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SURVIVAL OF PROBIOTIC *LACTOBACILLUS ACIDOPHILUS* IN ACIDIC ENVIRONMENT IS ENHANCED IN THE PRESENCE OF *SACCHAROMYCES CEREVISIAE*

Le Nguyen Han, Dong Thi Anh Dao

Ho Chi Minh City University of Technology

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SUMMARY

A number of health benefits have been proved for probiotic bacteria by many studies and probiotics are increasingly incorporated into foods. However, these market preparations have shown low viability of probiotics in human digestion. Therefore, providing viable probiotic cells to the colon and maintaining their metabolic activity against severe conditions of human digestion are increasingly interested by many recent scientific researches. In this trend, our research showed that by creating a physical barrier, the presence of *Saccharomyces cerevisiae* (SC) in *Lactobacillus acidophilus* (LA) suspension can effectively protect probiotic cells from stresses of digestion. After 150 minutes in simulated gastric juice, the survival of LA is significant improved ($p<0.05$) by forming cell-cell contact with SC cells. The LA-only cells show that most cells die with viability of 0% due to low pH medium, compared with 11.025 ± 1.127% of LA+SC mixture. Besides, we found that the cell concentration ratio at 1:10 between SC and LA cells performs highest protective effects on the probiotic in the acidic environment with 10.122 ± 1.348% LA viability. This concentration ratio is the critical value because when the SC concentration is increasingly higher (SC÷LA concentration ratios higher than 1÷10), LA viability shows no significantly different increase. We also found that yeast cells with oxidized carbohydrates on cell's surface have many adverse impacts on co-aggregation (4.003 ± 0.115% after 240-minute treatment) while non-viable yeast cells with damaged and denatured protein on cell's surface still maintains a high percentage of co-aggregation with LA (26.050 ± 0.259% after 240-minute treatment).

Keywords: Co-aggregation, *Lactobacillus acidophilus*, *Saccharomyces cerevisiae*, simulated gastric juice, survival

INTRODUCTION

Probiotics are living microorganisms which once consumed with adequate intake through digestion will bring positive effects on the host's activity of intestinal microflora and improve its health. Several studies claimed that probiotics contribute to decrease of serum cholesterol and blood pressure, prevention of vaginitis, decreased incidence and duration of diarrhea etc. (Klaenhammer *et al.,* 1999; Lee *et al.,* 2008). Probiotics, most of which belong to lactic acid bacteria (LAB) and *bifidobacteria*, proved to have most positive effects on maintaining the intestinal ecosystem (Picot *et al*., 2004).

Several studies employing various techniques such as encapsulation, probiotic training for low pH environment resistant strains, nutrient supplementation, etc. have been conducted and shown positive results at varied degrees in terms of enhancing probiotic viability (Michida *et al.*, 2006; Özer *et al.*, 2005; Picot *et al.*, 2004; Sultana *et al.*, 2000). More recently, another technique called co-aggregation is considered an innovative in this field. Co-aggregation is defined as a process in which genetically-distinct microorganisms adhere to others' surface via specific molecules or some links, forming complex multispecies biofilms. Aggregation can occur among microbial cells of the same species (auto-aggregation) or different ones (co-aggregation) and this combination has been reported to improve probiotic strength in extreme condition (Collado *et al*., 2007).

Based on the co-aggregation mentioned above, a number of studies have discussed the roles of some kinds of yeast in maintaining probiotic viability in milk culture in several months (Graham *et al.*, 1943). *Torulopsis sp.*, a type of yeast as Soulides (1955) pointed out the increase of *S. thermophilus* and *L.*

bulgaricus survival in milk culture in proximately 5-8 months. Also, an American patent (Hsia, 2001) described a method for maintaining probiotic viability in nutrient supplement by adding non-viable yeast cells and protein. As this patent claimed, non-viable yeast cells functioned as yeast extract which supplies nutrition like vitamins to probiotic bacteria. Ningning *et al.* (2011) also reported that the presence of yeast cells *Saccharomyces cerevisiae* isolated from kefir could improve the survival of *L. paracasei* H9 via forming co-aggregation. However, those studies were preliminary steps and still unable to determine which bio-chemical characteristics of yeast cells contributed to enhancing probiotic viability. In this regard, the current study is to further address this issue through additional experiments.

