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Use of Brewery waste amended spent malt as substrate for mass production of *Trichoderma*

C. GOPALAKRISHNAN*, B. RAMANUJAM, R. D. PRASAD, N. S. RAO and R. J. RABINDRA
Project Directorate of Biological Control (ICAR)
P. B. No. 2491, H. A. Farm Post, Bellary Road
Bangalore 560 024, Karnataka, India
E-mail. pdblc@pdblc.com

ABSTRACT: **Among four different levels of brewers' yeast tested, 3g / 100 g of spent malt was found to be optimum for the growth of *Trichoderma harzianum* in solid-state fermentation. Spent malt brewers' yeast medium supported all the species namely, *T. harzianum*, *T. viride* and *T. virens*. *T. harzianum* recorded the highest number of viable propagules after twelve days of incubation. All the treatments recorded more number of viable propagules after twelve days of incubation than after seven days of incubation.**

KEY WORDS: Brewers' yeast, solid state, spent malt, *Trichoderma harzianum*

INTRODUCTION

Management of soil borne pathogens by using biocontrol agents is an efficient and ecofriendly approach. *Trichoderma harzianum* Rifai is an effective biocontrol agent of many economically important plant diseases. Mass production of this fungus has become an important aspect of research in an effort to find alternatives to harmful fungicides. Any media used for mass production of *Trichoderma* species should be economical, easily available and be able to support production of large quantities of biomass and viable propagules. Solid fermentation is a very common method of mass production of *Trichoderma* spp. in laboratory experimentations. Various cheap, agriculture wastes and byproducts were tried successfully for the mass production of antagonistic fungi by various workers. The common substrates which were utilized for solid state fermentation of *Trichoderma* were grain bran (Wells *et al.*, 1972), coconut water amended coir pith (Kumar *et al.*, 2000), shelled maize cob powder and black gram shell powder (Gandhikumar *et al.*, 2001), tea waste (Prakash *et al.*, 1999) and oven dried and powdered orange peel (Godwin-Egein and Arinze, 2000). Sawant and Sawant (1996) reported that coffee waste is the best medium for the growth of *Trichoderma*. In the present study, an attempt was made to utilize the spent malt as carbon source and brewers' yeast as nitrogen source (cheap brewery wastes) substrates for solid-state fermentation of *T. harzianum*. Three different species of *Trichoderma, viz., T. harzianum, T. viride* and *T. virens* have also been tried to find out the efficacy of different *Trichoderma* species to utilize the spent malt as a carbon source.

* Agricultural Research Station, TNAU, Bhavanisagar, 638451, Erode District, Tamil Nadu

MATERIALS AND METHODS

Organism and Media

Trichoderma harzianum (PDBCTH 10), *T. viride*, and *T. virens* were obtained from culture collection of Project Directorate of Biological Control, Bangalore. An attempt was made in this study to evaluate brewery waste, which is available in plenty in Bangalore, as substrate for mass production (solid state) of *Trichoderma*. Barley grains are used as raw material in beer industry. Spent malt is barley grains obtained after distillation as a brewery waste, which is not only available in plenty in and around Bangalore, but also disposal of these wastes involves huge costs. Brewers' yeast tried in this study was previously used for fermentation of barley grains and obtained from the wastes after brewing. It is used in distilling industry to brew the barley grains. The spent malt collected from these units was shade dried and made into fine pieces using mixie and stored in airtight polythene bags for further studies. In the first experiment, *T. harzianum* was tested in spent malt- brewers' yeast medium. Since spent malt alone did not support good growth and spread of the antagonistic fungi, brewers' yeast, which was in active dry form, was tried at four different levels along with spent malt. The medium with 100g of spent malt and brewers' yeast at four levels, namely, 2,3,4 and 5g were tried to find out the optimum level of brewers' yeast for amending spent malt for the growth of antagonistic fungi. In the second experiment, three different species of *Trichoderma, viz., T. harzianum, T. viride* and *T. virens* were tested for their growth in spent malt medium, which contains 3 per cent of brewers' yeast. The moisture level of spent malt was maintained at 52 per cent by adding sterile distilled water.

