Acid tolerance response of *Bifidobacterium animalis*

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Introduction

Probiotics have been known to contact acidic environment during fermentation, storage, and digestion in the stomach. In order for probiotics to provide a beneficial effect, it must survive the acidic environment of the gastric juice (pH2.0) and obtain sufficient numbers of live bacteria to colonize in the intestinal tract. The acid tolerance of many enterobacteria involves the production of certain acid resistance proteins, which increases the acid tolerance ability of the bacteria. Previous study has shown that *Bifidobacterium animalis* could increase acid tolerance ability after pre-adapting to mild acidic environment. The main objective of this study was to understand the acid tolerance response of *B. animalis* and examine whether certain acid resistance proteins were produced during acid adaption and acid shock environment.

Methods

B. animalis was grown in normal MRS broth with or without chloramphenicol, a protein synthesis inhibitor, and growth was allowed until it reached stationary phase. The bacteria were then treated for acid adaption by transferring the bacteria into pH 5.0 and 4.0 MRS broth adjusted with hydrochloric acid. During acid adaption, protein inhibition was also applied by the addition of chloramphenicol. Acid tolerance ability was determined by transferring bacteria into an acid shock environment of pH2.0 for 30 minutes with or without chloramphenicol added, and viable counts were determined with three repeats for each treatment and the viable counts were compared among different treatments (Fig 1).

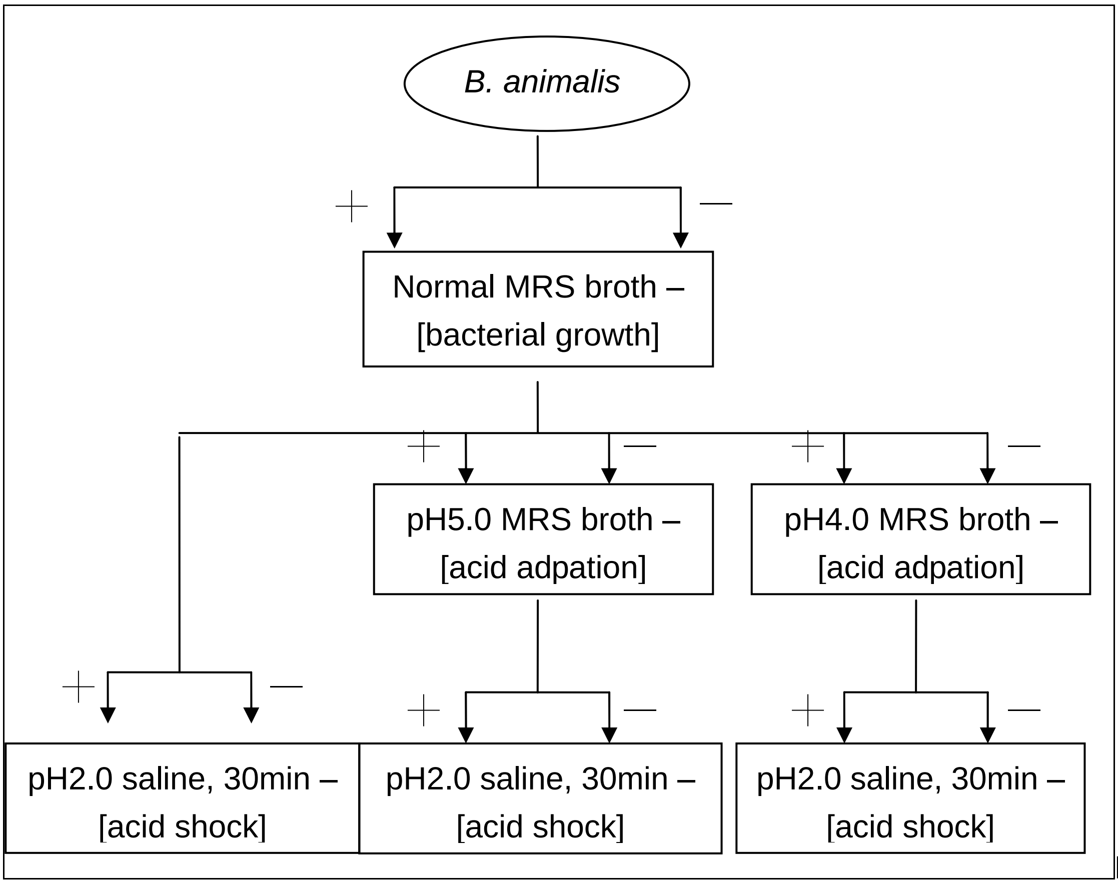

Fig. 1

Experimental flow chart.