MATERIALS AND METHODS

Microorganisms, cultivation conditions, and enumeration

Lactobacillus acidophilus

ATCC 43121 (LA) was used in this study. Freeze-dried cells were rehydrated in 5 mL MRS broth and then incubated in conditions appropriate for their growth (37^{o}C in 18 h). After that, cultures were moved into liquid MRS broth and grown in the same condition above until reaching the concentration of 10^{10} CFU mL^{-1}. Collection process was conducted at 5000rpm centrifugation for 5 minutes at low temperature (4°C). Cells collected from MRS broth were washed twice with a solution of sodium chloride 0.9%. The washed cells then were selected for later experiments. The concentration of living cells was determined by pour plate method in MRS agar. Plates were also incubated in the same conditions mentioned above (Chávarri *et al.*, 2010).

Saccharomyces cereviciae

BY 4741 (SC) were also rehydrated in 5 mL of YM broth and adjusted to pH 5.0 with 1 M HCl. Then, the inoculated broths were move to liquid YM broth at 30^{o}C for 24 hours to collect stationary phase with cell concentration of 10^{8} CFU mL^{-1} (Lim *et al.*, 2015). Cells collected from YM broth were washed twice with a solution of sodium chloride 0.9%. The washed cells then were selected for later experiments and cells concentration was determined by pour plating in YM agar. Plates were also incubated in the same conditions mentioned above.

In this study, in order to evaluate the enhancing effects of probiotics in co-aggregation with yeast, natamycin (Natamax, Danisco) was used at final concentration of 50ppm to inactivate yeast growth when pouring plating at 37^{o}C in 48 h (Liu *et al.,* 2009).

The data is reported in the current study are the average values of triplicate determinations (plating) from separate experiments.

SC and LAB treatment

SC and LA cells were treated according to the method in Golowczyc *et al,* (2009). To denature protein molecules on surface of SC cells, SC suspensions were sterile in autoclave in 121^{o}C in 30 minutes to make all cells die completely (non-viable SC (NSC)). To oxidize carbohydrates on surface of SC cells, after washed with a solution of sodium chloride 0.9%, SC cells were dissolved in sodium periodate 0.05M and incubated in 30 minutes to form oxidized SC cells (OSC). Initial SC cells without any treatment were called viable SC cells (VSC).

Preparation of simulated gastrointestinal juices (SGJ)

SGJ were used as environment stressing factor on the survival of LA in this study. SGJ was prepared by following method that previously used in Michida *et al.* (2006). Suspending pepsin (P7000, 1:10.000) was dissolved into a solution of sodium chloride (NaCl 0.5% w/v) so that its concentration reached 3g L^{-1}. Using concentrated HCl or NaOH 0.1 mol L^{-1} to make a solution having a desirable pH.

Effect of SC concentration on probiotic's viability

LA concentration was initially fixed at 6.5 × 10^{6} CFU mL^{-1}. SC concentration was based on this LA concentration and prepared with varied ratios. Two suspensions (20 mL each) were combined to form 40 mL cell mixture which was then incubated at 37^{o}C for 20 minutes. The control sample was prepared with only 20mL LA suspension at 6.5 × 10^{6} CFU mL^{-1} added with 20 mL of sodium chloride 0.9%. Afterwards, SGJ pH 2.0 previously prepared was used to cause stress on the mixed culture in 150 minutes. After 150-minute treatment, pour plate method was conducted to identify LA viability. The optimal SC and LA ratio found in this examination was used for later ones.

Effect of varied pH values on enhancement effect of SC on LA viability

The optimal SC and LA ratio mentioned above was chosen to conduct this experiment. 20 mL of each suspension at this ratio was combined to form 40 mL cell mixture and then incubated at 37^{o}C for 20 minutes. The control sample was prepared as mentioned above. Afterwards, SGJ with varied pH values: 5.8, 3.5, 3.0, 2.5, 2,0 was used to cause stress on the mixed culture in 150 minutes. Samples were taken each 30 minutes for pour plate method to identify LA viability.

Effect of SC viability on survival of LA

VSC and NSC were prepared as mentioned above. 20 mL of each was combined with 20 mL LA suspension according to the optimal ratio above. After that, the prepared SGJ pH 2.0 was used to cause stress on the mixed culture in 150 minutes. Samples were taken each 30 minutes for pour plate method to identify LA viability.

