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In vitro Suppression against Streptococcal Bacteria and Health-Promoting Effects of Probiotic Bacillus polyfermenticus in Tilapia (Oreochromis niloticus)

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Original Article

In vitro Suppression against Streptococcal Bacteria and Health-Promoting Effects of Probiotic *Bacillus polyfermenticus* in Tilapia (*Oreochromis niloticus*)

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Abstract

In vitro antibacterial activity of *Bacillus polyfermenticus* (BP) against tilapia streptococcal pathogen was evaluated using the agar dilution method and co-incubation method. Both *in vitro* tests confirmed the inhibitory effect of *B. polyfermenticus* on the growth of *Streptococcus agalactiae*. The suppression of *S. agalactiae* was observed in the test system containing *B. polyfermenticus* and the inhibition was more evident in the system with high density of *B. polyfermenticus*. Subsequently, feeding administration of BP was employed in tilapia broodstocks (12 months old) and fry (37.33 ± 1.37 mg body weight) to examine the effect of BP on health performance. Significantly higher amount of fertilized eggs was recorded in the tilapia broodstocks fed BP supplemented diet for 5 months as compared with the control groups ($p < 0.05$); however no significant differences were found in the survival of broodstocks between the treatment and control groups. The tilapia fry fed on BP diet for 28 days also displayed significantly improved feed conversion ratio, average daily weight gain and survival rate. The study demonstrated *in vitro* anti-streptococcal potency of *B. polyfermenticus* and the potential of BP dietary supplement in promoting tilapia health performance.

Keywords: *Bacillus polyfermenticus*, streptococcosis, tilapia

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Introduction

Tilapia (*Oreochromis niloticus*) are being increasingly cultured as food fish in many countries. Among the diseases affecting intensive tilapia farming, streptococcosis causes the greatest reduction in stocks of tilapia and has economic consequences on fisheries in many areas of the world (Shoemaker and Klesius, 1997; Amal and Zamri-Saad, 2011; Yanong and Francis-Floyd, 2013). It has been found that there is a greater incidence of streptococcal infection in tilapia than any other cultured fish. With the intensive growth of tilapia cultures in Thailand, streptococcal infections are now a primary constraint on the tilapia industry (Maisak et al., 2008; Jantrakajorn et al., 2014). Infection is most severe when water temperature rises above 28°C and other environmental stressors are present (El-Sayed, 2006). The current control regimen in Thai fish farming is mainly through the application of antimicrobials and chemotherapeutics. However, the use of antimicrobials and chemotherapeutics in aquaculture is becoming a threat to consumer health (FAO/WHO/OIE, 2006; 2008). Negative consequences of antimicrobial residues in aquaculture products and antimicrobial resistance genes developed in aquatic bacteria following exposure to antimicrobial agents are well documented (Teale, 2002; Smith, 2008; Tuševljak et al., 2013). The use of probiotics to control pathogens is increasingly applied as an alternative to antimicrobial treatment (Tuan et al., 2013). Many recent studies have demonstrated the efficacy of probiotic application in disease control, in enhancing health performance and improving water quality in aquaculture (Verschuere et al., 2000; Welker and Lim, 2011; Cruz et al., 2012; Tuan et al., 2013; Newaj-Fyzul et al., 2014). While probiotic strains have been isolated from different populations of microbiota, microorganisms from the genus *Bacillus* have been reported as putative probiotics and the use of microorganisms is regarded as one of the most promising preventive methods for disease control in aquaculture (Balcázar et al., 2006; Kesarcodi-Watson et al., 2008; Cutting, 2011). Although the mechanism of the action of probiotics remains to be further determined, competitive exclusion of pathogenic bacteria and immunostimulating effect are suggested as possible benefits linked to the administration of the probiotic bacterium from the genus *Bacillus* (Leonel Ochoa-Solano and Olmos-Soto, 2006; Cutting, 2011). *Bacillus polyfermenticus* is a gram-positive rod shape, endospore bacterial probiotic. It was isolated from air sample and cultured in general laboratory media (Lee et al., 2001). This probiotic survives in the gastrointestinal environment and it is currently a commercial probiotic for the treatment of intestinal disorders in human and nutritional supplement for human and animals (Lee et al., 2001; Kim et al., 2009; Cutting, 2011; Jung et al., 2012). The purposes of this study were to examine the *in vitro* inhibitory potency of *B. polyfermenticus* against tilapia streptococcal pathogen and to evaluate the *in vivo* efficacy of dietary supplementation in health enhancement of tilapia fry and broodstocks.

