Journal of Animal and Veterinary Advances 11 (24): 4615-4616, 2012
ISSN: 1680-5593

Influence of Wheat Bran Particle Size for Spore Production from *Bacillus coagulans* by Solid State Fermentation

[1, 2]Liu Yang, [1]Hua Zheng, [1]Yi-Fan Zhang, [2]Fei-Yun Yang, [1]Jin-Long Yang and [1]Li-Zhi Fu
[1]Chongqing Academy of Animal Sciences,
[2]Key Laboratory of Pig Industry Sciences, Ministry of Agriculture,
Rongchang, 402460 Chongqing, China

Abstract: Wheat bran was selected as the substrate from agricultural byproducts for spore production of *Bacillus coagulans* in Solid State Fermentation (SSF). Its particle size has an obvious influence on spore production in SSF conducted by incubation 60% moisture at 37°C for 48 h. The maximum yield (2.85×10^{10} cfu g^{-1}) was achieved using the wheat bran particle passed through a 20 mesh sieves without any additional nutrient source to be supplemented.

Key words: *Bacillus coagulans*, wheat bran, substrate, particle size, solid state fermentation

INTRODUCTION

Bacillus coagulans, a Gram-positive spore-forming bacterium is one of the most widely used biological agents in feed additives (http://www.aafco.org/). Its bacterial spore has strong resistant to harsh environments and can be stored for a long time (Hong *et al*., 2005). As one of Bacillus probiotic products, *B. coagulans* is often produced using submerged fermentation. While in recent years, SSF has gained new attention for the researchers especially in developing countries due to that this way not only requires inexpensive equipment, uses cheap agricultural byproduct but also does not produce any waste (Adams *et al*., 2002; El-Bendary, 2006). The present investigation compared three agricultural byproduct wheat bran, rice bran and rice straw powder commonly available in western China as nutrient sources for spore production of *B. coagulans*. The main objective of this study was to determine which agricultural byproduct was the most suitable substrate to produce spores and explore the influence of the selected substrate particle size for spore production from *B. coagulans* using SSF.

MATERIALS AND METHODS

Microorganisms: *B. coagulans* CQ1, isolated from excreta of pigs in Chongqing China was used in the present investigation.

Substrate: Agriculture byproduct (wheat bran, rice bran and rice straw powder) bought from the local market was used as substrate for Solid State Fermentation (SSF). The most suitable substrate was selected to be processed passed through 10, 20, 30, 40, 50, 60 standard mesh sieves to obtain various particle size powder forms and stored in polyethylene bag before use.

Solid state fermentation: About 20 g of substrate was taken in 250 mL conical flasks and was mixed with water to reach the required relative moisture contents and autoclaved at 121°C 30 min at 1 kgf/cm^2. After cooling the flasks to room temperature, the treated substrate was inoculated with 2 mL of *B. coagulans* 24 h grown culture broth under sterile conditions. Flasks were placed at 37°C for 48 h.

Enumeration of spores: Spores were enumerated as described by Zhao *et al*. (2008).

RESULTS AND DISCUSSION

Evaluation of different agricultural byproducts material for spore production: Table 1 indicated the effect of three types substrates on spore production of *B. coagulans*, the statistical values showed that spore production varied obviously with the difference of the substrate types

Table 1: Effect of different substrates on spore production by *B. coagulans* in SSF

Substrate	Spores ($\times10^9$ cfu g^{-1})
Wheat bran	2.33±0.25
Rice straw powder	0.21±0.05
Rice bran	0.13±0.06

SSF was conducted by incubation at 37°C for 48 h. The medium was the substrates without any nutrition source to be supplemented. Spores values are the average values of three determinations with standard deviation

Corresponding Author: Li-Zhi Fu, Chongqing Academy of Animal Sciences, Rongchang, 402460 Chongqing, China