Effect of cell component on the co-aggregation between SC and LA

Co-aggregation assays were conducted in accordance with Bao *et al.* (2010) with some modifications. 20 mL of each suspension which followed the optimal SC÷LA ratio was combined to form 40 mL cell mixture which was then incubated at 37^{o}C from 0 to 240 minutes in SGJ pH 7.2. Then, spectrophotometer (UNICO 2150 Spectrophotometer, China) was used to determine optical density of each and mixed suspensions. Co-aggregation between SC and LA was calculated according to the following equation:

$$A\% = (\frac{A_{LA}+A_{SC}}{2} - A_{mix})/(\frac{A_{LA}+A_{SC}}{2}) \times 100$$

Where A_{LA} and A_{SC} represent optical density of separate LA and SC suspensions at 600nm. A_{mix} is the absorbance of the mixed LA and SC suspension. A_{LA}, A_{SC} and A_{mix} were calculated according to the following equation:

$$A\% = 1 - \frac{A_t}{A_i} \times 100$$

Where A_t is the optical density of microbial suspension at test time and A_i is optical density of the initial suspension.

RESULTS AND DISCUSSION

Effect of SC concentration on probiotic's viability

As indicated in Fig. 1, SC concentration significantly improves LA viability. Control sample (no added SC) and SC÷LA concentration at 1÷50 shows nearly no presence of viable LA cells after 150-minute treatment in SGJ pH 2. From 1÷40 to 1÷30 SC÷LA ratios, SC presence has higher effect on LA viability at respectively 0.298 ± 0.109% and 2.084 ± 0.511% LA viability. SC÷LA concentration at 1÷20 shows a significant improvement on LA protection, at 7.055 ± 0,740% LA viability. However, when the SC concentration is increasingly higher (SC÷LA concentration ratios higher than 1÷10), LA viability shows no significantly different increase from 10.122 ± 1.348% to 11.201 ± 1.243% (P>0.05).

Hence, this suggests that each certain LA concentration requires a critical SC concentration which once is surpassed, LA viability shows no significantly different improvement. SC÷LA concentration at 1:10 which proves the most effective ratio for viability enhancing effect of SC was chosen as the optimal ratio for later experiments.

So far, there have been different reports on finding a suitable concentration ratio between yeast and LAB, greatly depending on varying microorganisms examined and also shown dissimilar results. For instance, Phebe *et al.* (2015) demonstrated that in Mcllvaine's buffer solution pH = 2, 1÷1 ratio of *L. rhamnosus* HN001 to viable SC concentration is needed to effectively protect LAB in acidic environment. In their studies, the initial *L. rhamnosus* 8.45 ± 0.07 Log CFU mL^{-1} fell to 7.28 ± 0,31 Log CFU mL^{-1}, equivalent to 6.76% viability. Meanwhile, Ningning *et al.,* (2011) reported that they only needed 5 Log CFU mL^{-1} SC cells for 8 Log CFU mL^{-1} *L.paracasei*, equivalent to 1÷1000 of SC and *L.paracasei*. However, after 60-minute treatment, *L.paracasei* viability remained only 5.98 Log CFU mL^{-1} in comparison to 8 Log CFU mL^{-1} of initial concentration, equivalent to 1% protective effect. All the differences above prove that each species of

probiotic when in contact with the same SC yeast needs certain amount of SC cells which can be explained by the different in structure's cell surface and dimension between varied probiotic strains.

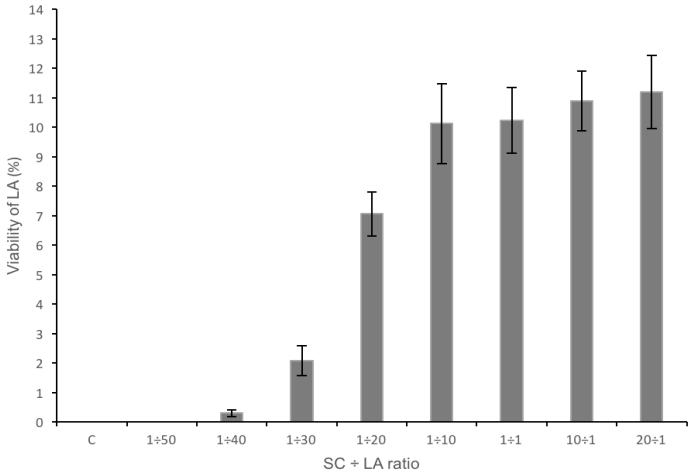

Figure 1. Effect of different SC concentrations on viability of LA. Cell counts are the mean of three experiments (n=3), with error bars representing the standard deviation of the mean. "C" sample means control (No added SC).

