Journal of Innovations in Pharmaceutical and Biological Sciences (JIPBS)
ISSN: 2349-2759
Available online at www.jipbs.com

Research article

Evaluation of probiotic potential of dairy propionibacteria

Mohamed K. Ibrahim[1], Baher A. M. Effat[2], Nabil F. Tawfik[2], Nayra Sh. Mehanna[2], Nariman R. Soliman[2]*

[1]*Microbiology Dept., Faculty of Science, Ain Shams University, Egypt.*
[2]*Dairy Sci. Dept., National Research Center, Dokki, Cairo, Egypt.*

Key words: Dairy propionibacteria, probiotic, antimicrobial activity, bile salt.

***Corresponding Author: Nariman R. Soliman,** Dairy Sci. Dept., National Research Center, Dokki, Cairo, Egypt.

Abstract

According to propionibacteria natural habitats, nine species have been recognized to belong to the genus *Propionibacterium* and grouped to dairy propionibacteria. These strains were comprising *Propionibacterium thoenii* (3), *Propionibacterium acidipropionici* (2), *Propionibacterium freudenreichii* ssp. *shermanii* (1), *Propionibacteriun freudenreichii* (1), *Propionibacterium freudenreichii* ssp. *freudenreichii* (1) and *Propionibacterium shermanii* (1). All nine strains were subjected to in *vitro* analysis to assess their probiotic potential. Eight strains of *Propionbacterium* were fully tolerant to 0.3% and 0.5% bile salts and were able to survive at pH 3 for 3hrs while at pH 2 a few strains were able to survive. Moreover, good growth was observed in the presence of 0.3% phenol. Antimicrobial activity of *Propionibacterium* strains was found to be good against most of tested strains. In addition, all tested strains were sensitive to tertracyclin and trimethoprime/sulfamethoxazole. Technological and productive characteristics tests showed that tested strains disaplayed various behaviours in their acidifying activity and four strains produced exopolysaccharide. In conclusion, eight strains *P. thoenii* P15, *P. thoenii* TL18, *P. freudenreichii* ssp. *shermanii* ATCC1907, *P. freudenreichii* 169TM, *P. freudenreichii* ssp. *freudenreichii* 111, *P. shermanii* B-123, *P. acidipropinici* TL2 and *P. acidipropionici* P124I were able to pass all the tests and were considered as novel putative probiotic propionibacteria.

Introduction

Microorganisms play a very important role in human health and nutrition. They are involved in the production of various bio-molecules, fermentation of milk and reside symbiotically in gut benefiting the host. Propionibacteria are one such important class of organisms. Propionibacteria are mesophilic, Gram positive, catalase positive, non-motile pleomorphic rods, non-sporeforming, and anaerobic to aerotolerant bacteria. Some cells may be elongated, bifid or arranged in "Chinese characters" [1]. The optimal growth temperature of these bacteria is between 30°C and 37°C [2]. Traditionally, propionibacteria are divided into two main groups: "the classical or dairy" and "cutaneous" propioni bacteria. The dairy species includes *Propionibacterium acidipropionici, P. j*ensenii, *P. thoenii* and both subspecies of *P. freudenreichii.* Propionibacteria play an important role in the production of flavor compounds and in the ripening of Swiss type cheese.

The literature concerning the potential probiotic properties of propionibacteria is very limited compared to that about lactobacilli and bifidobacteria. However, in recent years an increasing number of reports on the potential health benefits of propionbacteria have been published. The dairy propionibacteria have a number of properties that make them good probiotic candidates [3]. In this sense, *Propionibacterium* ssp. are able to produce a wide variety of biological compounds that enhance the human health like folic acid, proline, conjugated linoleic acid and vitamin B_{12} [4], and synthesize several different bio-protective compounds such as bacteriocins or antifungal compounds [5]. Moreover, many members of the dairy group have a long history of safe use in food manufacturing [6].

Some strains of propionibacteria have a potential to be used as probiotic cultures. These strains produce bifidogenic compounds and show the ability to survive and maintain activity during passage of the digestive tract [2]. Furthermore, propionibacteria are able to stimulate the immune system and limit cancer progression although the mechanism involved is not defined. Cousin *et al.* [2] reported that dairy propionibacteria are able to prevent infections and allergies, promote immune system maturation, and reduce the risk of cancer because they bind carcinogenic compounds (mycotoxins, plants lactins and heavy metals). The search for strains which show resistance to biological barriers of the human gastrointestinal tract, and which possess physiological characteristics compatible with probiotic properties among propionibacteria, may eventually lead to the finding of new probiotic strains for functional food products. As part of the selection of new probiotic candidates, nine propionibacteria strains were subjected to a series of *in vitro* analysis to assess their probiotic properties.

Experimental

Microorganisms and growth conditions

Table (1) reports the species and strains of propionibacteria and indictor strains and their sources. To prepare active cultures, *Propionibacterium* strains were activated by their successive transfers every 48 h in yeast extract /lactate broth (YELB) medium under anaerobic conditions at 30°C [7,8]. Stock cultures were prepared by maintaining strains in YELB containing 30% glycerol at -80°C for further use. All indicator organisms were routinely prepared in Tryptone Soya Broth (Oxoid, Basingstoke, England) and grown at 30°C for 24 h with exception of the mold strains, which were incubated for 72 h. The indicator strains were maintained as frozen stock at -80°C in the presence of 150 ml/l glycerol as cryoprotective agent. All working cultures were prepared from stock cultures through subcultured twice before use in the manipulations.

