BACTERIOLOGICAL STUDIES ON DOMIATI CHEESE

By

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SUMMARY

Lactobacillus farciminis (16 isolates), **Lactobacillus alimentarius** (10 isolates), **Lactobacillus casei** (1 isolate), **Enterococcus faecalis** (3 isolates), **Enterococcus faecium** (2 isolates), **Propionibacterium jensenii** (1 isolate), **Microbacterium lacticum** (7 isolates), and **Brevibacterium linens** (7 isolates) were isolated on their specific media from 10 samples of Domiati cheese.

INTRODUCTION

Different desirable bacterial species had been isolated from Domiati cheese by many investigators e.g., **Micrococcus (M.) lutus** (Shehata et al. 1984) ; **M. halobius, M. caseolyticus M. varians, M. roseus, Streptococcus (Str.) faecalis, Str. cremoris, Str. lactis, Str. lactis var. diacetylactis, Str. thermophilus, Str. durans, Str. bovis, Str. liquefaciens, Lactobacillus (Lb.) casei, Lb. casei var. casei, Lb. casei var. alatosous, Lb. plantarum, Lb. fermentum and Lb. bulgaricus** (Shehata et al. 1975; Fahmy and Youssef, 1978).

However, the previous bacterial isolates were used as starter cultures during the manufacture of Domiati cheese, little attention was directed towards their role on the formation of cheese flavour.

In the present paper, many desirable bacteria were isolated and identified, and their role in flavour development will be presented in a subsequent paper.

MATERIALS AND METHODS

Ten samples of Domiati cheese (fresh or 3 months-ripened) were made from raw or pasteurized milk. One gram of cheese sample was taken out under aseptic conditions and grounded in a sterilized mortar with 1 of sterile sodium citrate solution 200 gL^{-1} and 8 ml sterile saline solution (8.5 gL^{-1} NaCl). Serial dilutions were then made and cultured under the optimum conditions for each bacterial species. MRS-medium (De Man et al. 1960), Elliker medium (Elliker et al. 1956), pH- medium (Pearce and Halligan, 1978). nutrient agar medium + 60 gL^{-1} NaCl, yeast extract-sodium lactate medium (Malik et al. 1968), corynebacterium-selective (Merck), yeast extract milk medium (Harrigan and McCane, 1976) and tryptone soy agar medium + 40 gL^{-1} 1 NaCl (El-Erian, 1969) were used for isolation of **Lactobacillus, Streptococcus, Leuconostoc, Micrococcus, Propionibacterium, Corynebacterium, Microbacterium** and **Brevibacterium** species in order. The cultures were purified by streaking three times on their specific media.

Lactobacillus spp. were identified according to Kandler and Weiss (1986) and API 50 CH system (API-Biomèrieux, France). **Streptococcus** spp. were identified according to Schleifer (1986) and API 20 STREP system (API-Biomèrieux). **Propionibacterium, Microbacterium** and **Brevibacterium** spp. were identified according to Jones and Collins (1986).

RESULTS AND DISCUSSION

Forty seven bacterial species were isolated from 10 Domiati cheese samples. The bacterial isolates were firstly identified according to their Gram-stain, spore formation, shapes, catalase test, O_2-requirements and gas formation from glucose anaerobically, into : 1) 27 **Lactobacillus** spp. (Gram-positive, non-sporeforming rods, facultative in their O_2-requirements and catalase negative), 2) 5 **Enterococcus** spp. (Gram-positive cocci in pairs, non-sporeforming, facultative anaerobes, catalase-negative and homofermentative) and 3) 15 **Propionibacterium, Microbacterium** and/or **Previbacterium** spp. (Gram-positive and non-sporeforming irregular rods).

Identification of Lactobacillus

Lactobacillus isolates had circular, convex, entire, smooth glistening, opaque, butyrous, greyish white colonies (on MRS-agar) and formed pin-point colonies on nutrient agar.

The isolates were unable to reduce nitrate, produce indole, liquefy gelatin, produce CO_2 from glucose, form levan and dextran from sucrose, ferment glycerol ; erythritol; D-arabinose ; D-xylose ; L-xylose ; adonitol ; β-methyl-xyloside ; rhamnose ; dulictol ; melibiose ; inulin ; D-raffinose ; starch ; glycogen ; xylitol ; D-turanose ; D-lyxose ; D-fucose ; L-fucose ; D-arabitol ; L-arabitol ; 2-ceto-gluconate and 5-ceto-gluconate. However, they able to ferment D-glucose, D-fructose, D-mannose, N-acetylglucosamine, trehalose and β-gentiobiose.