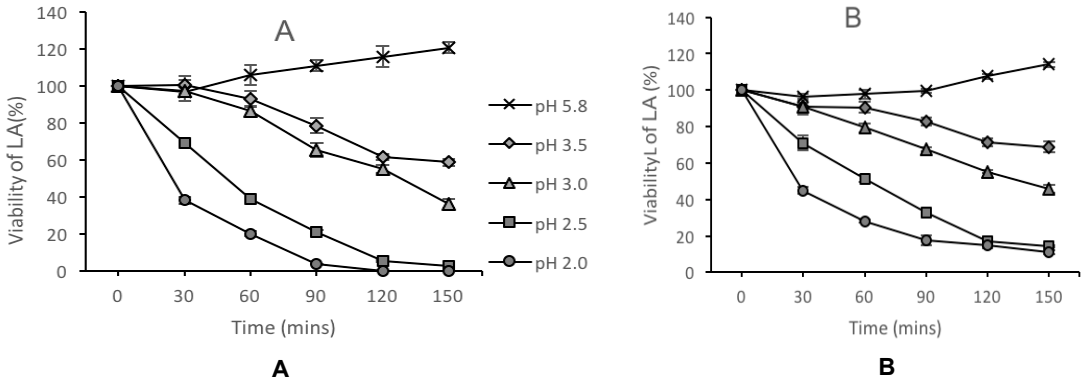

Figure 2. Survival of LA-only (A) and LA in combination with SC (LA+SC) (B). Cell counts are the mean of three experiments (n=3), with error bars representing the standard deviation of the mean.

Effect of varied pH values on enhancement effect of SC on LA viability

1÷10 ratio of SC÷LA concentration was chosen to conduct this experiment. Most of the current probiotic preparations are taken in via digestion. With 2 liters of gastric juice daily released and very low pH, humans' stomach forms a barrier that kills most probiotics. Fig.2A shows that SGJ pH 2 and pH 2.5 eliminates most LA cells (in LA-only sample) and so does SGJ pH 3.0 and pH 3.5 though less seriously. As for controlled pH, cell viability tends to increase due to substrate-rich medium and appropriate-for-growing pH (pH=5.8), consequently cell survival significantly rose after 150 minutes. According to Fig. 2, the LA-only increases to 20.7% compared with 14.09% of LA cells in (LA+SC) at this control pH value. The LA-only cells have more interaction with substrates while LA cells in (SC+LA) have less because of lower nutrition competition of the LA-only sample.

In SGJ pH 2, the LA-only cells with initial viability of 100%, after 30 and 60 minutes of treatment, has lower survival of 38.322 ± 1.745% and 20.408 ± 1.483% respectively. With 90-minute

treatment, this figure plummeted at only 3.968 ± 0.196%. This result is quite congruent with those presented by Ashraf *et al.* (2009) as they asserted that SGJ pH 2 is screening value for probiotic characteristic of *Lactobacillus* since on this condition, *L. acidophilus*, *L. delbrucekii*, *L. rhamnosus* show a sharp decrease in cell viability from 90 minutes.

Survival of the LA-only cells in pH 2.5 after 150 minutes declines at 2.721 ± 0.589%. Applying treatment of 120 and 150 minutes, most cells die with viability of 0% due to low pH medium, causing intracellular pH to decrease accordingly and so does the difference in pH inside probiotic cell walls. This result leads to the fact that probiotic cells cannot synthesize ATP due to electrochemical gradient loss. In addition, acidification inside cells reduces activities of several enzymes sensitive to acid, causing confusion in biosynthesis of DNA and protein. Also, according to Presser *et al.* (1997), the presence of several non-crossed-linked anion of organic acids can cause random contact among several particles occurring inside cells, significantly impacting cells' bio-physical activities.

With the presence of SC, LA survival at pH 2 & pH 2.5 is considerably improved. Treatment of 120 minutes and pH 2 also proves itself where most free LA cannot survive while viability of LA cells in (SC+LA) reaches 14.294 ± 0.775% and this figure is 11.125 ± 1.127% for treatment of 150 minutes as indicated in Fig. 2.

Based on the results compared above, it is well-grounded to assert viability enhancing-effect of SC on LA in severe condition.