Table 1. *Propionibacterium* strains and indicator strains used in this study

Bacterial strain	Source
Propionibacterium thoenii P15 *Propionibacterium thoenii* P127	Department of food technology, Iowa State University (ISU).
Propionibacterium thoenii TL18 *Propionibacterium acidipropionici* TL2	Institute National de la Recherache Agronomique (INRA).
Propionibacterium freudenreichii ssp. shermanii ATCC 1907	American Type Culture Collection (ATCC), Manasses, USA.
Propionibacterium freudenreichii 169TM	Bio.Vet.Inc.USA.
Propionibacterium freudenreichii ssp. freudenreichii 111	University of Warmia and Mazury (Olsztn,Poland)
Propionibacterium shermanii B-123	Department of Food Techn., propionibacteria culture collection, Iwa State Univ.
Propionibacterium acidipropionici P124I *Bacillus subtililus* *Bacillus cereus* *Escherichia coli* *Staphylococcus aureus* *Pseudomonas aeruginosa* *Saccharomyces cervisiae* *Candida albicans* *Alternaria sp* *Aspergillus niger* *Fusarium sp*	Dairy Microbiology Laboratory, National Research Center, Dokki, Giza, Egypt.

Confirmation of *Propionibacterium* strains

Cultures of propionibacteria were routinely grown at 30°C in YELB for 48 h under anaerobic conditions. After growing, morphological characteristics were determined with bright-field microscopy of Gram stained preparations, catalase activity and oxidase test, gas produced from glucose, growth in 20% bile salt, motility after 48 h in MRS medium [9] and colony pigmentation after 10 days on yeast extract /lactate agar (YELA) plates [8]. The strains were tested for production of acids from carbohydrates and related compounds by using API 50 CH strips (bio-Merieux, Fance). The tests were carried out according to the instructions of the manufacturer and results read after incubation of strains at 30°C for 2, 4 and 7 days.

Evaluation of probiotic properties

Acid tolerance

The experiment for tolerance of propionibacteria to pH 2, 3, 3.5 and 7(control) was performed following the method described by Sahadeva *et al.* [10] and Yuksedag *et al.* [8]. Viable cell counts were determined on YELA at various times (0, 1, 2 and 3 h) of incubation at 37°C. The plates were incubated at 30°C for 2 days under anaerobic conditions. Acid tolerance was estimated by comparing the growth of viable cell counts in all the YELA plates after 48 h.

Resistance to bile salts

To study the effect of bile salts on the growth of propionibacteria strains, a method described by Sahadeva *et al.* [10] was employed. All cultures were grown in YELB for 24 h at 30°C. Bile resistance was determined on YELA containing different concentrations of oxal bile salts (Oxoid) (YEL-bile) (0, 0.1, 0.2, 0.3, 0.5 and 1% w/v). Viable counts were determined on YEL- bile agar and YELA (control) by plating serial dilutions in saline (0.9 % NaCl). Plates were incubated for 48 h at 30°C anaerobically and only plates with 30 to 300 colonies were considered.

Antimicrobial activity

All propionibacteria strains were tested for antimicrobial activity against indicator strains using the agar diffusion method [9]. Test microorganisms were propagated twice and then grown for 18- 24 h. Propionibacteria species were inoculated in reconstituted skim milk powder (RSMP) supplemented with 1% yeast extract and 0.5% glucose (pH 6.8) at 30°C for 8 days under anaerobic conditions. Activated cultures were centrifuged at 4000 rpm for 15 min and the clear supernatant was sterilized by filtration (0.45 µl), thus obtaining cell free filtrates.

An initial inoculum of approximately 10^6 cfu/ml of the target strain was incorporated into soft agar (1% w/v) plates of the appropriate for the target strain medium. Cell free filtrates (100 µl) were transferred in the holes (5mm diameter) drilled into the agar. The plates were incubated at 30°C, depending on the target strain, and antimicrobial activity was recorded at growth free inhibition zones (diameter) around the well.

Fermentation of carbohydrates

Carbohydrate fermentation profiles of the different strains were established using commercial API 50 CH system (bio Merieux, Marcyl' Etoile, France). Following the manufactures instructions [11].

Phenol Resistance

Tolerance to phenol was investigated using YELA containing 0, 0.1, 0.2, 0.3, 0.4 and 0.5% phenol [12]. Plates were incubated anaerobically at 30°C for 48 h and viable counts were determined.

Acid production

A time-course experiment to study acid production profiles of *Propionibacterium* was carried out as described by Arici *et al.* [13]. The strains were grown in 100 ml of heat treated (5 min at 121°C) 11% (w/v) reconstituted skim milk. Flasks were removed after 18and 36 h of incubation at 30°C. The pH of fermented milk was measured directly by using a pH meter (Hanna, model 211).

Exopolysaccharide (EPS) production

Propionibacteria strains were screened for (EPS) production according to the method described by Mabrouk *et al.* [14]. Strains were grown on YELA medium supplemented with 100 g/l sucrose. The poured plates were incubated for 48 h at 30°C under anaerobic conditions. At the end of incubation period, the bigger size and mucus colonies were tested for compactness or ropiness by touching them with a sterile metal loop and observed the formation of slime or strings.

