Investigation on the suitable isolate and medium for production of *Bacillus thuringiensis*

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ABSTRACT

Bacillus thuringiensis bioinsecticide has been widely used on crops worldwide to replace chemical pesticides. *B. thuringiensis* production by solid-state fermentation requires less capital investment and modest technical skills. The method is often considered unsuitable for growth of aerobic organisms. However, optimization of *Bt* production using solid-state fermentation can effectively contribute to promote use of this bacterium in insect pest management programs. Research into suitable nutrient concentrations of different media and characteristics of bacterial growth on these has enabled use of several agricultural or industrial by-products for mass production of several *Bt* strains. These materials include wheat bran, rice bran, rice crumb, and remaining barley from feeding of *Sitotroga serealella*. Wheat bran was the best of media for production of *B. thuringiensis*.

Key words: *Bacillus thuringiensis*, media, semi-solid production, wheat bran

INTRODUCTION

About 3 million tons of chemical pesticides which cost 20 billion dollars are yearly sold all over the world. Only half of milliard dollars of pesticides' trade belongs to biopesticides and more than 60% of this trade is related to the commercial products of *Bacillus thuringiensis* (*Bt*). More than fifty species of pests, sensitive to *Bt* which belongs to three order of insects namely Lepidoptera, Coleoptera and Diptera have been reported. Now a day, products of *Bt* included biopesticides and transgenic plants are a firm basis in Integrated Pests Management. Dulmage and Rhodes (1971) reminded that *Bt* could be produced in liquid and semi-solid culture media. Ross (1974) discusses that biological products of *Bt* is produced widely in a lot of countries such as America, France, Germany, Czechoslovakia, Yugoslavia etc. and these bioproducts are sold with different brands. Morris *et al.* (1996) studied the effect of medium on the amount of spore and crystal of *Bt*.

Since about 1960, several studies have been done in China to produce and use *Bt* and also different methods have been applied to expand and complete the technology of fermentation and semi industrial production. According to Tianjin (1994) report in China, the agriculture byproducts such as soybean, cotton seed and extracted peanut are used as the basis *Bt* production. Ziwen (1994) also mentions the different stages of fermentation as primary inoculums' reproduction, medium selection and control of fermentation process and recycled it. Most commercial products of *Bt* are produced by liquid fermentation which have some disadvantages such as difficult mass production, high investment, problems of storekeeping and unstable quality. Dulmage (1983) states that although solid fermentation is an easy way for *Bt* production it needs expert staff to avoid the final product contamination. Kelly Cheng (2000) the manager of GCTG Co. a Chinese company produced *Bt* in solid fermentation with potential 12000-40000 (IU/mg). Solid fermentation is easy to produce, involves low investment (only 1/4 of liquid fermentation), easy storekeeping and high toxicity of products, which are the advantages of solid fermentation. In China and Brazil, farmers begin to produce biopesticide (Bernhard and Utz, 1993; Capalbo, 1995; Salama and Morris 1993). Capalbo *et al*. (2001) successfully applied *Bt* tolworthi to control *Caradrina exigua*, using solid fermentation in Brazil. Bactospein is the first microbial insecticide registered in Iran (*Bt* product,

Wp 90%), which was applied on pests of forest trees. Since to be 1970 various experiments have been done on *Bt* for pests control, eg., that of Safar Alizadeh (1976) on *Tortrix viridana*, Daniali *et al*. (1993) and Adldoost *et al*. (1995) on *Helicoverpa armigera*, Askari (1992) on *Ostrinia nubilalis*, Javan Moghaddam *et al*. (1995) on *Lymantria dispar* and Marzban (1997) on *Plodia interpunctella*.

Detection of ß-Exotoxin in commercial products of *Bt* by HPLC and bioassay method and isolation of native strains of *Bt* from Agriculture soils of Iran are other recent researches in Iran (Marzban and Tajbaksh, 2004). Very recently coconut (*Cocos nucifera*) cake powder, neem (*Azadirachta indica*) cake powder, and groundnut (*Arachis hypogea*) cake powder were used for the production of *Bt* in India (Subbiah Poopathi and Archana, 2012). The purpose of this project is determine to suitable isolate and culture media of *B. thuringiensis* based on native strains and applied plans' results in study to develop technical knowledge production of *Bt*.