Materials and Methods

In vitro* inhibitory potency of *Bacillus polyfermenticus* against *Streptococcus agalactiae

Bacterial isolates: *B. polyfermenticus* KJS-2 (US 2010/0040761A1) was provided by CTCBIO Inc. (Seoul, Republic of Korea) in lyophilized preparation. The bacterial isolate was genetically confirmed to be *B. polyfermenticus* using the Polymerase Chain Reaction (PCR) technique as previously described (Mo et al., 2010). *B. polyfermenticus* was propagated in Tryptic Soy Broth (TSB; OXOID®, UK) in a shaking incubator (SI4, Shel Lab, Cornelius, OR, USA) at 30°C 100 *rpm* for 14 h. *B. polyfermenticus* cells were harvested by centrifugation (5,500 g) (Sigma 4-16PK, Sartorius AG, Gottingen, Germany) at 4°C for 15 min and resuspended in sterile normal saline solution (NSS). The standard plate counting method was used for determination of total cells in suspension.

Five clinical isolates of *S. agalactiae* obtained from diseased cases occurring in Thailand during 2012 were used in this study. Identification of bacterial isolates was performed using API 20 STREP (BioMerieux®, France) and PCR as previously described (Martinez et al., 2001). All bacterial isolates were stored in maintenance broth containing 20% glycerol at -70°C. Before each experiment for the inhibitory test, the stored bacterial isolates were transferred to Tryptic Soy Agar (TSA; OXOID®, UK) supplemented with 5% sheep blood. After incubation at 30°C for 18-24 h, colonies from the pure culture material were transferred for the procedure. Bacterial colonies were picked from a pure culture material, resolved in 4 ml volume of sterile NSS. The bacterial cell density was adjusted to 4 McFarland standard or approximately 10^9 colony-forming units (CFU)/ml. The inocula were then diluted ten-fold in sterile NSS, giving a final cell density of approximately 10^8 CFU/ml.

Agar dilution method: The procedure followed the international recommendations provided by the Clinical and Laboratory Standards Institute (CLSI, 2012). Mueller Hinton Agar (MHA; OXOID®, UK) plates, containing serial ten-fold dilutions of the *B. polyfermenticus* (10^1-10^5 CFU/ml), were inoculated with a standardized inoculum of the test isolate (*S. agalactiae* 10^8 CFU/ml). Using a standard multipoint inoculator, *S. agalactiae* from mature cultures were allocated at approximately 10^4 CFU/spot on the surface of MHA. After 18-24 h incubation, result was recorded as densities of *B. polyfermenticus* with no visible growth of *S. agalactiae*. The growth of *S. agalactiae* on the MHA surface without *B. polyfermenticus* and the growth of *B. polyfermenticus* in MHA were recorded as controls for the system.

Co-incubation method: The method was modified from the study by Vaseeharan and Ramasamy (2003). A stock solution of *B. polyfermenticus* was adjusted to different densities in NSS, yielding 4×10^5, 4×10^4, 4×10^3 and 4×10^2 CFU/ml. Fifty ml of *B. polyfermenticus*, at each density, was mixed with 50 ml of 4×10^3 CFU/ml streptococcus suspension and incubated at 30°C. The comparative visible growth of

streptococcal bacteria at 0, 2 and 4 h after the incubation was determined to evaluate the inhibitory effect of *B. polyfermenticus* on *S. agalactiae*. The suspension of streptococcal bacteria in NSS (2×10^3 CFU/ml) and *B. polyfermenticus* in NSS (2×10^2, 2×10^3, 2×10^4 and 2×10^5 CFU/ml) was plated onto MHA and observed as controls of the system.