Antibiotic susceptibility

The antibiotic susceptibility of propionibacteria strains were assessed using antibiotic discs diffusion method on Mueller-Hinton agar plates. Broth cultures of propionibacteria was prepared using YELB and adjusted to 10^6 cfu/ml dilution of bacteria. A suspension of freshly grown *Propionibacterium* cultures of this concentration was spread on the surface of Mueller-Hinton agar plates. Two antibiotic discs (Oxoid) were placed on the surface of agar and the plates were incubated at 30°C for 4 days, under anaerobic conditions. The susceptibility or resistance of the examined strains against the used antibiotics was assessed by measuring the diameter of the inhibition zone around the discs in mm. Strains were classified as resistant (-), moderately susceptible (+), susceptible (++), according to the recommendations published by the Clinical and Laboratory Standards Institute (2012) [15]. The Susceptibility pattern was assessed using Nalidixic acid (NA 30µg/ml), Tetracyclins (TET 30µg/ml), Trimethoprime/ Sulfamethoxazole (SXT 1.25/23.75µg), Polymyxin B (PB 30U/ml), Nitrofurantoin (F 300µg/ml), Clindamycin (CLi 2µg/ml), Imienem (Imi 10µg/ml), Aztreonam (Azt 30µg/ml), Norfloxacin (Nor 10µg), Cefazidime (Cef 30µg/ml), Streptomycin (S 10µg/ml), Kanamycin (K 30µg/ml), Gentamycin (CN 10µg/ml), PenicillinG (P 10u/ml), Ampicillin (Amp 10µg/ml), Chloroamphenicol (C 30µg/ml).

All the experiments were performed in triplicates and the data shown are the average of all repetitions.

Statistical analysis

Statistical analysis was carried out using Costat software program. Standard Error Mean (SEM) was carried out according to Fisher [16], LSD (List Significant Difference) test was used to compare the significant difference between means of treatment [17].

Results and Discussion

Confirmation for *Propionibacterium* strains

All the tested cultures were Gram positive in nature, catalase positive and short or filamentous rods in arrangements that resemble V, Y or Chinese characters. According to these morphological characteristics and as a result of the API identification tests all strains were grouped as dairy propionibacteria. Based on these results and the schemes for identifying species developed by Martinez *et al.* [18] and API 50 STREP, 9 strains were identified as 3 *P. thoenii*, 2 *P. freudenreichii*, 2 *P. acidipropionici*, and 1 for each *P. shermanii* and *P. freudenreichii* spp. *shermanii*. So this confirmed that all strains resembled the characteristics of genus *Propionibacterium* as described by Cummins and Johnson [19].

