
International Journal of Advanced Research in Biological Sciences

ISSN: 2348-8069

www.ijarbs.com

DOI: 10.22192/ijarbs

Coden: IJARQG(USA)

Volume 6, Issue 8 -2019

Research Article



DOI: <http://dx.doi.org/10.22192/ijarbs.2019.06.08.014>

Effect of Different Prebiotics on Survival and Viability of Some Dairy Propionibacteria

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Abstract

The effect of seven different prebiotics (inulin, litesse, xylitol, lactitol, dextrin, fructofin, fructo oligosaccharides) were investigated in concentrations 1, 3, 5 % on survival and viability of eight selected probiotic *Propionibacterium* 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. acidipropionici* TL2 and *P. cidipropionici* P124I). The results obtained could be summarized as follows: When the percentage of different prebiotics was increased from 1 to 3 %, the survival and viability of the probiotic *Propionibacterium* strains were increased. However, it is also observed that supplemented skim with 3% dextrin resulted in cultures having the highest viability % within the same 8 probiotic *Propionibacterium* strains tested. A slightly decrease of viability % was observed as a results of increasing the concentration from 3 to 5% of prebiotics added. This decrease in the viability % when the percentage of the different prebiotic increased to 5% could be attributed to the addition decrease in pH values as compared with 1 or 3 % of prebiotics. Also, the survival (log cfu/ ml) of all cultures showed a decrease at the end of storage period (30 days).

Keywords: Prebiotics – Probionibacteria – Probiotic Propionibacteria

Introduction

Prebiotic is “a non-digestible food ingredient that beneficially affects the host by selectively stimulating the growth and/or activity of one or a limited number of bacteria in the colon” (Gibson and Roberfroid, 1995, Gibson et al., 2017). Recently, the term of ‘prebiotic’ was changed to be “a selectively fermented ingredient that results in specific changes in the composition and/or activity of the gastrointestinal microbiota”, thus conferring benefits upon host health. There has been a considerable interest in the use of prebiotic to enhance the survival and colonization of

probiotic bacteria that are added in food (Ziemer and Gibbson, 1998). Salem et al. (2007) added 1 % inulin as prebiotic in the manufacture of labneh cheese and mentioned that all strains showed good growth and survival in the presence of prebiotic and increased the soluble nitrogen, total volatile fatty acids, acetaldehyde and acetyl. Buddington and Weber (1999) reported that the fermentable fibers increased the number of beneficial bacteria, stimulated the growth and function of the healthy intestine. Moreover, Frank (1999) pointed out that the use of different prebiotics such as inulin stimulate the absorption of several minerals e.g. Ca, Mg and

improve mineralization of bone. In this respect, **Mohanty et al. (2018)** stated that, prebiotics especially inulin-type fructans (also synbiotics) are good candidates to be classified as functional food. In another study, **Schley and Field (2002)** mentioned that fermentable prebiotics can modulate various properties of the immune system; including those of the gut associated lymphoid tissue (GALT). **Abou Ayana et al. (2016)** manufactured four kareish cheeses using (1) *L. lactis* subsp. *Lactis* served as a control, (2) *L. lactis* subsp. *Lactis* and *P. acidipropionici* 117 (LPA), (3) *L. lactis* subsp. *lactis* and *P. jensenii* 118 (LPJ) and (4) *L. lactis* subsp. *lactis* and *P. thoenii* 119 (LPT), and found that The LPT cheese achieved the greatest of yield and sensory evaluation and decreased The populations of *Aspergillus flavus* and *Candida albicans*. In recent years, a new strategy has been developed using probiotics in combination with prebiotics, such as oligosaccharides resulting in the so-called "synbiotics" (**Singh et al., 2018**). Therefore, the aim of

the present work is to investigate the effect of different types and its concentrations of prebiotics on the survival and activity of some probiotics *Propionibacterium* strains.

Materials and Methods

There are 7 different prebiotics were used in this study (Inulin, Litesse, Xylitol, Dextrin, Lactitol, fructofin, Fructooligosaccharides) Each prebiotic was added at different concentrations 1,3 and 5% (w / v) to 12% RSMP and RSMP not containing any prebiotics which used as control and pasteurized at 110oC for 10 min. The pasteurized RSMP were inoculated with 8 probiotic dairy propionibacteria (Table 1) and cultured at 30oC for 24h then stored at the refrigerator at 7±2oC for 30 days (**Mabrouk, 2009**). Serial dilutions were performed and enumerate each culture using the pour plate method on YELA media, the plates were incubated at 30oC for 48h.

