Bioencapsulation of Lactic Acid Bacteria Using Inulin and Banana Powder as Excipients

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Introduction

Inulin has attracted much attention recently as nonabsorbable carbohydrate with prebiotic properties, stimulate the growth and/or activity of indigenous probiotic bacteria in the intestinal tract, decrease fecal odor components, reduce blood cholesterol, prevent or inhibit the occurrence of some types of cancer, enhance vitamin synthesis, increase mineral absorption, and stimulate the immune system (Jenkins et al., 1999). Inulin is naturally present in several fruits and vegetables, the most common sources being wheat, chicory onion, banana, garlic and leek (Van Loo et al., 1995). Chicory inulin currently used in the food industry (De Bruyn et al., 1992).

Banana is the world's most widely consumed fruit. According to the United Nations Food and Agriculture Organization, bananas are the world's fourth most important food corp. In the Asia-Pacific region, banana is the most widely produced fruit in Philippines, Thailand, Indonesia, and India. In Thailand, there are several species and varieties of banana. Among these, both unprocessed banana and processed banana products are generally consumed. Ripe banana is not only cheap but also claimed with its health benefits (Kruawan et al., 2004). Pannangpetch et al. (2001) found that the extracts of two different species of banana, Hom (*Musa sapientum* Linn.) and Palo (*Musa paradisiaca*) could protect the stomach from the indomethacin-induced injuries. They also presented that different kinds of banana have varying gastroprotective qualities. Whereas, Mukhopadhyaya et al. (1987) reported that the banana powder (*Musa sapientum* Linn.var.*paradiscica*) treatment was observed to strengthen gastric mucosal resistance in rats. Moreover, pharmacological studies showed that banana had antifungal (Ranasinghe et al., 2002) and antibacterial effects (Ono et al., 1998). It is also very interesting to explore other benefits of ripe banana, a cheap fruit commonly consumed by Thai people. Therefore, it is of relevance to evaluate the potential of inulin and banana powder as alternative excipients for tableting of the probiotic bacteria, *Lactobacillus acidophilus* and *L.fermentum* to improve their survival in gastric condition.

Materials and Methods

Bacterial strains and preparation of powder culture

LAB used in this work was *Lactobacillus acidophilus* 72-4 and *Lactobacillus fermentum* 2311. Lyophilisate form of *L.acidophilus* 72-4 was supplied from Chr.hansen Biosystems (Horsholm, Denmark). *L.fermentum* 2311, isolated from fermented food, was lyophilized by a lyophilizer model Chirst 1-4 (Chirst, Osterode, Germany).

Tablet production

Raftiline® (Orafti Active Food Ingredients, Oreye, Belgium), a commercial food grade of inulin was used in this study. Edible portion of ripe banana was cut and subsequently lyophilized. The lyophilized banana was following ground to fine powder. The formulations of LAB with/without the prebiotics (as shown in Table 1) were compressed into tablets using an instrumented single punch tablet press (Korch EKO, Berlin, Germany) with the compression force of 8 kN under

constant environmental conditions (30% RH; 25-27^{o}C). All tablets had a plane surface with a diameter of 10 mm.

Table 1 Tablet formulation

Formulations	Amount (%)				
	1	2	3	4	5
LAB	10	10	10	10	10
Avicel		19	-	-	-
$CaHPO_4$	-	70	-	-	-
Inulin	89	-	79	-	-
Banana powder	-	-	-	89	79
Lactose	-	-	10	-	10
Mg stearate	0.1	0.1	0.1	0.1	0.1
Talcum	0.9	0.9	0.9	0.9	0.9
Tablet weight (mg)	385	300	365	385	365

