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Original article

Survival and shelf life of *Lactobacillus lactis* 1464 in shrimp feed pellet after fluidized bed drying

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ABSTRACT

In the present study, *Lactobacillus lactis* 1464 was attempted to be incorporated in shrimp feed pellets. The fresh culture (25% volume per weight) with and without pH adjustment was mixed into feed ingredients prior to the pelleting process at ambient temperature. The wet pellets were dried using a fluidized bed dryer at 50 °C, 60 °C, 70 °C and 80 °C to achieve a moisture content below 11%. The results indicated that the strain viability depended on the drying temperature with a viable cell number of approximately 10^6–10^8 CFU/g and the pH of the culture was found to affect the strain viability during drying. At all drying temperatures, the strain survival after drying ranged from 75.94% to 92.28% at pH 3.8 and from 89.54% to 96.87% at pH 7.0. Moreover, the addition of protectants was found to enhance the strain survival during drying. In particular, milk powder and monosodium glutamate (MSG) exhibited significant ($p < 0.05$) protective effect on the viability at a high temperature of 80 °C. During storage at 30 °C, a high survival rate was found for the strain with MSG and acacia gum. Furthermore, the prediction model for long-term storage stability of the strain was found to validate only at a low temperature of 4 °C, in comparison to a high temperature of 30 °C.

Introduction

Nowadays, probiotics offer a promising alternative approach for controlling shrimp diseases and improving shrimp health through their potential to control pathogens, to stimulate the immune response, to improve water quality and to enhance nutrition through the production of digestive enzymes (Verschuere et al., 2000; Gullian et al., 2004; Wang, 2007). To achieve health benefits, probiotic bacteria must be viable and available at high concentration, typically 10^6–10^7 CFU/g of product (Kosin and Rakshit, 2006). Furthermore, incorporation into feed pellets is more effective in conveying probiotics into animals compared to direct application into rearing systems. It is also applicable for intensive aquaculture and requires no additional labor or shrimp handling (Gómez et al., 2007). The viability and stability of probiotics have been a technological challenge in feed manufacturing because probiotics, including *Lactobacillus, Bacillus* and yeast, are susceptible to the high temperature of the pelleting and drying process. According to Biourge et al. (1998), *Bacillus* CIP5832 spores in dog diet were found to have in excess of 99% loss after the extrusion, expansion and drying processes. Furthermore, the viable count of yeast in shrimp feed pellet decreased by 10^5 fold after extruding through a meat grinder at 72 °C for 31 s followed by drying at 65 °C for 6 h (Aguirre-Guzmán et al., 2002).

Fluidized bed drying is extensively used for drying wet particulate and granular materials. In a fluidized bed dryer, the probiotic cell suspension is mixed with a vibrating bed of absorbers or matrix molecules which helps to form capsules by adherence (Nag and Das, 2013). This process is comparatively economical. It involves low energy consumption, high throughput and imparts moderate heat stress to the bacterial cells (Beker and Rapoport, 1987; Nag and Das, 2013). Furthermore, this process was successfully used for the preparation of dried granules or powders containing lactic acid bacteria (Santivarangkna et al., 2007; Nag and Das, 2013). According to Nag and Das (2013), fluidized bed drying was able to retain viability of *Lactobacillus casei* CRL 431 of more than 7.7 log CFU/g during storage at 25 °C for 12 wk. Mille et al. (2004) revealed that the *Lactobacillus plantarum* viability in casein powder was up to 80% after fluidized bed drying at 35 °C for 30 min. Correspondingly, the

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survival of *Lactobacillus brevis* in fish feed was 99% (10^8–10^9 CFU/g) after fluidized bed drying at 40 °C for 40–60 min with a moisture content of 5% (Toledo et al., 2010). Several studies have reported that the viability of lactic acid bacteria during drying and storage was enhanced by the addition of protective agents such as trehalose, skim milk, whey protein, soy protein isolate, monosodium glutamate, sucrose, lactose, sorbitol and polymers such as carboxymethyl cellulose, dextran and acacia gum (Morgan et al., 2006; Santivarangkna et al., 2007; Golowczyc et al., 2011; Lapsiri et al., 2013). In the present study, an overnight culture of *Lactobacillus lactis* 1464 was incorporated into shrimp feed pellets prior to the pelleting process at ambient temperature and dried in a fluidized bed dryer to achieve a moisture content lower than 11%. The effect of the drying temperature, culture pH and protectants on the strain survival during drying was determined. Additionally, the storage stability of the strain in the pellets at 4 °C and 30 °C was also evaluated.

Materials and methods

Preparation of soymilk medium

Soymilk medium was prepared as described by Wang et al. (2002). Soybeans were washed and soaked overnight in distilled water. The soaked soybeans were blended with distilled water (soybean:water = 1:10 w/v) for 3 min and then filtered through a double-layer cheesecloth to obtain soymilk. 1% (w/v) glucose (Ajax Finechem; Taren Point, NSW, Australia) was added into the soymilk before sterilization at 121 °C for 15 min.