Table 1. Sources of *Propionibacterium* strains

Bacterial strain	Source
<i>Propionibacterium thoenii</i> P15	Department of food technology, Iowa State University (ISU).
<i>Propionibacterium thoenii</i> TL18	Institute National de la RechercheAgronomique (INRA).
<i>Propionibacterium acidipropionici</i> TL2	
<i>Propionibacterium freudenreichii</i> ssp. <i>Shermanii</i> ATCC 1907	American Type Culture Collection (ATCC), Manasses , USA .
<i>Propionibacterium freudenreichii</i> 169TM	Bio.Vet.Inc.USA.
<i>Propionibacterium freudenreichii</i> ssp. <i>Freudenreichii</i> 111	University of Warmia and Mazury (Olsztn,Poland)
<i>Ropionibacterium shermanii</i> B-123	Department of Food Techn., propionibacteria culture collection, Iwa State Univ.

The percentage of the viability of each culture was calculated according to this equation (**Desai et al., 2004**):

$$\% \text{ viability} = \frac{\log \text{ cfuml-1 after 30 days storage}}{\log \text{ cfuml-1}} \times 100$$

Litesse™ (Poly dextrose), lactitol and fructofin were obtained from Danisco sweeteners, Finland. Dextrin wheat pact was obtained from SD Fine-Chemlimited. Inulin powder BDH chemical ltd., Liverpool, UK.Fructooligodaccharides, Jarrow formula.

Results and Discussion

The effect of seven different prebiotics (inulin, litesse, xylitol, lactitol, dextrin, fructofin, fructooligosaccharides) were investigated in concentrations 1, 3 and 5% on survival and viability of eight selected *Propionibacterium* 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. acidipropionici* TL2 and *P. acidipropionici* P124I).

Survival (log cfu ml⁻¹) and viability (%) of 8 tested probiotic *Propionibacterium* strains grown in skim milk supplemented with 1% different 7 prebiotics after storage at 7°C for 30 days are presented in **Figure (1)**. The results revealed that the difference between different prebiotics at concentration 1% and storage period were significant (P < 0.05). Besides, the results showed that, the survival (log cfu ml⁻¹) of all 8 probiotic *Propionibacterium* strains grown in skim milk supplemented with 1% different 7 prebiotics in

zero time was ranged from 6.95 (*P.thoenii*P15 with Litesse) to 8.20 (*P. acidipropionici* P124I without prebiotic). However, at the end of storage at 7 °C for 30 days, the survival (log cfu/ml) of *Propionibacterium* strains were ranged from 4.30 (*P. thoenii* P15 with fructofin) to 6.81 (*P. freudenreichii* ssp. *Shermanii* (ATCC1907) with dextrin). It is clear from the data that the different used prebiotics at concentration 1% have a different effect on the tested organisms. In the presence of lactitol and fructofin, *P. shermanii* B-123 showed a slight reduction in viability and the count decreased about 2 log cycle at the end of storage. Besides, it is obvious that in the presence of inulin and fructooligosaccharide some reduction of viability of *P. fredenreichii* ssp. *shermanii* (ATCC 1907) could be observed. Also, *P. acidopropionici* TL2 show the same behavior with fructofin and fructooligosaccharide. On the other hand, it is clear that dextrin support the viability of all tested *Propionibacterium* ssp. except *P. thoenii* P15 loss of viability could be observed.