In vivo efficacy of *Bacillus polyfermenticus* on tilapia health performance

Diet preparation: The proximate compositions of commercial basal diet (GROBEST Co., Ltd, Bangkok, Thailand) and concentrations of *B. polyfermenticus* mixed with feed are shown in Table 1. The bacterial suspensions were mixed with commercial basal diet to obtain a final concentration of *B. polyfermenticus* 1×10^4 CFU/g feed (Kim et al., 2009), and air dried for 24 h before application. The diets were freshly prepared for daily administration.

Experimental design in tilapia broodstocks: Animal management was approved by the ethics committee of Chulalongkorn University Animal Care and Use Committee (CU-ACUC; Approval No. 12310086). The experiments were conducted in 3 control groups and 3 treatment groups, with an individual cement pond for each treatment. Water quality was maintained as dissolved oxygen (5.5-6.5 mg/l), pH (7.0-7.5), ammonia ($\leq$0.1 mg/l), and alkalinity (<100 mg/ml). Water temperature was recorded and managed to be within 25-32°C. Each pond (150 m^3) was stocked with two hundred tilapia broodstocks (12 months old, approximately 500 g body weight) in a ratio of 3 females per 1 male brooder. The fish were fed once a day (8.00 am) at a daily rate of 0.5% of body weight (BW).The control groups were fed on commercial basal diet and the treatment groups were fed on supplemented diet containing *B. polyfermenticus* 1×10^4 CFU/g feed. The effect of BP dietary supplement on the health performance of tilapia broodstocks was evaluated from the amount of fertilized eggs yield and survival rates during 5 months of the dietary administration.

Experimental design in tilapia fry: One-day-old feed fry from the hatchery were randomly divided into 3 control and 3 treatment groups, each consisting of 1,000 fry. The fish fry (37.33 ± 1.37 mg) were placed in a flow through system and the stocking density was 1,000 fry/50-L tank. Water was changed daily (25%) and to maintain water quality during the study oxygen (dissolved oxygen ranging from 6.5-7.0 mg/l), water temperature (28-32°C), pH (7.0-7.5), ammonia ($\leq$0.1 mg/l) and alkalinity (<100 mg/ml) were controlled. The tilapia fry were fed approximately 7% of BW/day, in 3 equal rations at 9.00 am, 1.00 pm and 5.00 pm. The feeding experiment was observed in the control groups fed basal diet and the treatment groups fed diet supplemented with *B. polyfermenticus* 1×10^4 CFU/g. Feeding rate was adjusted weekly with the increased body weight. After 28 days of feeding, health performance of the tilapia fry was determined on survival rate, average daily gain (ADG) and feed conversion ratio (FCR). The fry were weighed by batch weighing of each tank and an average BW/fish was used for calculation. The calculations were as the following formulae (Abdelhamid, 2009):

$$\mathrm{ADG} = \frac{(\mathrm{BWt} - \mathrm{BW0})}{t}$$

$$\mathrm{FCR} = \frac{\mathrm{FI}}{(\mathrm{BWt} - \mathrm{BW0})}$$

BW_t is the weight of fish at day t. BW_0 is the initial weight of fish. t is the duration of feeding (in days). FI is total feed intake during t.

Statistical analysis: Weight of fertilized eggs, body weight, ADG and FCR of the treatment groups were compared using 2-sample t-tests. Survival rates were compared using a nonparametric test. Treatment effects were considered significant at P = 0.05 (SPSS statistical software version 17.0; SPSS Inc., Chicago, IL, USA).

Table 1 Composition of basal diet (% dry matter) and *Bacillus polyfermenticus* concentration (CFU/g feed)

Composition	Broodstocks		Fry	
	Control	Treatment	Control	Treatment
Protein (minimum)	25	25	32	32
Fat (minimum)	3	3	3	3
Fiber (maximum)	8	8	8	8
Moisture (maximum)	12	12	12	12
Bacillus polyfermenticus	0	1×10^4	0	1×10^4

Results

The agar dilution test using *S. agalactiae* isolates spotted on the surface of MHA (10^4 CFU/spot) containing different density of *B. polyfermenticus* (10^3-10^5 CFU/ml) showed the inhibitory effect of *B. polyfermenticus* on *S. agalactiae* growth. The addition of *B. polyfermenticus* to MHA inhibited the growth of *S. agalactiae*. The inhibition gradually increased with the density of *B. polyfermenticus* and barely visible growth of *S. agalactiae* was observed at 10^5 CFU/ml of *B. polyfermenticus* (Fig 1). Similar inhibitory results were observed in the co-incubation method. The viable *S. agalactiae* cells were determined at various times using the standard plate counting method. The inhibition of *S. agalactiae* through the presence of *B. polyfermenticus* in the incubation was clearly observed in the systems with higher density of *B. polyfermenticus* compared to that of *S. agalactiae* (2×10^3 CFU/ml) (Figs 2-3).