Evaluation of probiotic properties

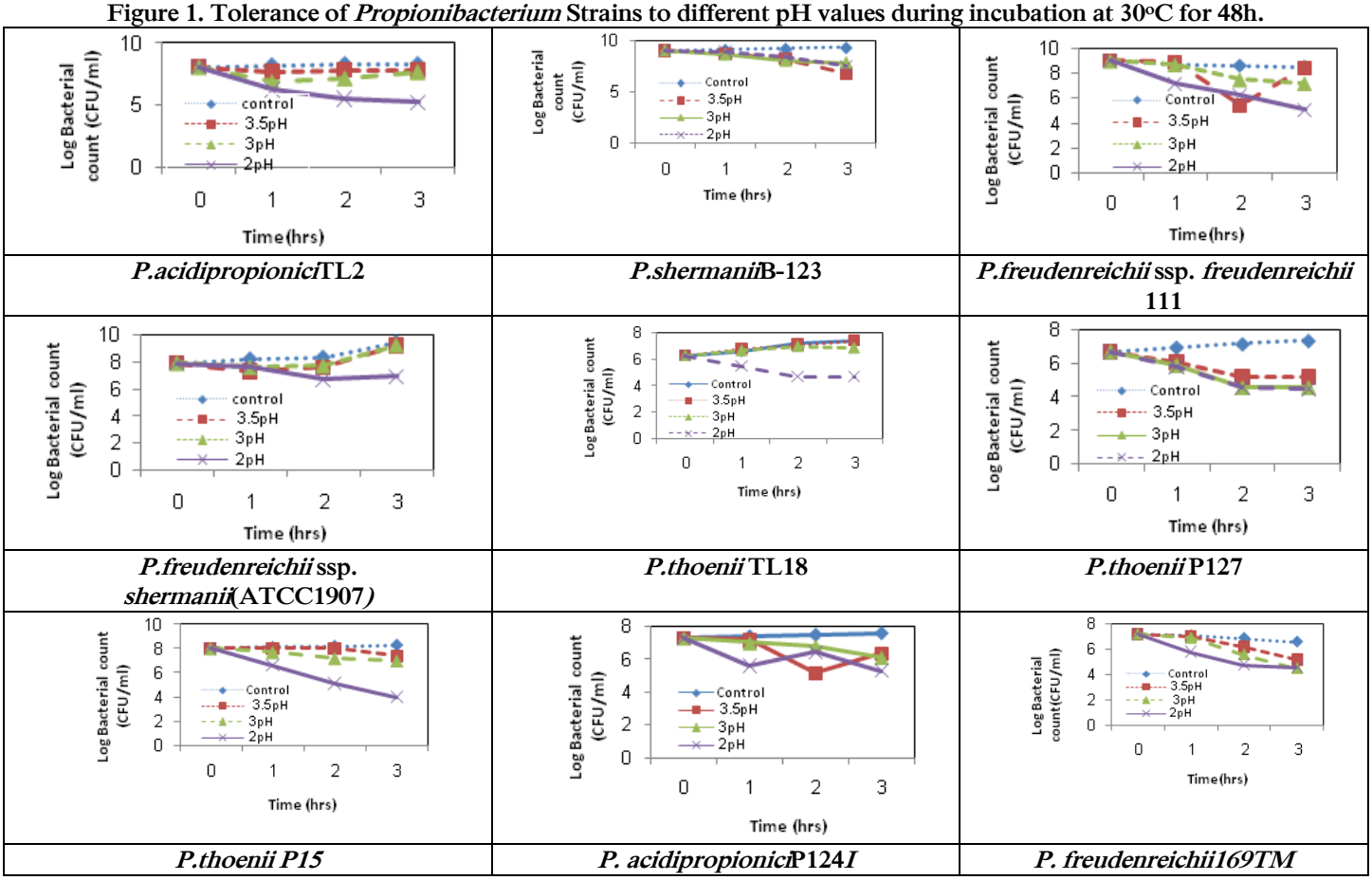
Acid Tolerance

The ability of the *Propionibacterium* strains to tolerate acid is commonly used as one of the preliminary selection criteria for potential probiotic candidates. It was found that 8 *Propionibacterium* strains (89 %) out of 9 strains were able to retain viable counts above 6 log cfu/ml in pH 3 and 3.5 after 3 hours of incubation (Figure 1). Reduction in counts of between 0.4 and one logarithmic unit were commonly recorded for all strains, except *P. thoenii* P127, which showed more reduction in counts with a 1.5 and 2.1 -log reduction in viability at this pH. At pH 2.0, all tested strains showed progressive reduction in resistance during incubation, especially the strains of *P. thoenii* TL18, *P. acidipropionici* TL2, *P. acidipropionici* P124I, *P. freudenreichii* ssp. *freudenreichii* 111, *P. freudenreichii* 169TM, *P. thoenii* P15 and *P. thoenii* P127 which decreased by about 1.6 to 4 log units after 3 hours. Only two strains *(P.freudenreichii* ssp. *shermanii* (ATCC1907*)* and *P. shermanii* B-123) showed a high level of survival above 83% after 3 h of incubation (Figure 1).

From these results it is clear that more loss of viability of propionibacteria at pH 2.5 after 3 h could be observed. In this respect, Suoumalainen *et al.* [20] recorded that counts of propionibacteria was significantly reduced after 3h at pH 2

but not at pH 3 or 4.In the same line Darilmaz and Beyetli [21] found a significant reduction in cell count at pH 2 and 3 for *P. freudenreichii* ssp. *freudenreichii* and *P. jensenii.* On the other hand, Campaniello *et al.* [1] reported that at pH2.5 strains of propionibacteria did not show any kind of viability loss after 3 h. In the same point, Zarate *et al.* [22] mentioned that 4 *Propionibacterium* strains were shown to tolerate well at pH 4 and one strain lost viability at pH 3. While, all tested strains lost viability at pH 2. The difference between tested strains found could be due to the strong strain –specificity at acidic pH [1].

Figure 1. Tolerance of *Propionibacterium* Strains to different pH values during incubation at 30°C for 48h.



***P.acidipropionici*TL2** | ***P.shermanii*B-123** | ***P.freudenreichii* ssp. *freudenreichii* 111**

***P.freudenreichii* ssp. *shermanii*(ATCC1907)** | ***P.thoenii* TL18** | ***P.thoenii* P127**

P.thoenii P15 | ***P. acidipropionici*P124I** | ***P. freudenreichii169TM***

Bile tolerance

Bile tolerance is essential for probiotic strains to colonize in the small intestine [23]. Our strains of propionibacteria were suspended in sodium lactate agar media containing 0.1, 0.2, 0.3 and 0.5 % oxgall and without as control. Survival of bacteria was determined after 48 hrs at 30°C. The statistical analysis of Table (2) showed that the concentrations of bile salts had not significant effects on the nine *Propioni-bacterium* strains tested.

As shown in Table (2) all tested strains showed a good survival at 0.1, 0.2, 0.3 and 0.5 % bile salt with some minor difference. The loss of log count reached 0.4, 0.5, 0.2, 0.5, 0.3, 0.4, 0.3, 0.7 and 0.3 log count in case of *P. thoenii* TL18, *P. acidipropionici*TL2, *P. acidipropionici* P124I, *P. shermanii* B-123, *P. freudenreichii* ssp. *freudenreichii* 111, *P. freudenreichii* 169TM, *P. thoenii* P15, *P. freudenreichii* ssp. *shermanii* (ATCC1907), *P. thoenii* P127, respectively. On the other hand at concentration 1% , *P.thoenii* TL18, *P. acidipropionici* TL2, *P. freudenreichii* ssp. *freudenreichii* 111, *P. thoenii* P15 and *P. thoenii* P127 could not be detected after (48 hrs) this means a loss of viability. Other strains tested, *P. acidipropionici* P124I, *P. freudenreichii* 169TM, *P. freudenreichii* ssp. *shermanii* (ATCC1907) and *P. thoenii* P127 showed a good resistance as the loss of log count reached 0.4, 0.6, 0.5 and 0.8 log cfu ml^{-1} for these strains, respectively. From these results, it is clear that out of nine strains tested four strains showed high level of resistance while five strains have some resistance at concentration ranged from 0.1 to 0.5% of bile salt, but at 1.0 % strains *P. thoenii* TL18, *P. acidipropionici*TL2, *P. freudenreichii ssp. freudenreichii* 111, *P. thoenii* P15, *P. thoenii* P127were inhibited completely. In this respect, Darilmaz and Beyatli [21] tested the resistance of some strains of propionibacteria to bile salt at concentrations 0.06, 0.15, 0.3 and 0.6%, they found that the tested strains were highly susceptible to these concentrations. In Contrary, Campeniello *et al.* [1] recorded that at 0.3% bile salt, propionibacteria expressed an increase in the cell count

ranging from 0.6 to 1.3 log cfu/ml after 24 hrs of incubation at 30°C. In the same line Darilmaz and Beyatli [21] found that most of 29 strains of propionibacteria tested for their resistance to 0.6% bile salt had a high level of resistance. But only seven strains showed some reduction in their resistance in the presence of 0.3% bile salt. Moreover, Yuksekdag *et al.* [8] recorded that different bile salt concentrations have a little effect on the tolerance of dairy propionibacteria, they recorded that bile salt did not seem to be related to the species but to the strain.