MATERIALS AND METHODS

In the current study, three native strains and a standard strain (HD1) of *Bacillus thuringiensis* sub species *kurstaki* were used. These strains belong to the bank of Biological Control Research Department. All strains of *Bt* were grown in Nutrient Broth and four solid culture media. Treatments were composed of four media, including: Rice bran, Wheat bran, Rice flour and (Lepidoptera: Gelechiidae). Culture media enrich with mineral salts ($MnSO_4$, $MgSO_4$, $ZnSO_4$, and $FeSO_4$) and PH adjusted to 7.5 and was incubated in Erlen Meyer flask for 96 hrs in shaker-incubator at 30°C. For spore count all the strains were incubated in cultures media, centrifuged at 8000 rpm. The supernatant was discarded and the number of spore in pellets was counted and expressed in Colony Forming Units per gram (CFU/g). Also samples were taken from each solid culture media for the spore count and toxicity tests on *H. armigera* (4 day old larvae).

RESULT AND DISCUSSION

Three of native strains and a standard strain of *Bt* were used for the production on four culture media. These materials included wheat bran, rice bran, rice crumb, and remaining barley from feeding of *S. serealella* (Table 1). Wheat bran was the best of media for production of *Bt*. Whenever four culture media were used for production of standard strain, and finally product was used assayed for toxicity test on *H. armigera*, the result showed that there is significant difference in treatments ($F_{80, 11}$= 11509; $P < 0.000$. The native strains on four cultures media showed that there is significant difference too [KNO3 ($F_{80, 11}$=14120; $P < 0.000$), KN3 ($F_{80, 11}$=14120.5; $P < 0.000$) and KD2 ($F_{80, 11}$=14511.8; $P < 0.000$)]. Therefore, native strain, KNO3 is suitable than other strains, the standard strain and native strain.

Table 1. Production of *Bt* isolates on four different culture media and assayed on *H. armigera*

Strain	Culture media	CFU/g	Growth bacteria status	Mortality
HD1	Barley	4.3×10^{10}	Very good	67.45 ± 2.2^{c}
	Rice bran	3.2×10^{4}	Infirm	11.67 ± 1.7^{a}
	Wheat bran	7.8×10^{10}	Very good	87.39 ± 3.9^{d}
	Rice flour	6.5×10^{7}	Good	51.18 ± 2.7^{b}
KON3	Barley	5.4×10^{10}	Very good	74.63 ± 2.7^{c}
	Rice bran	2.2×10^{3}	Infirm	9.87 ± 1.5^{a}
	Wheat bran	9.1×10^{10}	Very good	92.89 ± 4.1^{d}
	Rice flour	6.9×10^{7}	Good	58.29 ± 2.3^{b}
KN3	Barley	6.3×10^{8}	Good	37.77 ± 2.4^{b}
	Rice bran	2.8×10^{4}	Infirm	10.97 ± 1.6^{a}
	Wheat bran	7.1×10^{8}	Very good	63.81 ± 2.9^{d}
	Rice flour	4.6×10^{5}	Intermediate	39.44 ± 1.8^{b}
KD2	Barley	8.3×10^{7}	Good	57.66 ± 2.1^{c}
	Rice bran	3.1×10^{4}	Infirm	12.33 ± 1.3^{a}
	Wheat bran	6.9×10^{9}	Very good	69.89 ± 2.5^{d}
	Rice flour	1.9×10^{6}	Intermediate	42.73 ± 2.4^{b}

B. thuringeinsis was grown on wheat bran media without enriching it by carbon and nitrogen source. The spore quantity was counted and this product was assayed on *H. armigera*, percent of mortality was 92.89%. According to the results, wheat bran is suitable culture medium as it contains carbon and nitrogen sources and can provide ventilation for optimum growth of *Bt*. In addition to the passages, wheat bran culture media can be used as carrier and adjoints materials in formulation of *Bt* biopesticide.

Wheat bran was used widely for production of pathogenic fungi's insects such as *Beauvaria bassiana* (Hussey and Tinsley, 1981), *Bt* (Subbiah Poopathi and Archana, 2012) and antagonist fungi of pathogenic herbaceous agents such as *Trichoderma* sp. (Jagadeesh and Geeta, 1994). However reports on the number of biopesticide production based on *Bacillus thuringiensis* is low. Wheat bran is brimful of vitamins, fibers, mineral compounds and a few saturated fats. According to study on standard culture media of *Bt*, carbon and nitrogen sources and mineral salt (Mg, Na, Ca), are needed for growth of bacteria. Bacteria can be breaking the present compound in wheat bran to be available to carbon and nitrogen sources.

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