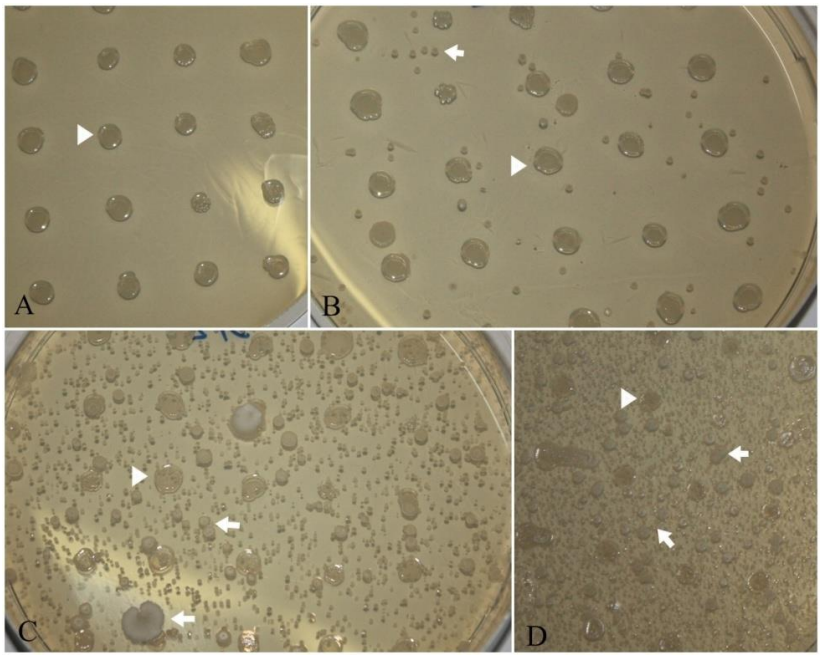

Figure 1 Spots of *Streptococcus agalactiae* (arrow head) growth were visible on MHA after 24 h incubation at 30ºC. The addition of *Bacillus polyfermenticus* (arrows) to MHA inhibited the growth of *S. agalactiae* and the inhibitory effect gradually increased with density of *B. polyfermenticus*. The photographs show test systems with different density of *B. polyfermenticus*; 0 CFU/ml (A), 10^2 CFU/ml (B), 10^3 CFU/ml (C) and 10^4 CFU/ml (D).

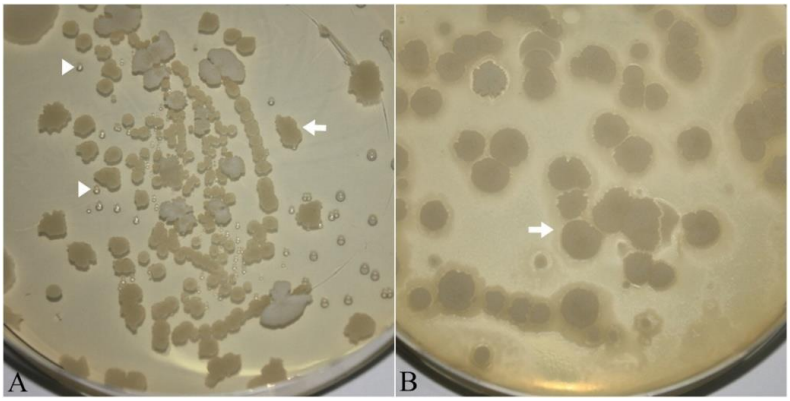

Figure 2 *In vitro* inhibitory effect of *Bacillus polyfermenticus* on *Streptococcus agalactiae* observed in the co-incubation system: *S. agalactiae* (4×10^3 CFU/ml) was incubated with *B. polyfermenticus* (4×10^5 CFU/ml) at 30ºC for 4 h; the mixed culture was then plated onto MHA to compare the visible growth of *S. agalactiae* and *B. polyfermenticus*. Colony forming of *S. agalactiae* noticeable as a white pin-point colony (arrow head) at the initial culture (A) was suppressed by the growth of *B. polyfermenticus* (arrows) following 4 h co-incubation (B).