Ten isolates were able to grow at 42°C, grow at 80 and 10 gL^{-1} NaCl, ferment ribose ; galactose ; esculin ; salicin and lactose. They were unable to produce NH_3 from arginine, ferment L-sorbose ; inositol ; mannitol ; sorbitol and melezitose. Some of them gave variable results for growth at 15°C, production of CO_2 from gluconate, growth at 120 gL^{-1} NaCl, fermentation of some sugars e.g., 4 isolates fermented L-arabinose and sucrose and 6 isolates fermented maltose. These ten isolates were identified as **L. alimentarius**. Kandler and Weiss (1986) mentioned that **L. alimentarius** gave variable results for arabinose fermentation, while Reuter (1983) recorded that L-arabinose was weakly fermented by **L. alimentarius.**

In contrast to the bacterial control (**L. alimentarius** NCIB 11994) and type strain of Kandler and Weiss (1986) . **L. alimentarius** isolates fermented lactose. This can be explained by that our strains are other type strains of **L. alimentarius.**

One isolate of lactobacilli was able to form CO_2 from gluconate, grow at 15, 42 and 45°C, grow at 80 gL^{-1} NaCl, ferment ribose ; galactose ; L-sorbose ; inositol ; mannitol ; amygdalin ; arbutin ; esculin ; salicin ; cellobiose ; lactose ; melezitose ; D-tagatose and gluconate. It was unable to grow at 8°C, grow at 100 and 120 gL^{-1} NaCl, produce NH_3 from arginine, ferment L-arabinose; α-methyl-D-mannoside; α-methyl-D-glucoside ; maltose and sucrose. Thus, this isolate was identified as **L. casei.** Moreover, this isolate is similar to **L. casei** subsp. **casei** (Kandler and Weiss, 1986) except, it was unable to ferment either maltose or sucrose.

Sixteen lactobacilli isolates were unable to grow at 8, 42 and 45°C, produce CO_2 from gluconate, ferment L-arabinose ; ribose ; L-sorbose ; inositol ; mannitol ; sorbitol ; α-methyl-D-mannoside ; α-methyl-D-glucoside ; melezitose and gluconate. Some of them gave variable results for growth at 15 and 42°C, growth at 120 gL^{-1} NaCl, production of NH_3 from arginine, fermentation of some sugars e.g., 11/16 isolates fermented amygdalin

and 5/16 isolates fermented maltose, thus, these isolates were identified as L. farciminis. Also, the bacterial control (**L. farciminis** LMG 9200) was unable to ferment amygdalin and weakly fermented maltose. Kandler and Weiss (1986), recorded the ability of **L. farciminis** to ferment amygdalin while, Reuter (1983) mentioned that maltose was weakly fermented by **L. farciminis.**

Identification of Enterococcus

Five isolates had circular, convex, entire, smooth surface, opaque, butyrous and white colonies. They were non-motile, able to grow at 10 and 45°C, grow in 65 gL^{-1} NaCl; pH 9.6 and 1 gL^{-1} methylene blue milk, produce NH_3 from arginine ; acetoin from glucose ; pyrrolidonylarylamidase ; leucine arylamidase and arginine dihydrolase, hydrolyze hippurate and esculin, ferment ribose ; lactose ; trehalose and starch, reduce tetrazolium chloride and potassium tellurite and survive 60°C/30 min. They were unable to produce β-glucuronidase, ferment inulin or glycogen, reduce nitrate or utilize citrate. These isolates gave variable results for alkaline phosphatse, gelatin liquefaction, protein hydrolysis, sugars fermentation (mannitol, sorbitol, melezitose and raffinose) and α-and β-galactosidase production.

The three isolates which fermented mannitol, sorbitol and melezitose were identified as **Enterococcus faecalis.** The other two isolates which fermented raffinose and melibiose and produced α-and β-galactosidase were identified as **Enterococcus faecium.**

Identification of Propionibacterium

One isolate had circular, convex, entire, smooth surface, opaque, butyrous and cream white colonies. It was Gram-positive, pleomorphic rods, non-sporeforming, non-motile and facultative anaerobes. Also, it was able to produce NH_3 from arginine, H_2S from cysteine, acid from amygdalin ; cellobiose ; esculin ; fructose ; galactose ; glucose ; glycerol ; inositol ; lactose ; maltose ; mannitol ; mannose ; rhamnose ; sorbitol ; starch ; sucrose and trehalose and hydrolyze esculin. However, it was unable to produce oxidase, catalase and/or indole, form acid from adonitol ; arabinose ; dulictol ; erythritiol ; glycogen ; inulin ; melezitose ; melibiose ; raffinose ; sorbose and xylose, hydrolyze starch, liquefy gelatin, reduce nitrate, form gas from glucose and grow at 200 gL^{-1} bile salts. This isolate was identified as **P. jensenii.**

These results are in agreement with Britz and Riedel (1991), who recorded the ability of **P. jensenii** to ferment inositol and maltose and its inability to ferment xylose, melezitose, melibiose and raffinose.

Identification of Microbacterium

Seven isolates had circular, convex, entire, smooth glistening surface, opaque, butyrous and yellow colonies. They were Gram-positive, short rods to coccoid, nonsporeforming, nonmotile, heat survival (63°C/30 min and 72°C/15 min), rod-coccus growth cycle-free, phosphatase positive, DNase positive, able to utilize acetate and lactate. Also, they were able to grow anaerobically, grow at 10°C, grow in the presence of 50 gL^{-1} NaCl. They produced acid from glucose, fructose, galactose, mannose, maltose, cellobiose, sucrose, trehalose, lactose, ribose, starch and amygdalin.