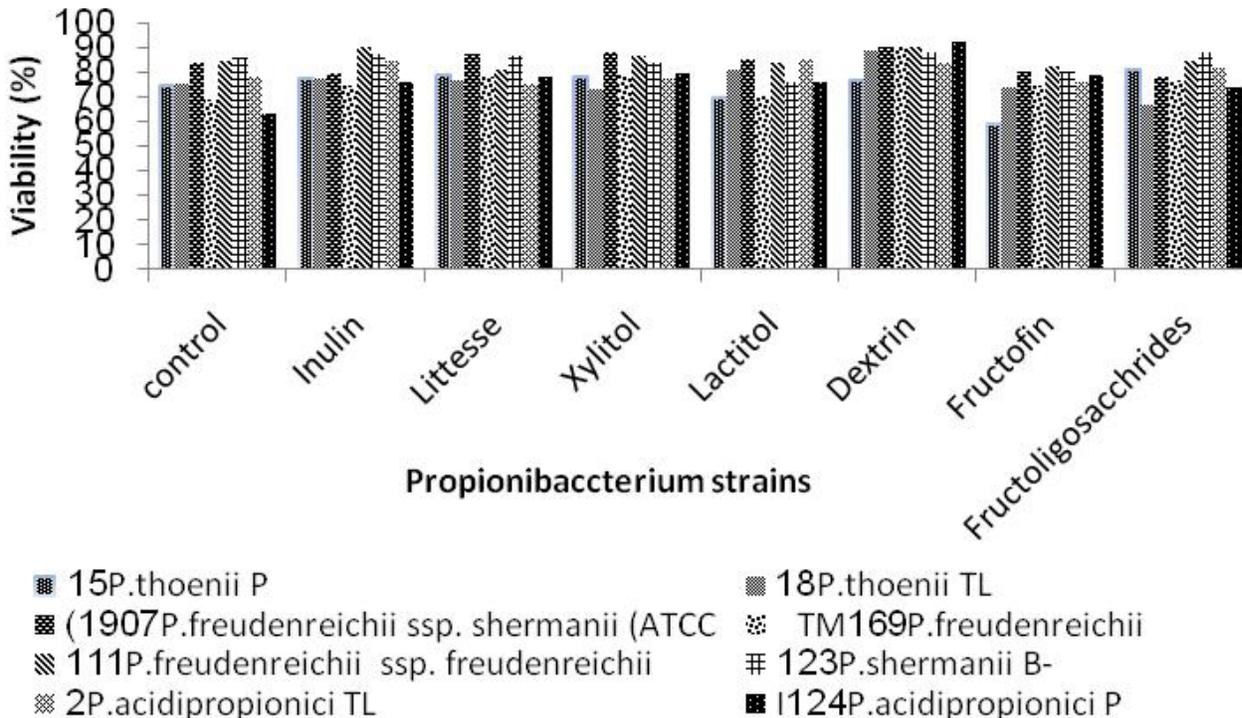


Figure (1). Survival and viability of 8 probiotic *Propionibacterium* strains grown in skim milk supplemented separately with 1% of 7 different prebiotics after refrigerated at 7°C for 30 days

Moreover, the survival (log cfu ml⁻¹) and viability (%) of 8 probiotic *Propionibacterium* strains grown in skim milk supplemented with 3% different prebiotics after storage at 7°C for 30 days are showed in **Figure (2)**. Survival and viability of all tested *Propionibacteria* grown in skim milk had significantly affected (P 0.05) with the addition of 3% different

prebiotics. Skim milk without prebiotics (control) had lower survival counts and viability of the eight probiotic *Propionibacterium* strains as compared with those supplemented with 3% different prebiotics during refrigerator storage for 30 days. Also, at the end of storage period, there was a decrease in the survival (log cfu ml⁻¹) for all strains were observed.