Effect of SC viability on survival of LA

The experimental results show that the presence of SC cells, despite whether they are viable (VSC) or not (NSC), has positive impact on improving LA viability. Fig. 3 shows that after 150-minute treatment, both VSC and NSC show almost the same degree in enhancing LA viability, at respectively 11.125 ± 1.127% and 10.252 ± 0.687% ($P<0.05$). This result is quite congruent with those presented by Phebe *et al.* (2015) and Ningning *et al.* (2011) as they asserted that there is no statistically significant result between the effect of VSC and NSC.

Effect of SC at various pretreated methods on co-aggregation between SC and LA was examined and results were shown in Table 1. Co-aggregation percentage between LA and OSC shows no significant change. Moreover, LA and OSC combination indicates lower aggregation ability than others. Meanwhile, 2 groups LA+VSC and LA+NSC have obviously higher co-aggregation percentage and there is no significant statistical difference between them. These results also coincide with the results in Fig. 3 in that the survival of LA when combined with VSC and NSC is the same.

The results also show that OSC cells with oxidized carbohydrates on cell's surface has many adverse impacts on co-aggregation. Meanwhile, NSC with damaged and denatured protein on cell's surface still maintains a high percentage of co-aggregation with LA. This is quite congruent with the hypothesis of Golowczyc *et al.* (2009) and Kogan *et al.* (2007) that the protein on bacteria's surface will link with polysaccharides on SC's surface. This kind of polysaccharides also proves their roles in adherent specificity to Caco-2 cell, the continuous cells of heterogeneous human epithelial colorectal adenocarcinoma cells.

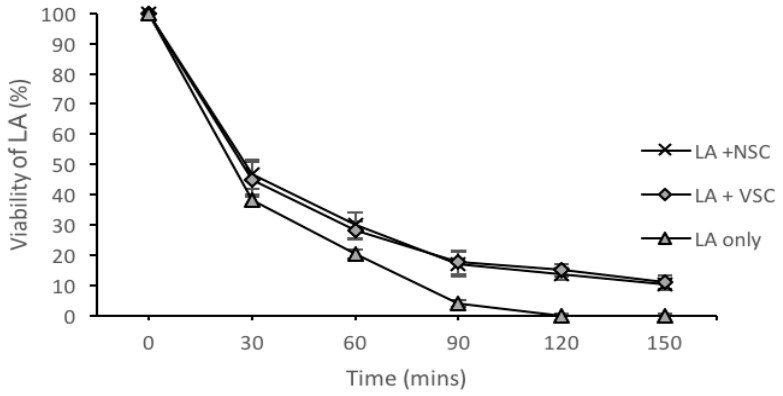

Figure 3. Effect of SC viability on survival of LA.

Effect of cell component on the co-aggregation between SC and LA

Besides, Golowczyc *et al.* (2009) affirmed that a lectin-like activity of proteins on bacteria's surface had an important role in connecting with SC cells to form co-aggregation. In their studies, LA cell's proteins on their surface were denatured by heat treatment and LA cell's polysaccharides on their surface were oxidized, but both of them showed no adhering ability to Caco-2 cells. Therefore, it is necessary to protect LA's proteins on cell's surface because they have an important role in adhering to Caco-2 cell and forming co-aggregation with SC cells. Co-aggregation between LA cells and SC cells means these important proteins are protected, indicating improvement in of probiotic survival in human digestion.

Table 1. Aggregation percentage of LA with different treated SC at pH 7.2

Time (mins)	Percentage of aggregation (%)			
	LA	LA+VSC	LA+NSC	LA+OSC
30	3.797 ± 0.199^{i}	13.030 ± 0.362^{de}	11.957 ± 0.332^{f}	2.33 ± 0.30^{j}
60	5.357 ± 0.206^{h}	13.287 ± 0.624^{de}	13.283 ± 0,404^{de}	2.10 ± 0.42^{j}
90	5.200 ± 0.495^{h}	16.460 ± 0.417^{c}	16.050 ± 0.250^{c}	3.077 ± 0.405^{k}
120	8.443 ± 0.518^{g}	23.190 ± 1.338^{b}	25.157 ± 1.246^{ab}	3.150 ± 0.276^{k}
180	12.200 ± 0.304^{f}	25.537 ± 0.400^{a}	25.810 ± 0.449^{a}	3.870 ± 0.114^{il}
240	11.943 ± 0.070^{f}	26.737 ± 0.645^{a}	26.050 ± 0.259^{a}	4.003 ± 0.115^{im}

Means with the same letter are not significantly different (P>0.05).

