed by incubation at 37°C (Abd-El-Malek and Gibson 1948). Cell growth was observed after 24 h of incubation.

**Growth at pH 9.6**

A 24 h culture of each suspected *Enterococcus* isolate grown in MRS broth was inoculated at 1% (v/v) into the same broth adjusted to pH 9.6, followed by incubation at 37°C (Sharpe 1979). Cell growth was observed after 24 h of incubation.

**Growth on bile aesculin agar (BAA) medium**

One hundred microliters of a 24 h culture of each suspected *Enterococcus* isolate grown in MRS broth were spread onto bile aesculin agar (BAA) (Oxoid), followed by incubation at 37°C for 24 h. The development of brown colored growth on BAA indicated a positive result of the test.

**Gas production from glucose**

A 24 h culture of each suspected *Enterococcus* isolate grown in MRS broth was inoculated at 1% (v/v) into MRS broth, followed by incubation at 37°C for 24 h (De Man et al. 1960). Gas production was indicated by bubble formation in the inverted Durham tube in the MRS broth.

**Biochemical speciation of *Enterococcus* isolates**

Confirmed *Enterococcus* isolates were further identified to the species level. They were grown in MRS broth at 37°C for 24 h and subjected to the following examinations:

**Carbohydrate fermentation**

*Enterococcus* cultures were inoculated at 1% (v/v) into nutrient broth (Oxoid) supplemented with 1% (w/v%) glucose, sucrose, lactose, arabinose, sorbitol, glycerol, or mannitol and 0.0018% (w/v%) bromocresol purple (BCP) (1 ml from 1.6% BCP solution in 95% ethanol was added to 900 ml nutrient broth). A positive reaction was recorded when the indicator changed from purple to yellow, which indicated acid production. Tubes were observed on a daily basis for 3 days (Facklam 1972).

**Pigment production**

One hundred microliters of each *Enterococcus* culture were spread onto tryptone soya agar (TSA) (Oxoid), followed by incubation at 37°C for 24 h. The development of yellow colored growth on TSA indicated a positive result of the test (Facklam and Collins 1989).

**Motility and hydrogen sulfide (H₂S) production**

*Enterococcus* cultures were inoculated into test tubes containing the sulfide-indole-motility (SIM) medium (Oxoid), followed by incubation at 37°C for 24 h (Harrigan and McCance 1966). Non-motile isolates showed growth only along the line of inoculation, whereas motile cultures had either a diffuse
even growth spreading from the inoculum, turbidity of the whole medium, or more rarely, localised outgrowths which were usually fan-shaped or occasionally nodular. Hydrogen sulfide production was indicated by blackening of the line of inoculation.

**Pyruvate utilization**

*Enterococcus* isolates were inoculated at 1% (v/v) into nutrient broth, to which 1% sodium salt of pyruvic acid and 0.004% bromothymol blue were added. Cultures were incubated for 16 to 18 h at 35°C and observed for colour changes. Colour turned into bright yellow in pyruvate-utilizing cultures (Gross et al. 1975).

**RESULTS AND DISCUSSION**

**Isolation of Enterococcus from traditional Egyptian dairy products**

The prevalence of *Enterococcus* in traditional Egyptian dairy products was studied. Samples of dairy products were randomly collected from local markets in Mansoura city and examined for the presence of *Enterococcus* by plating on Kanamycin Asculin Azid (KAA) agar. Suspected colonies were subjected to biochemical and physiological identification tests. Isolates that were found to be Gram-positive cocci, and gave a negative reaction in the catalase test were considered as potential *Enterococcus* cultures. Table 1 shows the number of examined dairy samples and potential *Enterococcus* isolates recovered from these samples. It could be seen that potential *Enterococcus* isolates could be cultured from all examined traditional Egyptian dairy products. Higher percentages of positive samples containing potential isolates of the organism were found with pickled Domiati cheese (71%) and Mish cheese (83%), compared with fresh Domiati cheese (60%), and Kariesh cheese (67%) (Table 1). Potential *Enterococcus* isolates were further examined for physiological traits shown in Table 2. Out of 77 potential *Enterococcus* isolates, 61 cultures could grow at 10°C, 45°C, and pH 9.6 and in the presence of 6.5% NaCl. (Table 2). They also gave positive results in the bile aesculin test, and fermented glucose without gas production (Table 2). Since these traits are typical phenotypic characteristics of the members of the *Enterococcus* genus (Sherman 1937; Stiles and Holzapfel 1997), those 61 cultures were confirmed as *Enterococcus* isolates.