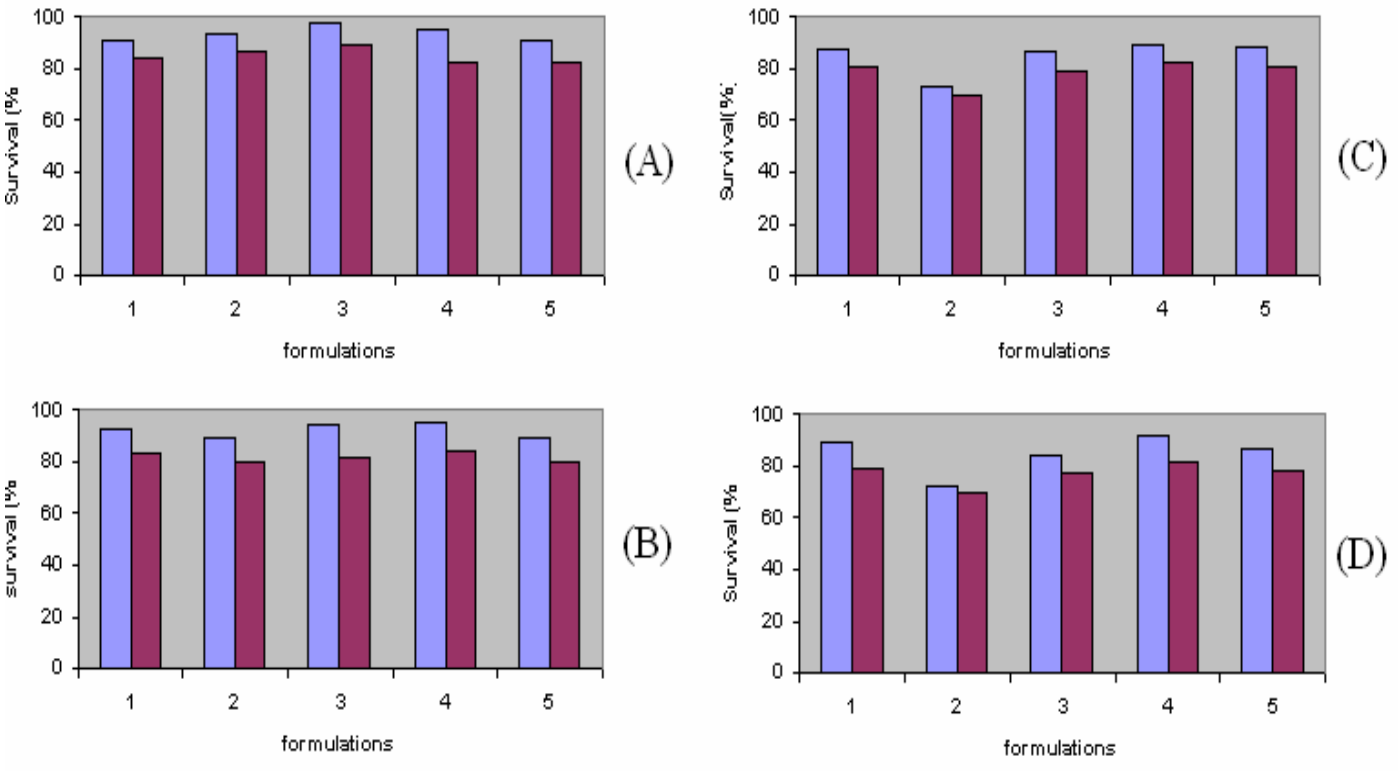

Figure 1. Survival rate of *L.acidophilu*s (A) and *L.fermentum* 2311 (B) during exposure to 0.04N HCl and survival rate of *L.acidophilus* (C) and *L.fermentum* 2311 (D) during exposure to 0.1N HCl.

Test for LAB survival in artificial gastric juice

The survival of LAB in tablet in acidic media of 0.04N HCl and 0.1N HCl were studied. All tests were carried out according to USP 23 paddle method at 100 rpm, 37 °C with 600 ml of medium (Chan & Zhang, 2005). The tablets were exposed to respective acidic media for 1 and 2 h before viability assays were carried out.

Results and Discussion

The survival of LAB after incubation in 0.04 N HCl and 0.1 N HCl was showed in Figure 1. It was found that LAB tested strains in five formulations showed different resistance to acidic conditions. The survival rate of all tablets containing inulin and banana powder remained relatively constant for three months at temperature of 10 ^{o}C. Storage at a temperature of 30 ^{o}C led to a slight decrease of survival rate (average 0.5 log-units after three months). Data of storage stability were shown in Table 2.

Table 2 Survival of LAB in various formulations after three month storage

Formulations	Initial population (log CFU/tablet)	Viability of LAB (log CFU/ tablet) after three months storage	
		10^{o}C	30^{o}C
LA1	9.230 ± 0.120	9.183 ± o.110	8.760 ± 0.107
LA2	9.041 ± 0.098	9.028 ± 0.141	8.481± 0.052
LA3	9.531 ± 0.114	9.509 ± 0.089	8.911 ± 0.087
LA4	9.193 ± 0.026	9.130 ± 0.123	8.743 ± 0.094
LA5	9.112 ± 0.056	9.008 ± 0.025	8.527 ± 0.084
LF1	8.980 ± 0.040	8.931 ± 0.045	8.590 ± 0.165
LF2	8.089 ± 0.065	8.062 ± 0.102	7.572 ± 0.096
LF3	8.373 ± 0.131	8.313 ± 0.056	7.673 ± 0.124
LF4	8.985 ± 0.168	8.953 ± 0.106	8.545 ± 0.046
LF5	8.981 ± 0.069	8.883 ± 0.098	8.471 ± 0.058

Note: LA = *Lactobacillus acidophilus* 72-4, LF = *L.fermentum* 2311
The log CFU/tablet were shown as mean ± standard deviation (n=3)

Conclusion

It was concluded that both inulin and banana powder have potential to protect the LAB in acidic gastric condition. Probiotic tablets containing inulin and banana powder might provide functional benefits as alternative synbiotic product.

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