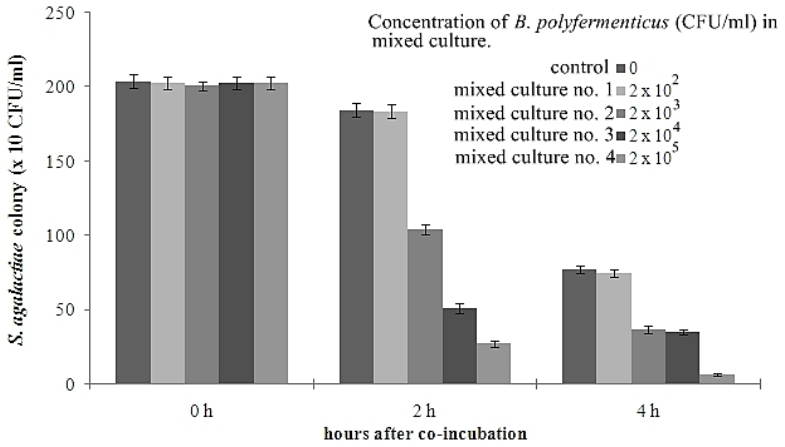

Figure 3 Inhibitory potency of *Bacillus polyfermenticus* against *Streptococcus agalactiae* was evaluated using co-incubation method. The comparative visible growth of *S. agalactiae* was determined by colony count during co-incubation.

In this study we further investigated the efficacy of *B. polyfermenticus* dietary administration on health promotion in tilapia broodstocks (12 months old) and tilapia fry (37.33 ± 1.37 mg body weight) fed on BP supplementation (10^4 CFU/g feed). The tilapia breeders were recorded for survival rate and fertilized egg yield during 5 months of the feeding treatment. The dietary administration of *B. polyfermenticus* could improve the reproductive performance of broodstocks determined by an average yield of fertilized eggs from the female breeders. The amount of fertilized eggs obtained from the treated groups (569.33 ± 17.93 g/month/150 females) was significantly higher than the yield from the control broodstocks fed basal diet (501.67 ± 24.69 g/month/150 females). The quality of the fertilized eggs determined by the hatching rate was found to be normal in both groups (approximately 70%). An improved survival rate was also observed in the broodstocks fed on the supplemented diet (98.83 ± 0.29%), although it is not significant compared to that of the control groups (98.33 ± 0.58%) (Table 2). The study in tilapia fry demonstrated the efficient ADG and FCR with BP dietary supplementation. After 28 days of feeding, significant decrease in FCR, with increase in ADG was observed in the treatment groups. A significant higher survival rate of tilapia fry was found in the fish fed supplemented diet (93.27 ± 1.99%) compared with the fish fed the control diet (86.43 ± 2.10%) (Table 3).

Table 2 Production of fertilized eggs and survival rate of broodstocks after 5 months of BP dietary supplementation

Experimental group	Weight of fertilized eggs (g/month/150 females)	Survival rate (%)
Treatment	569.33 ± 17.93[a]	98.83 ± 0.29
Control	501.67 ± 24.69[b]	98.33 ± 0.58

Different superscript letters are significantly different ($p< 0.05$).

Table 3 Body weight, ADG, FCR and survival rate of tilapia fry after 28 days of BP dietary supplementation

Experimental group	Average BW (mg/fish)		ADG (mg/day/ fish)	FCR	Survival rate (%)
	D0	D28			
Treatment	37.03 ± 2.00	175.49 ± 3.25[a]	4.94 ± 0.09 [a]	1.37 ± 0.03 [a]	93.27 ± 1.99 [a]
Control	37.63 ± 0.64	163.67 ± 6.03[b]	4.50 ± 0.20 [b]	1.51 ± 0.07 [b]	86.43 ± 2.10 [b]

Different superscript letters in columns are significantly different ($p< 0.05$).