**Biochemical speciation of Enterococcus isolates cultured from traditional Egyptian dairy products**

Confirmed *Enterococcus* isolates cultured from Egyptian dairy products were further identified to the species level based on their biochemical activities. This involved examining the ability of these isolates to ferment glucose, sucrose, lactose, sorbitol, and mannitol, produce pigment and hydrogen sulphide (H₂S), show motility, and utilize pyruvate (Table 3). The results of these biochemical examinations were interpreted according to Collins *et al.* (1986), Facklam and Collins (1989), and Day *et al.* (2001). It could be seen in Table 3 that *E. faecium* was the most frequently isolated *Enterococcus* species since 80% of the total isolates were found to belong to...
it. *E. faecium* isolates could be recovered from all examined traditional Egyptian dairy products (Table 3). Higher incidence rates of *E. faecium* were observed with fresh Domiati cheese (25 isolates) and pickled Domiati cheese (16 isolates), compared with Kariesh cheese (5 isolates) and Mish cheese (3 isolates). Other *Enterococcus* species including *E. faecalis*, *E. gallinarum*, and *E. malodoratus* also existed in the examined traditional Egyptian dairy products, but at minor incidence rates of 8%, 2%, and 10%, respectively (Table 3). *E. faecalis*, and *E. gallinarum* were isolated from Domiati cheese, but could not be detected in Kariesh cheese or Mish cheese. Whereas, *E. malodoratus* could be isolated from both Kariesh cheese and Mish cheese, but could not be cultured from pickled or fresh Domiati cheese.

Table 1: Association of *Enterococcus* with traditional Egyptian dairy products

<table>
<thead>
<tr>
<th>Samples</th>
<th>No. of Samples</th>
<th>No. of Positive Samples (%)</th>
<th>No. of potential <em>Enterococcus</em> isolates*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pickled Domiati cheese</td>
<td>7</td>
<td>5 (71%)</td>
<td>35</td>
</tr>
<tr>
<td>Fresh Domiati cheese</td>
<td>5</td>
<td>3 (60%)</td>
<td>28</td>
</tr>
<tr>
<td>Kariesh cheese</td>
<td>6</td>
<td>4 (67%)</td>
<td>7</td>
</tr>
<tr>
<td>Mish cheese</td>
<td>6</td>
<td>5 (83%)</td>
<td>7</td>
</tr>
<tr>
<td>Total</td>
<td>24</td>
<td>17 (71%)</td>
<td>77</td>
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</tbody>
</table>

*Isolates that were Gram-positive cocci and gave negative results in the catalase test.*

Table 2: Physiological confirmation of potential *Enterococcus* isolates recovered from traditional Egyptian dairy products

<table>
<thead>
<tr>
<th>Dairy product (Number of samples)</th>
<th>Number of confirmed/potential <em>Enterococcus</em> isolates (%)</th>
<th>Physiological Characteristics</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Growth at 10°C Growth at 45°C Growth in 6.5% NaCl Growth at pH 9.6 Bile-aesculin reaction Gas from glucose</td>
<td></td>
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<tr>
<td>Pickled Domiati Cheese</td>
<td>19/35 (54%)</td>
<td>+</td>
</tr>
<tr>
<td>Fresh Domiati Cheese</td>
<td>28/28 (100%)</td>
<td>+ + + + + -</td>
</tr>
<tr>
<td>Kariesh Cheese</td>
<td>7/7 (100%)</td>
<td></td>
</tr>
<tr>
<td>Mish cheese</td>
<td>7/7 (100%)</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>61</td>
<td></td>
</tr>
</tbody>
</table>
Table 3: Biochemical speciation of Enterococcus cultures isolated from traditional Egyptian dairy products

| Isolat un | Source (Number of isolates) | Source (Number of isolates) | Glucose | Sucrose | Lactose | Sorbitol | Mannitol | Pigment | H₂S | Mobility | Pyruvate | Identification
<table>
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</thead>
<tbody>
<tr>
<td>49 (80%)</td>
<td>Fresh Domiati cheese (25)</td>
<td></td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>E. faecium</td>
</tr>
</tbody>
</table>
|           | Pickled Domiati cheese (16) | Kariesh cheese (5)          |         |         |         | (2%)
|           | Mish cheese (3)             |                              |         |         |         | (96%)
|           |                             |                              |         |         |         | (4%)
| 5 (8%)    | Fresh Domiati cheese (2)    | Pickled Domiati cheese (3)  | +       | +       | +       | +        | -        | -       | -    | -        | +       | E. faecalis      |
| 1 (2%)    | Fresh Domiati cheese (1)    |                              | +       | +       | +       | +        | -        | -       | -    | +        | +       | E. gallinarum    |
| 6 (10%)   | Kariesh cheese (2)          | Mish cheese (4)             | +       | +       | +       | +        | -        | +       | +    | -        | -       | E. malodoratus   |

*Percent of total confirmed Enterococcus isolates.
*percent of isolates producing positive reaction.
*percent of isolates producing negative reaction.