Inoculation and Growth conditions

Since spent malt alone did not support the growth of *Trichoderma*, the brewers' yeast was added to assess the growth promoting ability. Autoclavable polypropylene bags (HIMEDIA) of size 12" X 10" were filled with 100 g spent malt and different levels of brewers' yeast. The bags were sealed air tightly using flames. A small hole was made at one corner of the bags for inoculation of fungal discs and was plugged with cotton. Bags were sterilized in an autoclave at 1 kg/m^2 for 30 minutes. The fungi were grown in Petri-plates containing PDA for seven days. Fungal discs from these plates (5mm) were inoculated in sterilized polypropylene bags containing 100g spent malt–brewers' yeast medium. After inoculation, all the cultures were incubated at 25°C for 12 days. The medium was mixed well aseptically using a sterile glass rod once in three days to enable uniform growth and spread of the fungi.

Harvesting and Assay

The presence of conidia and chlamydospores was assessed by microscopic observation of 10 samples taken randomly from each *Trichoderma* species. All the cultures were homogenized by blending in a mixie for one minute after incubation. One gram from each treatment was taken for estimation of colony forming units (cfus). The cfus were determined by plating serial dilutions of the homogenized suspensions on *Trichoderma* specific medium described by Elad and Chet, 1983. The cfu counts were done on seventh and twelfth days of incubation.

RESULTS AND DISCUSSION

Among the four different levels of brewers' yeast tested for replenishing spent malt medium, 5g/100g of spent malt recorded the highest cfu of 86.4 x 10^4 after 7 days of incubation. However, after 12 days of incubation 3, 4 and 5g were statistically on par, which were significantly superior to 2g of brewers' yeast treatment. Brewers' yeast at 2 per cent supported lesser cfu of 56.9 x 10^6 after 12 days, while other treatments recorded about 90 x 10^6 viable propagules. It may be concluded that brewers' yeast at 3g/100g spent malt is the optimum combination for the growth of *T. harzianum* in solid-state fermentation (Table 1). Previous report suggests (Prasad and Rangeshwaran, 2000) that additon of more nitrogen source beyond optimum level has not yielded corresponding increased cfu in liquid fermentation. The same trend was noticed in solid fermentation.

Table1. Effect of different levels of brewers' yeast on the growth of *T. harzianum* on spent malt

Medium	(cfu x 10^4) after 7 days	(cfu x 10^6) after 12 days
Brewers' yeast (2%)	48.40	56.90
Brewers' yeast (3%)	81.60	91.20
Brewers' yeast (4%)	85.70	90.50
Brewers' yeast (5%)	86.40	91.00
CD (P=0.05)	4.13	13.48

Table 2. Effect of spent malt on the growth of different *Trichoderma* spp. (solid state)

Trichoderma spp.	After 7 days (cfu x 10^4)	After12 days (cfu x 10^6)	Production of conidia	Production of chlamydospores
T. harzianum	84.2	88.1	+++	+
T. virens	104.7	75.8	++	-
T. viride	116.6	58.5	++	-
CD (P=0.05)	12.13	10.46		

- = less + = moderate ++ = high +++ = very high

Among the three different species of *Trichoderma* tested, *T. viride* recorded maximum number of viable propagules (116.6 x 10^4 cfu) at 7 days of incubation followed by *T. virens* recording 104.7 x 10^4 cfu (Table 2). However, after 12 days of incubation, *T. harzianum* produced the highest viable propagules (88.1 x 10^6) followed by *T. virens* (75.8 x 10^6). The present study indicates that 7 days of incubation has recorded lesser viable propagules compared to 12 days of incubation.

All the three species of *Trichoderma* produced more conidia and chlamydospores were produced only by *T. harzianum*. Since the costs of brewery wastes are very cheap (Re. 1 per kg of spent malt and Rs.30 per kg of brewers' yeast), the cost of production of *Trichoderma* on these substrates is very less. Commercial firms involved in mass production of *Trichoderma* can utilize these brewery wastes for large scale production. As these substrates are available in plenty with brewery industry, utilization of these wastes for mass production of *Trichoderma* will not only helps in proper disposal but also useful in plant disease control programme. However, the effect of residual nutrients from this substrate on pathogens has to be studied before going for field application.

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