Microorganism

L. lactis 1464 isolated from the sediment of a Nile tilapia fish pond with antimicrobial activity against shrimp pathogen was obtained from the Department of Biotechnology, Kasetsart University, Thailand. After two successive transfers of the strain in de Man, Rogosa and Sharpe (MRS) broth (Merck; Darmstadt, Germany) at 37 °C for 24 h, the activated culture was again inoculated into MRS broth at 37 °C for 24 h which served as the inoculum (Lapsiri et al., 2011). The overnight cultures were conducted in 250 mL Erlenmeyer flasks containing 120 mL of sterile soymilk and inoculated with 5 mL of the inoculum (approximately 10^9 CFU/mL). The sample was incubated at 37 °C for 24 h.

Preparation of shrimp feed pellets

The formulated shrimp feed consisted of 40% fish meal, 8% shrimp head meal, 20% rice bran, 10% wheat flour, 5% sago flour, 10% horse tamarind leaves powder, 5% soybean oil and 2% premix by weight. The feed mixture was sterilized at 121 °C for 30 min and dried overnight in a hot air oven (Memmert GmbH; Memmert, Germany) at 55 °C. Gelatin (3% w/v) as a feed binder was mixed with the dried mixture in a stand mixer. The overnight culture (25% v/w) of *L. lactis* 1464 with and without pH adjustment to 7.0 with 5 M NaOH (Ajax Finechem; Taren Point, NSW, Australia) was then added. After mixing for 3 min, soybean oil was then added and mixed for 3 min again. The feed mixture was pressed into pellets at ambient temperature using a laboratory pellet mill with a 2 mm diameter (California Pellet Mill Co.; Crawfordsville, IN, USA). The wet pellets (250 g) were dried in a laboratory fluidized bed dryer (Sherwood Scientific; Cambridge, UK) with a 5 L stainless chamber at various air inlet temperatures—50 °C, 60 °C, 70 °C and 80 °C. The fluidizing air flow velocity was held constant at 3.10 m/s. Samples were collected during drying to determine the viability of *L. lactis* 1464 and the moisture content. The specific rate of degradation (k) of *L. lactis* 1464 during drying at constant temperature was calculated as described by first-order kinetics as shown in Equation (1) (Desmond et al., 1998).

$$\log N = \log N_0 - kt \quad (1)$$

where N_0 is the number of initial viable cells and N is the number of viable cells at any time both expressed in colony forming units (CFU) per gram, k is the specific rate of degradation per minute and t is the drying time in minutes. A plot of the term of log N versus time (t) yields the estimate of k from the slope.

Effect of protective agents on viability of L. lactis 1464 after fluidized bed drying

Each protective agent (5% w/v) including, monosodium glutamate (MSG) (Ajinomoto; Bangkok, Thailand), milk powder (Dumex; Samut Prakan, Thailand), acacia gum (MT Instrument; Bangkok, Thailand), maltodextrin (Du Zhi Xue, China) was added into the overnight cultures of *L. lactis* 1464 prior to mix with the feed mixture. The shrimp feed pellets were prepared by the same manner as described in the preparation of the shrimp feed pellets. The wet pellets with an initial moisture content of approximately 26.5% were dried at various temperatures (50 °C, 60 °C, 70 °C and 80 °C) until the moisture content was below 11%.

Storage of dried shrimp feed pellets

Dried shrimp feed pellets (5 g) were placed into plastic zip bags and kept at 4 °C and 30 °C for 6 mth. The viable cell counts were determined every month. Each treatment was duplicated.

Accelerated storage test

Dried shrimp feed pellets were incubated in a hot air oven at 50 °C, 60 °C, 70 °C and 80 °C. At 50 °C, samples were taken after 24 h, 48 h, 72 h, 96 h and 120 h of exposure; at 60 °C, after 3 h, 6 h, 9 h, 12 h, 15 h and 18 h; and at 70 °C and 80 °C after 1 h, 2 h, 3 h, 4 h, 5 h and 6 h to determine the residue viable counts. The specific rate of degradation (k) was calculated.

Enumeration viable counts

Viable counts were enumerated using a pour plate technique. The sample of feed pellets (5 g) was rehydrated with 45 mL of sterile 0.85% NaCl (Ajax Finechem; Taren Point, NSW, Australia) to obtain 1:10 dilution and mixed in a stomacher (Seward Laboratory Systems Inc; Davie, FL, USA) for 1 min. Serial dilutions were made for each sample and plated on MRS agar containing 0.03% bromocresol purple (Ajax Finechem; Taren Point, NSW, Australia). Plates were incubated at 37 °C for 24 h and enumerated for colony forming unit per gram (CFU/g). Each treatment was duplicated. The survival rates were calculated as: Survival rate (%) = $(\log N/\log N_0) \times 100$, where N_0 is the number of initial viable cells and N is the number of viable cells at any time both expressed in CFU per gram (Reddy et al., 2009).

Moisture content

The moisture content of the feed pellets was determined according to the relevant international standard 6496 (International Standard Organization, 1999).

Statistical analysis

All experiments were carried out in duplicate. The data were statistically analyzed for analysis of variance in a completely