Antimicrobial activity

Naturally produced metabolic products of probiotic bacteria such as bacteriocins and organic acid are said to have inhibitory effect against some pathogenic microorganisms [24, 25]. Dairy propionibacteria produce antimicrobial agents that can inhibit the growth of spoilage and pathogenic microorganisms [26, 9, 24].

The statistical analysis of Table (3) showed a significant differences ($P < 0.05$) were recorded among nine *Propionibacterium* strains toward various indicator pathogenic strains. .From Table (3) it is clear that all tested *Propionibacterium* have an inhibitory effect against *B. cereus* but two strains only *P. acidipropionici* P124I and *P. freudenreichii* 169 TM did not inhibit the growth of *B. subtilis*. Only *P. thoenii* TL18 showed no inhibitory effect on *E. coli* and *Pseudomonas aeruginosa*. *P.acidipropionici* TL2 also didn't inhibit the growth *of Staphylococcus aureus*. On the other hand, other tested *Propionibacterium* strains can inhibit the growth of these organisms. *Saccharomyces cervisiae* was inhibited by *P.acidipropionici* TL2, , *P. freudenreichii* ssp. *shermanii* (ATCC1907), *P.freudenreichii* ssp. *freudenreichii* 111, *P.thoenii* P15, *P.thoenii* TL18 and *P. shermanii* B -123but *Candida albicans* could be inhibited by *P. thoenii* P127, *P. acidipropionici* TL2, *P. freudenreichii* ssp. *freudenreichii* 111, *P.shermanii B-123*, *P.freudenreichii* ssp . *shermanii* (ATCC1907). Other propioni bacteria tested did not affect the growth of yeasts. *Aspergillus niger* and *Alternaria* are more resistant to inhibit by these bacteria as two strains *P.thoenii* P15 and *P. shermanii* B -123 affect the growth of *A. niger*.

However, three strains (*P. acidipropionici* TL2, *P. freudenreichii* ssp. *shermanii* (ATCC1907) and *P. shermanii* B-123) showed antimicrobial properties against *Alternaria* spp. Lastly, strains *P. acidipropionici* TL2, *P. acidipropionici* P124I, *P. freudenrechii ssp. freudenrechii* 111, *P. freudenreichii ssp . shermanii* (ATCC1907), *P. shermanii* B-123 and *P. thoenii* P127 can inhibit the growth of *Fusarium spp*. It is clear that among 9 tested *Propioni bacterium* strains one strain *P. shermanii* B123 had a broad spectrum against pathogenic strains followed by *P. freudenreichii* ssp . *shermanii (ATCC1907)*. The most sensitive organisms tested were *B. cereus*, followed by *Pseudomonas aeruginosa and Staphylococcus aureus*. Our results are in the same line of Yuksekdag *et al.* [8] as they recorded that antimicrobial effect of *Propionibacterium* ssp. strains was more obvious against the Gram negative strains. They added that *S. aureus* and *B. cerues* were sensitive to the metabolites of *Propioni bacterium* ssp. It is obvious that, there are differences in the degree of antagonistic effect depending on the indicator organism. It is worthy to mention that Darilmaz and Beyatli [21] reported that the different pathogens generally inhibited by propionibacteria.

Fermentation of carbohydrates

Table (4) shows the strains ability to ferment various carbohydrates. All strains fermented glycerol, D-arabinose, galactose, glucose, fructose, mannose and sucrose. On the other hand, most strains showed positive results in the fermentation of the following sugars: ribose (eight of nine), melibiose (sex of nine), a-methyl D-mannoside and N-acethyl-glucosamine (five of nine). In addition, four strains fermented each of adonitol, sorbitol, lactose, rafinose, tagatose and gluconate. Sorbose fermented only with *P. acidipropionici* P124I, while turanose fermented with two strains (*P. acidipropionici* P124I and *P. acidipropionici* TL2). None of the nine propioni bacterium strains able to ferment a-methyl-D-glucoside and gentibiose. These results are similar to those obtained by Martinez *et al.* [18] when different potentially probiotic strains of propionibacteria were studied.

Table 2. Survival of the tested *Propionibacterium* strains in different concentrations of bile salts after incubation at 30°C for 48 hrs

Propionibacterial Strains	Control	Growth (cfu/ml) at different bile salt concentrations%				
		0.1	0.2	0.3	0.5	1
P.thoenii P15	7.6±.196^{a}	7.5±.203^{b}	7.4±.068^{bc}	7.3±.046^{c}	7.3±.017^{c}	n.d
P.thoenii P127	8.2±.196^{a}	8±.062^{b}	8±.007^{b}	8±0^{b}	7.9±.009^{b}	n.d
P.thoenii TL18	7.7±.196^{a}	7.6±.089^{ab}	7.4±.026^{bc}	7.4±.100^{c}	7.3±.096^{c}	n.d
P.freudenreichii ssp. shermanii(ATCC1907)	8.7±.196^{a}	8.3±.231^{ab}	8.2±.055^{ab}	8±.032^{ab}	8±.022^{b}	7.9±.015^{b}
*P.freudenreichii*169TM	8.2±.196^{a}	8.2±.165^{a}	8.1±.003^{a}	8±.156^{b}	7.8±.078^{c}	7.7±.038d
P.freudenrechii ssp. freudenreichii 111	7.6±.196^{a}	7.5±.029^{ab}	7.4±.093^{bc}	7.4±.046^{bc}	7.3±.049^{c}	n.d
P.shermanii B-123	8.5±.196^{a}	8.3±071^{a}	8.1±.017^{b}	8±.075^{b}	8±.015^{b}	7.9±.037^{c}
P.acidipropionici TL2	7.8±.196^{a}	7.7±.185^{b}	7.4±.009^{c}	7.4±.582^{c}	7.3±.075^{c}	n.d
*P.acidipropionici*P124I	7.8±.196^{a}	7.8±.047^{a}	7.7±.041^{b}	7.6±.025^{b}	7.6±.023^{b}	7.4±.020^{c}