The above results showed that diverse Enterococcus species including E. faecium, E. faecalis, E. gallinarum, and E. malodoratus could be isolated from traditional Egyptian dairy products, which was also reported in previous studies. For instance, El-Awady et al. (2011) could isolate 80 Enterococcus cultures from 100 samples of Kariesh cheese, Zabady, Laban Rayeb, and Ras cheese. Ayad et al. (2004) had also studied the technological characteristics of 333 Enterococcus isolates cultured from artisanal Egyptian dairy products including Domiati cheese, Ras cheese, Mish, Zabady and Laban Rayeb. Those isolates involved E. faecium (n= 255), E. durans (n= 48), E. faecalis (n= 23), E. avium (n= 3), and E. casseliflavus (n= 4). However, the authors did not describe the prevalence of each Enterococcus species in each examined dairy product. Süßmuth (1995) could isolate 300 isolates of lactic acid bacteria from Domiati cheese and Ras cheese, most of which were identified as Enterococcus with only 3.5% of the strains belonging to Lactococcus and 1.5% belonging to S. thermophilus and S. bovis. The frequent occurrence of Enterococcus strains in traditional Egyptian dairy products as shown in those studies and the present work could reflect the ability of Enterococcus to tolerate relatively high salt levels of Domiati and Mish cheeses, and acidity of Kariesh cheese. It was reported that Enterococcus strains could persist during the preparation and storage of dairy products.
products due to their ability to withstand adverse environmental conditions of extreme pH, temperatures, and salinity (Giraffa 2003).

The diversity of Enterococcus species in Egyptian dairy products examined in this study was consistent with other reports of Teuber et al. (1996), Giraffa et al., (1997), Franz et al. (1999), and Sarantinopoulos et al. (2001). In those studies, E. faecium, E. faecalis, and, to a lesser extent, E. durans were the most frequently Enterococcus species to be isolated from dairy products. In agreement with the present study, Jokovic et al. (2008) found that E. faecium was the most prevalent Enterococcus species in the Serbian fermented cream product, Kajmak.

In conclusion, this study presented Enterococcus as prevalent LAB in traditional Egyptian dairy products. It also showed that diverse Enterococcus species existed in these products, with E. faecium being the most frequently isolated enterococci.

REFERENCES


Nassib, T. A. et al.


تتواجد ميكروب الانتيروكوركس في منتجات الألبان المصرية

تم دراسة تواجد ميكروب الانتيروكوركس في منتجات الألبان المصرية التقليدية والتعريف
على أنواعه المختلفة بها. حيث تم تجميع 24 عينة من هذه المنتجات اللذيذة إشتملت على 3 عيان من الجين الدمياطي الخزين و5 عيان من الجين الدمياطي الطازج و5 عيان من الجين الفريش و2 عيان من الجين المش وذلك من الأسواق المحلية بمدينة المنصورة، وفحصت هذه العينات عن ميكروب الانتيروكوركس ريم التآكل تم أخذ عينة من هذه المنتجات وعزل 77 مزرعة محتملة من ميكروب الانتيروكوركس تم التأكد من أن 66 مزرعة منها تنتمي إلى هذا الجنس. وقد كانت مصادر هذه المزارع الممكنة هي الجين الدمياطي الخزين (19 عزلة)، والجين الدمياطي الطازج (28 عزلة)، والجين الفريش (7 عزلات)، والجين المش (7 عزلات). وقد تم فحص هذه المزارع لتقييم أنواعها عن طريق اختبار قدرتها على تخمير السكريات (الجلوكوز والسكرورز واللاكتوز والسوربيتيول والمانيتول) وقدرتها على الحركة واستخدام البروتينات، وقد أظهرت نتائج هذه الاختبارات الفيزيولوجية أن 80% من E. faecium تم عزلها من كل منتجات الألبان المصرية التليدية التي تم فحصها في هذه الدراسة. أما مزرعات E. faecium و E. gallinarum و E. faecalis تم عزلها من كل منتجات الألبان المصرية التقليدية التي تم فحصها في هذه الدراسة. أما مزرعات E. faecium و E. gallinarum و E. faecalis فقد تم عزلها من الجين الفريش ولكن لم تتم معرفة أنواعها من الجين الفريش من الجينات المش. بينما أمكن عزل E. malodoratus في الجينات المش ولم يتم عزله من الجينات الدمياطية الطازجة أو الخزين. أظهرت هذه النتائج انتشار ميكروب الانتيروكوركس وتنوعه كميكروبات بكتريا حامض اللاكتيك في منتجات الألبان المصرية التقليدية.

قام بتحكيم البحث

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