Discussion

Bacillus polyfermenticus is a commercial probiotic which has been widely used for the treatment of intestinal disorders in human (Lee et al., 2007; Ma et al., 2010; Jung et al., 2012). The intake of *B. polyfermenticus* as a nutritional supplement is known to provide beneficial effects in human gastrointestinal tract (Cutting, 2011). In addition to the ability to colonize and establish in the intestinal mucosa, *B. polyfermenticus* was reported to produce an antimicrobial agent, bacteriocin (Lee et al., 2001; Newaj-Fyzul et al., 2014). *B. polyfermenticus* is formulated as health promoting-feed additive for aquaculture applications in the United States (PatentedUS20100087516A1 – USPTO) and Europe (PatentedEP 2 433 631 A2 – EPC). *B. polyfermenticus* has recently been considered for use in finfish as probiotic feed additives (Kim et al., 2009). In this study, *B. polyfermenticus* demonstrated inhibitory potency against *S. agalactiae* in both test methods, the agar dilution and the co-incubation method.

In recent years, the administration for antibiotics to treat streptococcosis in farmed tilapia is very constricted and becomes an emotion issue for consumers (Welker and Lim, 2011). Consequently, the use of probiotics to enhance immunity and control infections, notably against bacterial pathogens, is an extensive interest in fish culture (Welker and Lim, 2011; Cruz et al., 2012; Tuan et al., 2013; Newaj-Fyzul et al., 2014). A wide range of gram-negative and gram-positive bacteria probiotics have been considered for use in aquaculture; the genus *Bacillus* are broadly used in fish aquaculture (Newaj-Fyzul et al., 2014). The ability to antagonize pathogens of probiotic bacteria includes the production of antimicrobial substances, bacteriocins. Many bacteriocins in the genus *Bacillus* have been characterized and their activity against gram-positive and gram-negative bacteria, yeast and molds was reported both in *in vitro* and *in vivo* studies. Many *in vitro* studies showed that *Bacillus* probiotic could inhibit pathogenic bacteria of aquatic animals. *B. subtilis* was reported to suppress growth of gram-negative and gram- positive pathogenic bacteria; *A. hydrophila* KJ459001 (CAHHI), *P. aeruginosa* ATCC 35072, *E. tarda* JX280148 (CETMTI), *V. parahaemolyticus* JF966211 (CPVP7), *F. columnare* KF051085 (CFCCO41) and *Staphylococcus aureus* (ATCC6538) (Das et al., 2014). *B. pulmilus* and *B. clausii* were described as inhibitory to Vibrios from grouper *Epinephelus coioides* (Sun et al., 2009). Antagonistic *Bacillus* spp. was shown to inhibit *V. harveyi* from Indian carp *Catla catla* (Mohideen et al., 2010), *E. tarda, S. iniae, Y. ruckeri* and *F. columnare* in channel catfish *Ictalurus punctatus* (Ran et al., 2012). The inhibitory potency of *B. polyfermenticus* against aquatic animal pathogens was reported in a few studies. Kim et al. (2009) reported the antagonistic activity of *B. polyfermenticus* against numbers of finfish bacterial pathogens, *S. iniae* ATCC29178, *S. parauberis* DSM6631, *V. harveyi* ATCC14126, *V. ordalii* KCCM41669, *V. vulnificus* ATCC27562, *Flexibacter tractuosus* KCTC2670, *Edwardesiella tarda* ATCC15947 and *Lactococcus graviae* KCCM40698.

The growth inhibition ability of probiotic against target pathogens *in vitro* is often a criterion for

the choice of a potential probiotic although this may not absolutely correlate with *in vivo* efficiency (Verschuere et al., 2000). Different inhibition test methods were employed to determine the growth inhibition ability of probiotic strains. Given the fact that effective inhibition depends on the antimicrobial substances produced by probiotics, initial probiotic density and sufficient time to produce the antimicrobial substances before the addition of target bacteria is critical (Vaseeharan and Ramasamy, 2003).