-Values that have the same small letters in the same row are not significantly different (p>0.05).- n.d: not detected

Table 3. Antimicrobial activity of *Propionibacterium* strains tested toward some indicator pathogenic strains

Propionibacteria species	Indicators strains									
	B. subtilis	*P. aeruginosa*	*E. coli*	*B. cereus*	*C. albicans*	*S. cervisiae*	*S. aureus*	*A. niger*	*Fusarium sp*	*Alternaria sp*
	Diameter of inhibition zone (mm)									
***P.thoenii* P15**	1±.115^{d}	1.5±.058^{bc}	2±.230^{b}	1.7±.153^{bc}	-ve	1.3±.173^{cd}	1±.058^{d}	3±.173^{a}	-ve	-ve
***P.thoenii* P127**	2.5±.120^{b}	1±.115^{d}	2±..115^{c}	2±.115^{c}	3±.058^{a}	-ve	2±.115^{c}	-ve	2.5±.289^{c}	-ve
***P.thoenii* TL18**	1.5±.202^{a}	-ve	-ve	1.5±.202^{a}	-ve	1±.115^{b}	1.5±0^{a}	-ve	-ve	-ve
***P.freudenreichii ssp. shermanii* (ATCC1907)**	2.3±.115^{bc}	1.3±.058de	1.5±.115^{d}	2.5±.202^{b}	2±.058^{c}	1.5±.173^{d}	3.5±.088^{a}	-ve	2±.208^{c}	2.5±.115^{b}
P.freudenreichii* 169 T*M	-ve	1.3±.115^{c}	1.3±.115^{c}	2.2±.058^{b}	-ve	-ve	3.5±.115^{a}	-ve	-ve	-ve
P.freudenreichii ssp. fredenreichii 11I	2±.173^{b}	1±.058^{d}	2±.115^{b}	2±.115^{b}	1.2±.115^{c}	1.8±.115^{bc}	2±.058^{b}	-ve	2.5±.251^{a}	-ve
P.shermanii B-123	3±.289^{a}	3.2±.115^{a}	2.2±.115^{c}	2.5±,089^{b}	2.3±.115^{c}	2.5±.058^{b}	3±.058^{a}	2.3±.115^{c}	3±.115^{a}	2.5±.058^{b}
***P.acidipropionici* TL2**	1.5±.120^{d}	2±.176^{c}	2.2±.176^{bc}	1.2±.115^{e}	1.5±.058^{d}	2.2±.115^{bc}	-ve	-ve	2.5±0^{b}	3±.152^{a}
P.acidipropionici P124I	-ve	2±.230^{c}	1±.346^{d}	1±.240^{d}	-ve	-ve	3.5±0^{a}	-ve	3±.152^{b}	-ve

-Values that have the same small letters in the same row are not significantly different (p>0.05).- n.d: not detected

Table 4. Fermentation of different carbohydrates by tested *Propionibacterium* strains using API 50CH

Carbohydrate / strain	GLY	ERY	DAR	RIB	ADO	GAL	GLU	FRU	MNE	SBE	INO	MAN	SOR	MDM	MDG	NAG	SAL	LAC	MEL	SAC	RAF	GEN	TUR	TAG	GNT
P.1	+	-	+	+	-	+	+	+	+	-	-	-	-	-	-	+	-	+	-	+	-	-	-	-	+
P.2	+	-	+	+	-	+	+	+	+	-	-	-	-	-	-	+	+	+	+	+	-	-	-	+	-
P.3	+	-	+	+	-	+	+	+	+	-	-	-	-	+	-	+	+	+	-	+	-	-	-	+	-
P.4	+	+	+	-	-	+	+	+	+	-	+	-	+	+	-	+	+	-	+	+	-	-	-	-	-
P.5	+	+	+	+	+	+	+	+	+	-	+	-	-	+	-	-	+	-	+	+	+	-	-	-	+
P.6	+	-	+	+	-	+	+	+	+	-	-	-	-	-	-	-	+	-	-	+	-	-	-	-	-
P.7	+	+	+	+	+	+	+	+	+	-	-	+	+	+	-	+	+	-	+	+	+	-	+	+	+
P.8	+	+	+	+	+	+	+	+	+	-	-	-	-	+	-	-	+	-	+	+	+	-	-	-	+
P.9	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	-	-	-	+	+	+	-	+	+	+

Key of the carbohydrates: GLY: glycerol, ERY: erythritol, DAR:D-arabinose, RIB: ribose, ADO: adonitol, GAL: galactose, GLU: glucose, FRU: fructose, MNE: mannose, SBE: sorbose, INO:inositol, MAN: mannitol, SOR: sorbitol, MDM: a-methylD-mannoside,MDG: a-methyl-D-glucoside, NAG: N-acethyl-glucosamine, SAL: salicin, MAL: maltose, LAC: lactose, MEL:melibiose, SAC: sucrose, RAF: raffinose, GEN: gentibiose, TUR: turanose, TAG: tagatose, GNT: gluconate.