CONCLUDING REMARKS

This study confirmed that SC cells in particular and yeast in general have positive effects on improving probiotic's survival. Our findings suggested the promising effectiveness of co-culture of two strains in enhancing viability of vulnerable microorganisms like probiotic. While other techniques show remarkable limits which prevent their widespread application (such as microencapsulation entailing high cost for materials and many steps which reduce probiotic strength, training low pH tolerant strains consuming time and effort etc), co-aggregation has a lot of potentials in producing functional foods with probiotic.

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REFERENCES

Ashraf M, Arshad M, Siddique M, Muhammad G (2009) In vitro screening of locally isolated *Lactobacillus* species for probiotic properties. *Pakistan Vet J* 29(4): 186–190.

Bao Y, Zhang Y, Zhang Y, Liu Y, Wang S, Dong X (2010) Screening of potential probiotic properties of *Lactobacillus fermentum* isolated from traditional dairy products. *Food Control* 21: 695–701.

Chávarri M, Marañón I, Ares R, Ibáñez FC, Marzo F, Villarán MC (2010) Microencapsulation of a probiotic and prebiotic in alginate-chitosan capsules improves survival in simulated gastrointestinal conditions. *Int J Food Microbiol* 142(1-2): 185–189.

Collado MC, Meriluoto J, Salminen S (2007) Development of new probiotics by strain combinations: Is it possible to improve the adhesion to intestinal mucus. *J Dairy Sci* 90: 2710–2716.

Golowczyc MA, Mobili P, Garrote GL, de los Angeles Serradell M, Abraham AG, De Antoni GL (2009) Interaction between *Lactobacillus* kefir and *Saccharomyces lipolytica* isolated from kefir grains: Evidence for lectin-like activity of bacterial surface proteins. *J Dairy Res* 76: 111–116.

Graham VE (1943) The maintenance of viability in *Lactobacillus bulgaricus* culture by growth in association with certain yeasts. *J Bacteriol* 45: 51.

Hsia HS (2001) Nutrition supplement containing *Lactobacillus acidophilus*, yeast and soy protein. Patent. US 6: 294,166.

Klaenhammer TR, Kullen MJ (1999) Selection and design of probiotics. *Int J Food Microbiol* 50(1–2): 45–57.

Kogan G, Kocher A (2007) Role of yeast cell wall polysaccharide in pig nutrition and health protection. *Livestock science* 109: 161–165.

Lee YK, Salminen S (2008) Handbook of probiotics and prebiotics, 2nd edition, Wiley Publisher, Hoboken, 596p.

Lim PL, Toh M, Liu SQ (2015) *Saccharomyces cerevisiae* EC-1118 enhances the survivability of probiotic *Lactobacillus rhamnosus* HN001 in an acidic environment. *Appl Microbiol and Biotechnol* 99: 6803–6811.

Liu SQ, Tsao M (2009) Enhancement of survival of probiotic and non-probiotic lactic acid bacteria by yeasts in fermented milk under non-refrigerated conditions. *Int J Food Microbiol* 135(1): 34–38.

Michida H, Tamalampudi S, Pandiella S, Webb C, Fukuda H, Kondo A (2006) Effect of cereal extracts and cereal fiber on viability of *L. plantarum* under gastrointestinal tract conditions. *Biochem Eng J* 28(1): 73–78.

Ningning X, Tong Z, Bo L (2011) Kefir yeasts enhance probiotic potentials of *Lactobacillus paracasei* H9: The positive effects of co-aggregation between the two strains. *Food Res Int* 45: 394–401.

Özer D, Akin S, Özer B (2005) Effect of inulin and lactulose on survival of *Lactobacillus Acidophilus* LA-5 and *Bifidobacterium Bifidum* BB-02 in *acidophilus bifidus* yoghurt. *Food Sci Technol Int* 11(1): 19–24.

Phebe LL, Mingzhan T, Shao QL (2015) *Saccharomyces cerevisiae* EC-1118 enhances the survivability of probiotic *Lactobacillus rhamnosus* HN001 in an acidic environment. *Appl Microbiol Biotechnol* 99(16): 6803–6811.

Picot A, Lacroix C (2004) Encapsulation of *bifidobacteria* in whey protein-based microcapsules and survival in simulated gastrointestinal conditions and in yoghurt. *Int Dairy J* 14: 505–515.