With the results in this study it was shown that both methods could be used for determining the antagonist potency. The inhibitory activity was clearly obtained when the probiotic density was higher than that of streptococcal bacteria and the degree of inhibition increases with the density of the antagonist (Figs 1-3). The antagonist effect was reported in the co-culture method whereby the probiotics (*Pseudomonas* spp. and *Bacillus* spp.) and *Vibrio* spp. were applied at the initial cell density of 10^3 CFU/ml (Hai et al., 2007). However, some studies suggested that higher density of probiotics was required to exhibit the inhibitory effect. The initial density of *B. subtilis* at 10^5 CFU/ml was found to inhibit *V. harveyi* (10^3 CFU/ml) (Vaseeharan and Ramasamy, 2003) and *Bacillus* spp. at 10^8 CFU/ml, and was reported to inhibit *V. alginolyticus* and *V. parahaemolyticus* at 10^8 CFU/ml using co-culture method (Banerjee et al., 2007).

It was recommended that the initial probiotic density must be higher than that of the pathogen to accurately determine the effectiveness of the probiotic against that bacterium (Vaseeharan and Ramasamy, 2003). It may be assumed that the probiotic must be present at significant density such that antimicrobial compounds are produced at a concentration to inhibit pathogenic bacteria. However, appropriate density of probiotics is necessary to avoid dominance of probiotic strains that may occur with an overload of probiotic, despite the antimicrobial compounds not producing at an adequate inhibitory level (Hai et al., 2007).

Between the two methods used in the current study, the co-incubation method was more suitable for determining the degree of inhibition compared to the agar dilution method. The co-incubation method uses a known cell density of the probiotic and the tested bacterial pathogen, and the inhibition can be assessed at different incubation times by total bacterial count (Fig 3). The method can be used to compare the inhibitory potency of different probiotics against particular pathogenic bacteria. Thus, it may provide appropriate density of probiotic for further application *in vivo*. It is also important to note that, this method is only suitable for testing specific probiotics against specific bacteria and an accurate cell count is necessary for evaluating effective inhibition.

Bacillus probiotic feeding supplementation is known to support growth in many cultured fish species, both fresh water and marine species. Health benefits of dietary *Bacillus* probiotic were reported in numerous publications. Dietary supplementation of *B. pulmilus* (10^8 CFU/g feed) or *B. clausii* (10^8 CFU/g feed) for 60 days was reported to improve FCR and immune responses (phagocytic activity, serum lysozyme activity, serum complement C3 activity and serum IgM level) of grouper (Sun et al., 2010). *B. subtilis* supplementation in diet at 1×10^7 CFU/g feed for 5 weeks increased weight gain and improved FCR of koi carp *Cyprinus carpio* (He et al., 2011). Dietary administration of *B. subtilis* at 1.5×10^7 CFU/g feed for 2 weeks enhanced resistance to *A. hydrophila* infection in Indian major carp *Labeo rohita* (Kumar et al., 2006), while the supplementation of *B. subtilis* at 8×10^7 CFU/g feed for 2 weeks enhanced resistance to *E. ictaluri* in striped catfish *Pangasianodon hypophthalmus* and channel catfish (Ran et al., 2012).

Dietary administration of probiotics allows probiotics to colonize in the intestinal tract. The colonization of *Bacillus* spp. in fish gut introduces intestinal microflora balance that benefits feed utilization through their production of digestive enzymes and essential nutrients (Newaj-Fyzul et al., 2014). Mechanism of microbiota induction by *Bacillus* probiotics includes competitive exclusion whereby the probiotic establishes in the digestive tract and interferes with the invasion of pathogens by direct competition of space and nutrient and the production of inhibitory substances. Our data indicated that the health benefit of BP dietary supplement in tilapia broodstocks was shown by the better yield of fertilized eggs and survival rate (Table 2). Likewise, the tilapia fry utilized dietary nutrient more effectively and the higher survival rate was observed when feeding was supplemented with *B. polyfermenticus* (10^4 CFU/g feed) for 28 days (Table 3). Although the effect of BP dietary in the resistance to streptococcal infection was not examined *in vivo,* the inhibitory potency to *S. agalactiae* demonstrated *in vitro* may suggest that an appropriate proportion of *B. polyfermenticus* in the gut could prevent the adhesion of pathogenic streptococcal bacteria. While the streptococcal bacteria are opportunistic pathogens that can seriously harm tilapia culture under stress conditions and the disease transmission is predominantly through an oral-fecal route (Bromage and Owens, 2002; Pasnik et al., 2009; Jantrakajorn et al., 2014), the competitive exclusion mechanism occurring with *Bacillus* colonization could provide resistance against streptococcal infection in tilapia fry. In this respect, it is determined that the absence of antigenic stimuli from the opportunistic streptococcal pathogens results in better absorption and utilization of the nutrients, which in turn contributes to the increased growth and survival of tilapia fry.