Strains : *P.1: P.thoenii* P15, *P.2:P.thoenii* P127 , *P.3: P.thoenii* TL18 , *P.4 : P.freudenreichii spp . shermanii* (ATCC1907), *P.5 : P.freudenreichii* 169 TM , *P.6 : P.freudenreichii ssp. freudenreichii ssp* 111, P7: *P.shermanii B-123* , P.8: *Pacidipropionici*TL2, P.9: *P.acidipropionici*P124I

Symbols: +: present character, - : absence character

Phenol resistance

Resistance to phenol was tested as an additional indicator for survival under intestinal conditions for the same nine probiotic candidate strains. Phenol is an intermediate of putrefactive process in the intestinal tract that may be formed by bacterial determination of some aromatic amino

acids derived from dietary or endogenous produced proteins [15]. Table (5) shows the survival of *Propionibacterium* strains at different concentrations of phenol. These strains showed different degrees of sensitivity towards this compound. It can be seen that 4 and 6 strains were completely inhibited at 0.4 % and 0.5% phenol, respectively. Statistical analysis proved that; phenol concentrations had significant effect ($P<0.05$) on the viability of all *Propioni bacterium* strains examined. In this respect, some authors suggest that a 0.4% concentration of phenol causes a bacteriostatic action in some microorganisms [27]**.** In contrast, 3 strains included *P. freudenreichii* ssp. *freudenreichii* 111*, P. shermanii* B-123 and *P. freudenreichii ssp. shermanii* (ATCC1907) exhibited a good phenol tolerance.

Acidification properties

The acidity developmentof tested *Propionibacterium* strains are presented in Table (6). The results showed that the tested nine strains displayed various behaviours in their acidification activity. The statistical analysis showed that no significant differences ($P<0.05$) were observed between the tested strains .The pH values ranged from 5.13 to 5.71 after 18h. After 36h of incubation at 30^{o}C the pH of milk inoculated with *Propionibcterium* was less than 5.40. In general, the results show that all the tested *Propionibacterium* strains acidified milk well. The acidification characteristy by propionibacteria strains is required for reducing fermentation time when using them in production of dairy products and reducing contamination by spoilage and/or pathogenic microorganisms. This result may be considered as a consequence of its obligatory homo-fermentative metabolism, the propionic fermentation leads to the production of short chain fatty acids- acetate and propionate [28].

Exopolysaccharide (EPS) production

The potential of propionibacteria to produce exopolysaccharide is displayed in Table (7). The results show that four strains *P. acidipropionici* TL2, *P. acidipropionici* P124I*, P. shermanii* B-123 and *P. freudenreichii ssp. shermanii* (ATCC1907) gave ropy colonies and were positive for production of exopolysaccharide. These results are similar to those reported by Gorret *et al.* [29] for *Propionibacterium* strains. Generally, the exopolysaccharide produced by probiotic strains is extracellularly secreted as microbial polysaccharide present on the surface of many probiotics. The capsular structure of exopolysaccharide has been found to protect strains from unfavorable environment. Furthermore, Ren *et al.* [30] reported that probiotic strains produced exopolysaccharides have an ability to exhibit acid and bile tolerance. In this study, the four strains produced exopolysaccharide exhibited acid and bile tolerance.

Antibiotic susceptibility

Because of the risk that any antibiotic resistance bacteria will spread to intestinal microbiota. The absence of antibiotic resistance for probiotic is an important criterion. *Propionibacterium* strains were tested for their sensitivity to antibiotics as this criterion is taken into account during the evaluation of probiotic strains. From Table (8) it is clear that all tested strains showed high sensitivity to Tetracyclins and Trimethoprime/Sulfamethoxazole. On the other hand, seven strains *P. thoenii* TL18, *P. acidipropionici* P124I, *P. shermanii* B-123, *P. freudenreichii ssp. freudenreichii* 111, P. *freudenreichii* 169TM, *P. freudenreichii ssp . shermanii* (ATCC1907), *P. thoenii* P127 could resist Ampicillin while strains *P. thoenii* TL18*, P. thoenii* P15, *P. freudenreichii* 169TM*, P. freudenreichii ssp. freudenreichii* 111 resist chloroamphenicol.

Table 5. Survival of the tested *Propionibacterium* strains in different concentrations of phenol after incubation at 30^{0}C for 48h

Strain	Control (no Phenol)	Phenol concentrations %				
		0.1	0.2	0.3	0.4	0.5
***P.thoenii* P15**	8±.200^{a}	7.6±.035^{b}	7.2±.020^{b}	6.9±.024^{c}	n.d	n.d
***P.thoenii* P127**	7.9±.036^{a}	7.6±.187^{b}	7.3±.108^{b}	6.4±.009^{c}	n.d	n.d
***P.thoenii* TL18**	8±.192^{a}	7.6±.040^{b}	7.3±.145^{c}	7.1±.026^{c}	6.4±.046^{d}	n.d
***P.freudenreichii ssp. shermanii* (ATCC1907)**	8±.045^{a}	7.7±.021^{b}	7.6±.073^{ac}	7.1±.022^{d}	6.6±.007^{e}	6.4±.049^{f}
***P.freudenreichii*169TM**	8±.035^{a}	7.8±.165^{a}	7.8±.165^{a}	6.9±.040^{b}	6.5±.030^{c}	n.d
***P.freudenrechii ssp. freudenrechii* 111**	7.9±.044^{a}	7.8±.025^{a}	7.5±.072^{b}	7.2±.003^{b}	7±.026^{c}	6.9±.018^{d}
***P.shermanii* B-123**	7.8±.026^{a}	7.4±.o46^{b}	7.3±.035^{b}	7±.055^{c}	6.8±.024^{d}	6.3±.031^{e}
***P.acidipropionici*TL2**	8±.076^{a}	7.7±.025^{b}	7.5±.108^{c}	6.9±.023^{d}	n.d	n.d
P.acidipropionici P124I	7.9±.076^{a}	7.7±.100^{b}	7.4±.036^{c}	6.3±.020^{d}	n.d	n.d