Presser KA, Ratkowsky DA, Ross T (1997) Modeling the growth rate of *Escherichia coli* as a function of pH and lactic acid concentration. *Appl Environ Microbiol* 63: 2355–2360.

Soulides DA (1955) A synergism between yoghurt bacteria and yeasts and the effect of their association upon the viability of the bacteria. *Appl Microbiol* 3: 129–131.

Sultana K, Godward G, Reynolds N, Arumugaswamy R, Peiris P, Kailasapathy K (2000) Encapsulation of probiotic bacteria with alginate-starch and evaluation of survival in simulated gastrointestinal conditions and in yoghurt. *Int J Food Microbiol* 62: 47–55.

Saarela M, Lathenmaki L, Crittenden R, Salminen S, Mattilasandholm T (2002) Gut bacteria and health foods - The European perspective. *Int J Food Microbiol.* 78: 99–117.

NÂNG CAO KHẢ NĂNG SỐNG CỦA PROBIOTIC *LACTOBACILLUS ACIDOPHILUS* TRONG MÔI TRƯỜNG ACID BẰNG SỰ HIỆN DIỆN CỦA *SACCHAROMYCES CEREVISIAE*

Lê Nguyên Hản✉, Đống Thị Anh Đào

Trường Đại học Bách khoa, Đại học Quốc gia Thành phố Hồ Chí Minh

TÓM TẮT

Vi sinh vật probiotic từ lâu đã được chứng minh bởi rất nhiều nghiên cứu về tác dụng của chúng đối với sức khoẻ của con người. Chính bởi các tác dụng có ích ấy, probiotic đã được nghiên cứu bổ sung vào nhiều loại thực phẩm khác nhau. Tuy nhiên, các sản phẩm này có một nhược điểm lớn là tỷ lệ sống của probiotic rất thấp khi đưa vào môi trường hệ tiêu hoá. Do đó, việc cung cấp các tế bào probiotic còn sống và vẫn duy trì được hoạt tính của chúng khi vào đại tràng trong những điều kiện khắc nghiệt của hệ tiêu hoá ngày càng được nhiều nghiên cứu trên thế giới quan tâm. Trong xu thế đó, nghiên cứu của chúng tôi chỉ ra rằng nhờ vào việc tạo ra một hàng rào bảo vệ, sự hiện diện của tế bào nấm men *Saccharomyces cerevisiae* (SC) trong huyền phù *Lactobacillus acidophilus* (LA) đã bảo vệ được tế bào probiotic trước những điều kiện khắc nghiệt của hệ tiêu hoá. Sau 150 phút trong môi trường dịch dạ dày giả lập, khả năng sống của LA được cải thiện rõ rệt ($p<0.05$) dựa vào tương tác trực tiếp giữa các tế bào probiotic và nấm men. Mẫu chỉ có tế bào LA cho kết quả hầu như tất cả các tế bào probiotic bị chết với tỉ lệ sống 0% so với 11.025 ± 1.127% tỉ lệ sống của LA trong huyền phù LA+SC. Bên cạnh đó, tỉ lệ tế bào 1:10 giữa hai loại tế bào SC và LA cho hiệu quả bảo vệ cao nhất trong môi trường pH thấp với tỷ lệ tế bào LA sống là 10.122 ± 1.348%. Tỷ lệ này được xem là giá trị tới hạn vì khi nồng độ tế bào SC tăng lên hơn nữa (tỷ lệ nồng độ SC:LA cao hơn 1:10) thì tỷ lệ sống của LA cũng tăng lên không có ý nghĩa thống kê. Chúng tôi cũng nhận thấy rằng tế bào nấm men với bề mặt carbohydrate bị oxy hoá sẽ ảnh hưởng rất nghiêm trọng đến khả năng kết tụ (tỷ lệ kết tụ là 4.003 ± 0.115% sau 240 phút khảo sát) trong khi các tế bào nấm men chết với các thành phần protein trên bề mặt bị phá huỷ và biến tính vẫn duy trì được tỷ lệ kết tụ rất cao với LA (tỷ lệ kết tụ là 26.050 ± 0.259% sau 240 phút khảo sát).

Từ khoá: *dịch dạ dày giả lập, kết tụ, khả năng sống, Lactobacillus acidophilus, Saccharomyces cerevisiae ,*

✉ *Author for correspondence:* E-mail*: lenguyenhan@gmail.com*