However, they were unable to hydrolyze cellulose or hippurate, liquefy gelatin, utilize formate, grow at 40°C and/or in the presence of 80 gL^{-1} NaCl, produce acid from inulin ; raffinose ; inositol ; sorbose ; erythritol ; xylose ; adonitol ; dulictol and sorbitol. Also, they gave negative results for indole and sulphatase tests. These isolates were identified as **M. lacticum.**

These results are in agreement with Yamada and Komagata (1972a), who recorded the ability of **M. lacticum** to form acid from sucrose and trehalose.

Three of the seven **M. lacticum** isolates hydrolyzed starch, utilized succinate and citrate as carbon source however, were unable to form acid from glycerol and glycogen. Also, Jones and Collins (1986) recorded the inability of **M. lacticum** to form acid from glycerol while, Keddie and Jones (1981) mentioned that **M. lacticum** contained diastatic activity for starch hydrolysis.

Identification of Brevibacterium

Seven isolates had circular, convex. entire, smooth glistening surface, opaqce, butyrous, orange (in day light) and pale-orange (in dark) colonies. They were Gram ; catalase ; oxidase and colour reactions positive, obligate aerobes, nonsporeforming and nonmotile they had rod-coccus growth cycle, liquefied gelatin and hydroylzed protein. These isolates were identified as **B. linens.** These results are in agreement with Meyer and Jones (1973) and Jones (1975), who mentioned that **B. linens** (ATCC 9174) was weakly oxidase positive and in disagreement with Sharpe et al. (1976), who mentioned that **Brevibacterium** was oxidase-negative.

B. linens isolates were able to grow in the presence of 60-180 gL^{-1} NaCl in Mineral Base E or in the growth media after 1-3 weeks at 30°C. Also, 2 isolates were able to grow in the presence of 200 gL^{-1} NaCl in the growth medium after 1 month at 30°C. These results are in agreement with Mulder et al. (1966), and Crombach (1974), who

mentioned that all the tested strains of **B. linens** were able to grow in basal medium of 80 and 120 gL^{-1} NaCl after 1 and 4 weeks at 25°C, respectively. Also, Sharpe et al. (1977) mentioned the same trend of results with 150 gL^{-1} NaCl.

B. linens isolates did not survive heat treatment of 60°C/30 min in nutrient broth, however they did in the same medium supplemented with 40 gL^{-1} NaCl. Mulder et al. (1966) and Jones (1975) found that brevibacteria were unable to survive heat treatment of 60°C/30 min. However, Sharpe et al. (1976, 1977, 1978) reported that most of the strains isolated from cheese and skin survived heating at 60°C/30 min.

The minimum and the maximum growth temperature were 10 and 37-40°C, respectively, in tryptone soya broth plus 40 gL^{-1} NaCl. The isolates failed to grow in NaCl-free tryptone soya broth medium either at 10 or 40°C.

B. linens isolates gave negative results with urease test, indole production, nitrate reduction and starch hydrolysis. While they produced methanethiol from L-methionine. These results are in agreement with Sharpe et al. (1976, 1977, 1978) and Pitcher and Noble (1978).

B. linens isolates were able to utilize a number of substances as carbon + energy sources such as glucose, lactate, glycerol, lactose, sucrose, inulin, acetate, citrate, fumarate and succinate. At the same time all the isolates were unable to utilize formate, malate and oxalate. Many investigators reported the utilization of the above substances by **Brevibacterium** e.g., Mulder et al. (1966), Bousfield (1972), and Crombach (1974).

However, **B. linens** isolates decomposed tyrosine, the brown pigment (melanin) was only formed by some isolates (4/7). These results are in agreement with Colwell et al. (1969), Jones (1975) and Sharpe et al. (1977, 1978) who found that tyrosine was decomposed to some extent by all strains, but melanin production had not been noted.

B. linens isolates were unable to hydrolyze starch or urea. Also, Colwell et al. (1969), Crombach (1974), Jones (1975) and Sharpe et al. (1976, 1977) reported the same results.

Also, all **B. linens** isolates were unable to grow in the presence of ammonium sulphate, ammonium sulphate plus glucose or L-methionine. However they grew in the presence of ammonium sulphate plus yeast extract.

At the same time, we failed to ioslate **Leuconostoc, Corynebacteium** and **Micrococcus** spp. from these samples on their specific medium.

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دراسات بكتريولوجية على الجبن الدميـــاطى

تم عـزل وتعريف ٤٧ ميكروب من ١٠ عينات جبن دميـــاطى وتم تعريفها الى
Lactobacillus (Lb.) farciminis (١٦ عزلة) و **Lb. alimentarius** (١٠ عزلات)
و **Lb. Casei** (عزلة واحـــدة) و **Enterococcus (Ent.) faecalis** (٣ عزلات)
و **Ent. faecium** (عزلتين) و **Propionibacterium jensenii** (عزلة واحـــدة
و **Microbacterium lacticum** (٧ عزلات) **Brevibacterium linens** (٧ عزلات) .