-Values that have the same small letters in the same row are not significantly different ($p>0.05$).-n.d: not detected

Table 6. Acid production by different tested *Propioni bacterium*

Propioni bacteria strain	Incubation period		
	0	18	36
	pH values		
P.thoenii P15	6.84±.063[a]	5.65±.013[b]	4.88±.133[c]
P.thoenii 127	7.12±.017[a]	5.69±.027[b]	5.10±.033[c]
P.thoenii TL1*8*	6.86±.080[a]	5.56[b]	4.83±.027[c]
P.freudenreichii ssp. *shermanii(A*TCC1907*)*	6.70±.035[a]	5.71±.033[b]	5.39±.050[c]
P.freudenreichii 169 T*M*	6.66±.046[a]	5.59±.020[b]	5.00±.040[c]
P.freudenreichii ssp. freudenreichii 111	6.77±.035[a]	5.61±.010[b]	5.40±.010[c]
P.shermanii B-123	6.62±.104[a]	5.57±.133[b]	4.93±.020[c]
*P.acidipropionici*TL2	6.62±.104[a]	5.13±.047[b]	4.63±.113[c]
P.acidipropionici P124I	6.75±.104[a]	5.26±.047[b]	4.91±.113[c]

-Values that have the same small letters in the same row are not significantly different (p>0.05)

Table 7. Exopolysaccharide production by different tested *Propionibacterium*

Propionibacteriaum strain	Exopolysacchride production
P.thoenii P1*5*	-
P.thoenii P12*7*	-
P.thoenii TL1*8*	-
P.freudenreichii ssp. shermanii (ATCC190*7*)	+
P.freudenreichii169TM	-
P.freudenrechii ssp. freudenrechii 111	-
P.shermanii B-12*3*	+
*P.acidipropionici*TL*2*	+
*P.acidipropionici*P124I	+

(+) The strain produce EPS, (-) The strain not produce EPS

Table 8. Antibiotic resistance profiles of the tested *Propionibacterium* strains after incubation at 30 °C for 48 hrs

Propionibacterial strain	Antibiotic conc.																
	NA	TET	SXT	PB	F	CLi	Imi	Azt	Nor	Cef	S	K	CN	P	Amp	C	
P.thoenii* P1*5	++	++	++	+	++	+	-	++	++	++	+	+	-	+	+	-	
P.thoenii* P12*7	++	++	++	+	+	-	++	++	++	++	-	-	++	+	-	++	
P.thoenii* TL1*8	++	++	++	++	++	++	+	++	++	++	-	-	++	_	-	-	
P.freudenreichii ssp. shermanii(ATCC1907)	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	
P.freudenreichii 169 TM	++	++	++	++	++	++	++	+	++	++	++	+	++	_	--	-	
***P.freudenrechii ssp. freudenrechii* 111**	++	++	++	++	++	++	++	+	++	++	++	++	++	+	-	-	
P.shermanii B-123	++	++	++	+	++	++	+	+	++	++	++	+	++	+	-	++	
P.acidipropionici*TL*2	++	++	++	++	+	-	-	+	++	-	++	++	++	++	-	+	
P.acidipropionici P124I	-	++	++	-	-	++	-	-	-	++	++	++	++	+	-	++	

Resistant (-), moderately susceptible (+), susceptible (++).

Nalidixic acid (NA 30µg/ml), Tetracyclins (TET 30µg/ml), Trimethoprime/Sulfamethoxazole(SXT 1.25/23.75µg), Polymyxin B (PB 30U/ml), Nitrofurantoin (F 300µg/ml), Clindamycin(CLi 2µg/ml), Imienem(Imi 10µg/ml), Aztreonam(Azt 30µg/ml), Norfloxacin(Nor 10µg), Cefazidime(Cef 30µg/ml), Streptomycin (S 10µg/ml), Kanamycin (K 30µg/ml), Gentamycin(CN 10µg/ml), PenicillinG(P 10u/ml), Ampicillin(Amp 10µg/ml), Chloroamphenicol(C 30µg/ml)

From the same Table, it could be recorded that *P. freudenreichii ssp .shermanii* (ATCC1907) did not show any resistance to antibiotic tested, while *P. acidipropionici* P124I, can resist 7 of these antibiotics. In this respect, Suoumalainen *et al.* [20] recorded that *P. freudenreichii ssp. shermanii* (ATCC1907) have a high minimal inhibitory concentration value for aminoglycosides, streptomycin, gentamycin, kanamycin. It is worthy to mention that some authors reported that the resistance to antibiotic is not problem for these microorganisms [31].

Conclusion

In conclusion, the data present in this study reveals that eight of tested *Propioni bacterium* strains were found to possess desirable *in vitro* probiotic properties. These strains are good candidates for further investigation to elucidate the effect of commercially available oligosaccharides as prebiotics on their survival and activity.

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