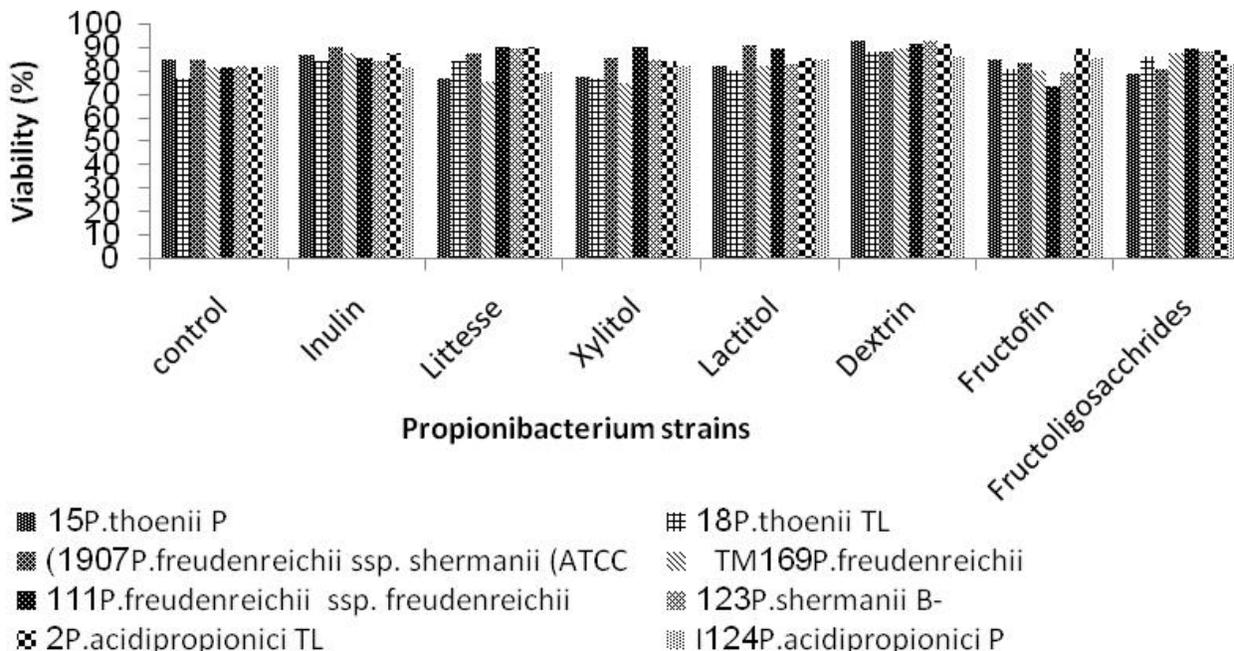


Figure (2). Survival and viability of 8 probiotic *Propionibacterium* strains grown in skim milk supplemented separately with 3% of 7 different prebiotics after refrigerated at 7°C for 30 days.

The survival (log CFU/ml) for the strains grown in skim milk supplemented with 3% different prebiotics in zero day was ranged from 6.95 (*P. freudenreichii* 169TM with lactitol) to 8.30 (*P. freudenreichii* ssp. *Shermanii* ATCC1907 with dextrin). At the end of storage at 7 C for 30 days, the survival (log cfuml-1) of all 8 probioic *Propionibacterium* strains were ranged from 5.32 (*P. freudenreichii* ssp. *freudenreichii* 111 with fructofin) to 7.38 (*P. acidipropionici* TL2 with dextrin). In the presence of 3% prebiotics, it is clear from the results that by increasing the concentration of lactitol from 1 to 3% the viability of *P. shermanii* B-123 was enhanced. However, it is also observed that addition of 3% dextrin enhanced the viability of this organism. In the presence of 3% dextrin, the viability increased as compared with control. In case of *P. thoenii* P15, the viability increased from 76.37% to 88.42%, *P. thoenii* TL18 (from 76.73% to 80.43%), *P. freudenreichii* ssp. *shermanii* ATCC1907 (from 84.65% to 88.07%), *P. freudenreichii* 169TM (from 81.11% to 89.50%), *P. freudenreichii* ssp. *freudenreichii* 111 (from 80.96% to 91.20 %),

P. shermanii B-123 (from 81.97% to 92.81 %), *P. acidipropionici* TL2 (from 81.37% to 91.44 %) and finally *P. acidipropionici* P124I (from 81.71 % to 86.22 %).

Figure (3) show the survival (log cfuml-1) and viability (%) of eight probioic *Propionibacterium* strains grown in skim milk supplemented with 5% different seven tested prebiotics after storage at 7 C for 30 days. Survival and viability for all *Propinibacterium* ssp. grown in skim milk had significantly affected (P 0.05) with the addition of 5% different prebiotics. Also by increasing the concentration from 3% to 5%, the viability of *Propionibacterium* ssp. decreased. The survival (log cfuml-1) of all *Propionibacterium* strains grown in skim milk supplemented with 5% different prebiotics in zero day was ranged from 7.04 (*P. shermanii* B-123with fructofin) to 8.57 (*P. acidipropionici* TL2with lactitol). At the end of storage at 7 C for 30 days, the survival (log cfuml-1) of all eight probioic *Propionibacterium* strains were ranged from 4.95 (*P. shermanii* B-123with inulin) to 7.00 (*P. acidipropionic* P124I with dextrin).