"+" protein synthesis allowed , "—" protein synthesis inhibited

Results and discussions

Table 1 Viable bacterial count (logCFU) for various acid and protein inhibition treatments

Treatment			logCFU	Treatment			logCFU
Growth	Adaption	Shock		Growth	Adaption	Shock	
+	X	X	7.95	+	+	+	7.86
+	X	+	7.42	+	+	—	7.77
+	X	—	7.39	—	+	+	8.32
—	X	X	6.02	—	+	—	8.32
—	X	+	5.04	+	—	+	4.85
—	X	—	5.10	+	—	—	4.75
+	+	X	8.81	—	—	+	3.25
+	—	X	7.89	—	—	—	2.26

"+" protein synthesis allowed, "—" protein synthesis inhibited, "X" treatment not tested

The viable bacterial counts for the bacteria in different treatments are shown in Table 1. The numbers are used for comparison of the relationship between acid tolerance ability and protein synthesis shown in Table 2 and Table 3.

The comparison of viable bacterial count for protein inhibition during a certain treatment is compared to that for protein synthesis allowed during all treatments. A decrease in viable bacterial counts indicates that protein synthesis is closely related to the viability of the bacteria during that treatment. A comparison between protein synthesis allowed at a certain treatment with protein synthesis inhibited during all treatments can also show that an increase in viable bacterial count means that protein synthesis is crucial to the bacteria during the treatment.

From Table 2, protein synthesis inhibition in all separate treatments including bacterial growth, acid adaption and acid shock, all lead to a decrease in bacterial surviving numbers after acid shock treatment. The results indicated that protein synthesis is closely related with the viability of the bacteria during all three treatments. Protein synthesis allowed in all specific treatments compared to protein synthesis inhibited at certain treatments also indicated the same results, an increase in viability when protein is allowed at a certain treatment.

Table 2 Comparison of viable bacteria count (logCFU) at different treatments of protein inhibition

Treatment of protein synthesis	Protein inhibition	Protein allowance
Growth	8.32*	4.75*
Adaption	4.85*	8.32*
Shock	7.77	3.25*
	Protein synthesis allowed during all stages	Protein synthesis inhibition during all stages
	7.86	2.26

*Shows significant difference between values.

Values were compared using t test with a significance level of 0.05.

From Table 2, we can know that protein synthesis during all treatment is closely related with the viability of the bacteria, but whether the proteins affect the acid tolerance ability of the bacteria is still in doubt. Therefore, the comparison of acid tolerance survival rate of the bacteria with and without protein synthesis during the three treatments is further investigated.

The acid tolerance survival rate is gained by dividing the viable bacterial count after acid shock treatment to the viable bacterial count before acid shock treatment, multiplied by 100%. By comparing the acid survival rate of protein synthesis allowed and protein synthesis inhibited treatment groups, we are able to neglect the decreasing effect caused by acid shock and concentrate on the effect from protein synthesis treatment. If protein synthesis is important for acid tolerance ability of the bacteria, viable bacteria counts should be significantly lowered when protein synthesis is inhibited than when protein synthesis is allowed.

Results from Table 3 clearly showed that the viable count during bacterial growth treatment for protein synthesis inhibited and non-inhibited group had the same amount of viable count. When protein synthesis is inhibited during acid adaption and acid shock environment, an obvious decline of bacterial survival rate was observed, suggesting that protein produced during the two treatments contributed to the acid tolerance ability of the bacteria, increasing the ability of the bacteria against acid stress.

Table 3 Comparison of acid tolerance survival rate (%) during different treatments of protein inhibition

Treatment of protein synthesis	Protein inhibition	Protein allowance
Growth	10.63%	10.76%
Adaption	3.92%*	<0.01%
Shock	29.15%*	0.07%

Survival rate was obtained by dividing the viable bacterial count before and after acid shock treatment.

* Shows significant difference between two values. Values were compared using t test with a significance level of 0.05.

Results of bacteria adapted to pH4.0 also indicated the same results (data not shown).

Conclusion

The acid tolerance response of *B. animalis* includes the production of acid resistance proteins induced in both mild acidic and extreme acid shock environment. The proteins are synthesized only when bacteria encounter low pH environment, and the synthesis of these proteins are crucial for the acid tolerance ability of *B. animalis*. Further research on the mechanism of this response is required.