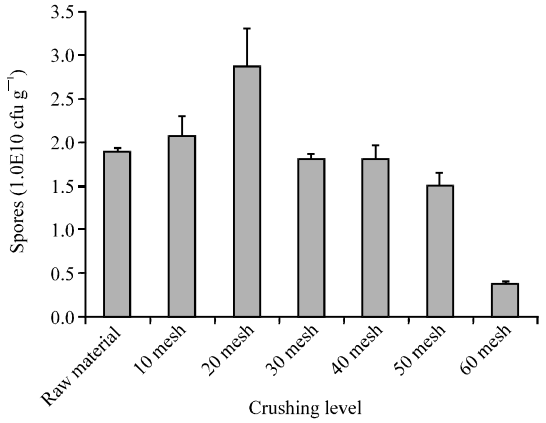

Fig. 1: Effect of different particle size wheat bran as substrate on spore production by *B. coagulans* in SSF conducted by incubation 60% moisture at 37°C for 48 h

wheat bran has the maximum of 2.33×10^9 spores g^{-1} dry substrate. Being solid substrate usually has dual roles to the microbial culture growing under SSF process that is solid substrate provided with not only the nutrients but also the anchorage sites for the growing cell (Prakasham *et al*., 2006). The results showed that wheat bran was a better substrate/anchorage material comparing to the other two substrates. Considering that the nutrients including carbon source, nitrogen source, basal salts (i.e., Mg, Na and Ca) and good aeration are the primary requirements for bacterial growth, the data revealed wheat bran is not only a good nutrient source but also provides the consistency suitable for providing sufficient air supply to support *B. coagulans* growth and that *B. coagulans* easily breakdown wheat bran to unitizing the carbon and nitrogen sources exist in the wheat bran for its growth. Finally, according to the cost and availability of the substrate materials, researchers selected wheat bran as an ideal substrate material for spore production of *B. coagulans*.

Influence of wheat bran particle size: Figure 1 displayed the influence of wheat bran particle size on spore production of *B. coagulans* using SSF conducted by incubation 60% moisture at 37°C for 48 h. The results showed spore production is closely related to substrate particle size. It can be observed that initially the spore production yields increased by increasing particle fineness up to wheat bran passing through a 20 mesh sieve but decreased thereafter. Fermentation of solid substrate passed through 20 mesh sieve has the maximum spore production (2.85×10^{10} cfu g^{-1}). These observations maybe reflect the fact that this particle size wheat bran material is the optimal for *B. coagulans* growth to provide with intra-particulate associated aeration, available surface area for microbial attachment and substrate mass transfer and subsequent spore production.

CONCLUSION

Spore production of *B. coagulans* using SSF was influenced by physiological and chemical nature of the agriculture byproduct. The growth of *B. coagulans* on wheat bran medium was the most efficacious in terms of spore yield when compare to that on the mediums of rice bran and rice straw powder using SSF. Particle size of wheat bran was observed to have important influence to achieve maximum spore production yields and enhanced spore production yields of *B. coagulans* could be gained by selecting the particle size wheat bran passed through a 20 mesh sieve.

ACKNOWLEDGEMENTS

This research was supported by the National Key Technology Research and Development Program of the Ministry of Science and Technology of China (Grant No. 2011BAD26B01); Fund for Chongqing Agriculture Development (11409); Science and Technology Development Program of Chongqing (CSTC 2011AB1078) and Chongqing Key Programs for Science and Technology Development (Application technology research and development, cstc2012gg-yyjsB80005).

REFERENCES

Adams, T.T., M.A. Eiteman and B.M. Hanel, 2002. Solid state fermentation of broiler litter for production of biocontrol agents. Bioresour. Technol., 82: 33-41.

El-Bendary, M.A., 2006. *Bacillus thuringiensis* and *Bacillus sphaericus* biopesticides production. J. Basic Microbiol., 46: 158-170.

Hong, H.A., L.H. Duc and S.M. Cutting, 2005. The use of bacterial spore formers as probiotics. FEMS Microbiol. Rev., 29: 813-835.

Prakasham, R.S., C.H. Subba Rao and P.N. Sarma, 2006. Green gram husk: An inexpensive substrate for alkaline protease production by *Bacillus* sp. in solid-state fermentation. Bioresour. Technol., 97: 1449-1454.

Zhao, S., N. Hu, J. Huang, Y. Liang and B. Zhao, 2008. High-yield spore production from *Bacillus licheniform* by solid state fermentation. Biotechnol. Lett., 30: 295-297.