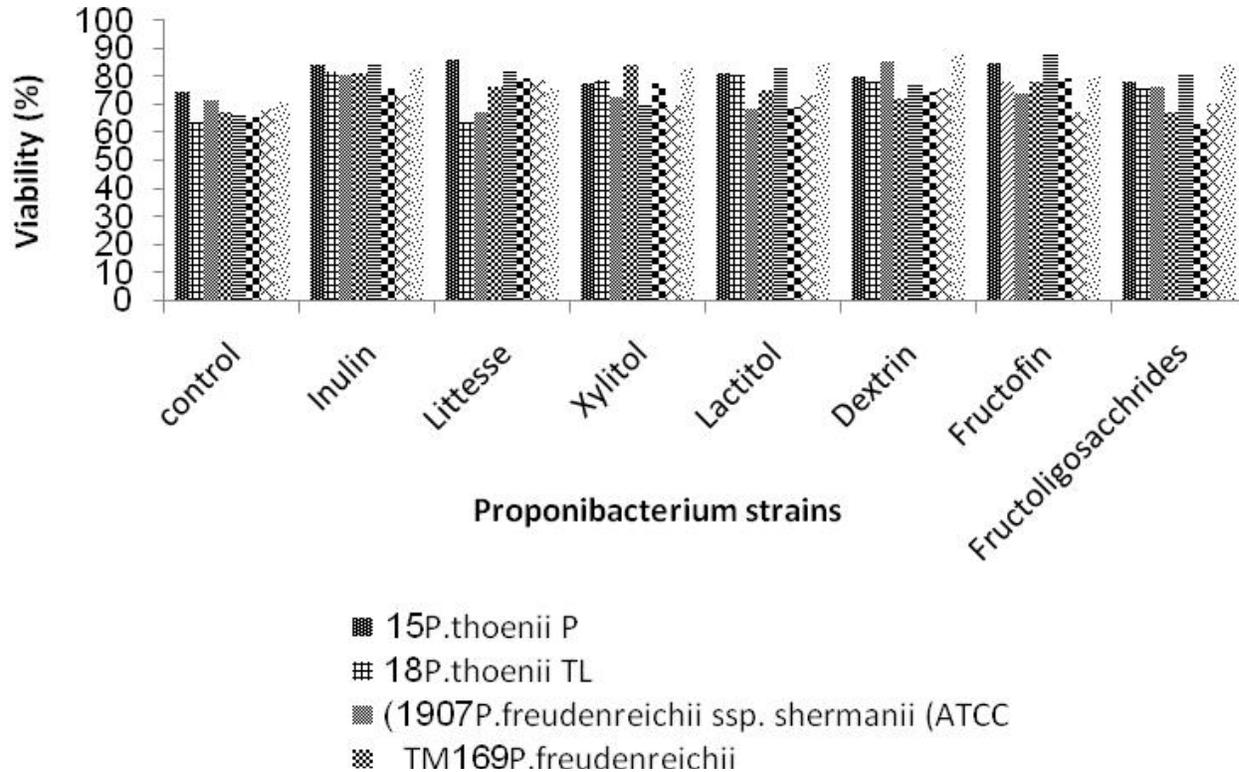


Figure (3). Survival and viability of 8 probiotic *Propionibacterium* strains grown in skim milk supplemented separately with 5% of 7 different prebiotics after refrigerated at 7 °C for 30 days.

At concentration 1% dextrin exhibited the highest survival (6.81 log cfu ml⁻¹) with the strain *P. freudenreichii* ssp. *Shermanii* (ATCC1907). While dextrin exhibited the highest survival at concentration 3% and 5% dextrin with the strain *P. acidipropionici* TL2 (7.38 log cfu ml⁻¹) and (7.00 log cfu ml⁻¹) *P. acidipropionic* P124I, respectively. From these results it is clear that the survival of propionibacteria was depend on the type and the concentration of the used prebiotics. By increasing the concentration of prebiotics from 1% to 3%, the log cfuml⁻¹ increased from 6.79 to 7.38, i.e., the count increased 0.59 and that may be due to increasing the concentration leading to increase the positive effect of prebiotics and these agree with those obtained by **Mumcu and Temiz (2014)**. On the other hand, it was observed that by increasing the concentration of prebiotics from 3% to 5%, the log cfu ml⁻¹ decreased from 7.38 to 7.00, i.e, the count decreased 0.38 and this may be attributed to the decrease in the pH value.

From such results it could be concluded that the growth of *Propionibacterium* strains was supported when the skim milk was supplemented with dextrin more than any other prebiotics were be used. **Slavin**

et al., (2009) reported that dextrin is a soluble fiber which utilized by bacteria in the lower intestine producing (SCFAS) like butyrate acetate and propionate which used as energy source and promote growth of bacterial flora so has a prebiotic effect. Moreover, **Knapp et al. (2013)** mentioned that dextrin increase propionate and decreased butyrate (SCFAS). The increase in propionate is an indication to the stimulation of *Propionibacterium* strains growth.

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How to cite this article:

Baher A. M. Effat, Mohamed K. Ibrahim, Nabil F. Tawfik, Nayra Sh. Mehanna, Nariman R. Soliman. (2019). Effect of Different Prebiotics on Survival and Viability of Some Dairy Propionibacteria. *Int. J. Adv. Res. Biol. Sci.* 6(8): 99-104.
DOI: <http://dx.doi.org/10.22192/ijarbs.2019.06.08.014>