Screening and Isolation of Bacteria from Thua-nao for Improving Nutritive Values of Fermented Soybean Meal in Animal Feed

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Abstract

Soybean meal (SBM) is the most widely used protein source in the animal feed industry. However, a variety of antinutritional factors, such as allergenic proteins and non-starch polysaccharides (NSPs), have limited the application of soybean meal in animal feed. Microbial fermentation of SBM is expected to increase nutritional values and also eliminate the antinutritional factors. The aim of this research was to screen and isolate bacteria with high protein- and NSPs degrading enzymes from Thua-nao, traditional fermented soybean food in northern Thailand for improving nutritive values of SBM. A total of 28 isolates showed high proteolytic activity which was indicated by clear zone formation on skim milk agar plate. Among these isolates, only 5 isolates namely, S1/1A, S1/3A, S5/1C, S5/2C and S6/1D exhibited high NSPs degrading enzyme activities (cellulase and mannanase). These 5 isolates were identified to be a strain of Bacillus, according to their morphological characteristics.

Keywords: animal feed, non-starch polysaccharide, protease producing bacteria, Thua-nao, soybean meal

Introduction

Soybean meal (SBM), the by-product after oil extraction of soybeans has become increasingly important as a feed component and is used in variable amounts in the feeding of all species in animal production to replace fishmeal and skim milk in the diets (Pettersson and Pontoppidan, 2013; Wongputtisin et al., 2012). Its high...
contents of crude protein, excellent amino acid profile, carbohydrates and isoflavones are just some of the benefits of using soybeans. Unfortunately, SBM contains several anti-nutritional factors such as large proteins, allergenic proteins and indigestible non-starch polysaccharides (NSPs), i.e. cellulose and mannan that resist to digestive enzymes, decrease digestibility and depress growth performance in animal (Pettersson and Pontoppidan, 2013; Wongputtisin et al., 2012; 2014). Microbial fermentation of SBM is interesting way for reducing these undesirable factors. Using microorganism with high protease and NSPs degrading enzymes activities can improve the nutritional value of SBM through the reduction of large allergenic proteins, and NSPs. Therefore, isolation of new food-derived bacteria that are capable of effective SBM fermentation will be beneficial to the animal production industry. This study aimed to screen and isolate for a suitable fermentative bacteria with high protease and NSPs degrading enzymes activities from Thua-nao, traditional fermented soybean food in northern font of Thailand. Thua-nao, fermented by many Bacillus spp. has been reported on nutritional enhancement of fermented soybean food for human consumption (Kiers et al., 2000; Wongputtisin et al., 2007; 2012). Therefore, fermentation of SBM using effective Bacillus spp. isolated from Thua-nao is very interesting as an alternative fermentative microorganism for improving nutritive value of SBM.

**Materials and Methods**

Thua-nao samples were collected from different local markets in northern font of Thailand. Bacteria were firstly screened and isolated for protease activity using a serial dilution method. Samples were inoculated on soybean meal agar plates that supplemented with skim milk (2%) for screening of protease activity, and then incubated at 37°C for 24 h. Colonies that were able to grow on this media and were able to demonstrate a clear zone around its colony were selected for the second round of screening. The colonies were transferred and isolated more than seven times to insure the purity and protein degrading ability of isolates. Thereafter, isolated protease producing bacteria were transferred to soybean meal agar plate that supplemented with each of 1% (w/v) locust bean gum (LBG) and carboxymethyl cellulose (CMC) separately, for screening of mannanase and cellulase activities, respectively, and incubated at 37°C for 24 h and subsequently developed by congo red staining and 1 M NaCl washing. The formed clear zone represented the enzyme activity. To select new bacteria producing protease and non-starch polysaccharides degrading enzymes activities, pure isolated strains from Thau-nao samples were compared to Bacillus subtilis TISTR 001. B. subtilis TISTR 001 purchased from Thailand Institute of Scientific and Technological Research (TISTR) was used as a positive control due to its previous observation for fermentation of soybean and mungbean. The potent protease and NSPs degrading enzymes producing bacteria have been identified based on morphological and physiological characteristics.

**Results and Discussion**

Thau-nao samples were collected from different local markets in northern font of Thailand for screening and isolation of protease and NSPs degrading enzymes producing bacteria for improving nutritive values of fermented soybean meal. Screening of protease producing bacteria was performed using soybean meal agar plates that supplemented with skim milk. Clear zone forming colonies were sub-cultured for the purification of the isolates. Clear zones were formed because of the hydrolysis of casein by protease produced from the isolates. Repeated subcultures were performed up to uniform colonies were found. Finally, the bigger clear zone forming 28 isolates were selected. The radius of each clearance zones was measured and shown in Figure 1. The wider clear zone might be assumed as higher activity production. Therefore, there were 9 isolates, i.e. S1/1, S3/1, S3/2, S5/1, S5/2, S6/1, S6/3, S9/1 and S9/7 that showed satisfactory clear zone diameter which were higher than that of B. subtilis TISTR 001, the reference strain. Thereafter, 28 isolated
Protease producing bacteria were transferred to soybean meal agar plate that supplemented with each of LBG and CMC separately, for screening of mannanase and cellulase activities, respectively. For mannanase screening, all of the isolates tested showed clear zone forming on LBG plate, indicating mannanase activity. Especially, S1/4, S5/1, S5/2, S7/2 and S9/1 demonstrated significantly higher than the reference strain (Figure 2). For cellulase screening, only 5 isolates, S1/1, S1/3, S5/1, S5/2 and S6/1 were able to demonstrate satisfactory clear zone, reflecting high cellulase producing ability while B. subtilis TISTR 001 could not form clear zone on CMC plate (Figure 3). Therefore, only 5 isolates, S1/1, S1/3, S5/1, S5/2 and S6/1 showing high protease, mannanase and cellulase activities, are promising candidates for improving nutritive values of fermented SBM.

The 5 promising candidates were identified based on morphological and physiological characteristics. These isolates are rod shaped and spore-forming bacterium. For Gram stain test, S1/3, S5/1 and S5/2 are Gram-positive while S1/1 and S6/1 are Gram-variable. The optimum temperature for growth of these isolates is 37°C, but S5/1C, S5/2C can also grow at 50°C. The morphological and physiological characteristics of these isolates are coincided with Bacillus. Therefore, these isolates were identified to be a strain of Bacillus.

Summary

Microbial fermentation of SBM is alternative way for eliminating the anti-nutritional factors in animals feed and improving growth performance. This study aims to screen and isolate suitable fermentative bacteria with high protease and NSPs degrading enzymes activities from Thua nao samples for improving nutritive values of fermented SBM. From the results, 5 isolates namely, S1/1, S1/3, S5/1, S5/2 and S6/1 showing high protease, mannanase and cellulase activities, are promising candidates for improving nutritive values of fermented SBM. These 5 isolates were identified to be a strain of Bacillus, according to the morphological characteristics.

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Literature cited

Figure 1: Size of clearance zone formed by action of protease produced by isolated bacteria.

Figure 2: Size of clearance zone formed by action of mannanase produced by isolated bacteria.

Figure 3: Size of clearance zone formed by action of cellulase produced by isolated bacteria.