Although there has been considerable success with the use of *Bacillus* probiotics as feed additives for fish culture, specific microbial species that are suitable for dietary supplement in tilapia have not yet been determined (Welker and Lim, 2011). Our study considered the use of *B. polyfermenticus* probiotic because the anti-pathogen efficacy and safety of BP dietary supplementation were commonly approved in human health (Cutting, 2011). The study presents the benefits with the use of *B. polyfermenticus* as feed additives for tilapia culture. The better growth and health performance observed in both tilapia fry and brooders may reflect the action of *B. polyfermenticus* on improving feed utilization and infection control which may impact positively on the health of animal, and consequently grant an efficient growth, survival and production yield.

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บทคัดย่อ

ประสิทธิภาพของโปรไบโอติก บาซิลัส โพลีเฟอเมนติคัสในการยับยั้งการเจริญของเชื้อแบคทีเรียสเตรปโตคอคคัสและผลเสริมสุขภาพปลานิล (*Oreochromis niloticus*)

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ทดสอบเชื้อบาซิลัส โพลีเฟอเมนติคัสในการยับยั้งการเจริญของเชื้อสเตรปโตคอคคัสจากปลานิลป่วยด้วยวิธี agar dilution และวิธี co-incubation พบว่าทั้งสองวิธีทดสอบให้ผลสอดคล้องกัน คือ เชื้อบาซิลัส โพลีเฟอเมนติคัสสามารถยับยั้งการเจริญของเชื้อสเตรปโตคอคคัสอกาแลคติเอ้ และผลการยับยั้งมีประสิทธิภาพมากขึ้นตามปริมาณของเชื้อบาซิลัส โพลีเฟอเมนติคัส ทดลองนำเชื้อบาซิลัส โพลีเฟอเมนติคัสผสมอาหารให้ปลานิลพ่อแม่พันธุ์ (อายุ 12 เดือน) และลูกปลานิล (น้ำหนัก 37.33 ± 1.37 มิลลิกรัมต่อตัว) พบว่าปลาแม่พันธุ์ที่กินอาหารผสมเชื้อบาซิลัส โพลีเฟอเมนติคัสเป็นเวลา 5 เดือนให้ไข่ที่ได้รับการผสมมากขึ้นอย่างมีนัยสำคัญเมื่อเทียบกับกลุ่มควบคุม ($p < 0.05$) อย่างไรก็ตาม อัตรารอดของพ่อแม่พันธุ์ในกลุ่มทดลองและกลุ่มควบคุมไม่มีความแตกต่างกัน นอกจากนี้ยังพบว่าลูกปลานิลที่เลี้ยงด้วยอาหารเสริมบาซิลัส โพลีเฟอเมนติคัสเป็นเวลา 28 วันมีอัตราการเปลี่ยนอาหารเป็นเนื้อ การเพิ่มของน้ำหนักตัวเฉลี่ยต่อวัน และอัตรารอดชีวิตสูงกว่ากลุ่มควบคุมอย่างมีนัยสำคัญ การศึกษานี้แสดงประสิทธิภาพของเชื้อบาซิลัส โพลีเฟอเมนติคัสในการยับยั้งการเจริญของเชื้อสเตรปโตคอคคัสและประสิทธิผลในการเป็นสารเสริมสุขภาพผสมอาหารสำหรับปลานิล

คำสำคัญ: บาซิลัส โพลีเฟอเมนติคัส สเตรปโตคอคโคซิส ปลานิล

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