Research Article
CODEN (USA): IJPLCP

[Babu & Pallavi, 4(1): Jan., 2013]
ISSN: 0976-7126

INTERNATIONAL JOURNAL OF PHARMACY & LIFE SCIENCES

Isolation, identification and mass multiplication of Trichoderma- an important bio-control agent

K. Nagur Babu* and P.N. Pallavi
Department of Biotechnology, S.V. University, Tirupati, (A.P.) - India

Abstract

Recently, the environmental contamination caused by excessive use of chemical pesticides increased the interests in integrated pest management, where chemical pesticides are substituted by bio-pesticides to control plant pests and plant diseases. *Trichoderma candidum* is a potential fungal bio-control agent against a range of plant pathogens. The major issue involved in mass production and utilization of biocontrol agent are selection of effective strains, development cost effective methods, for mass multiplication, effective methods for storage, shipment and its formulation. Present study deals with use of household waste, vegetable waste and other wastes and assessment for their suitability as substrates for mass multiplication *Trichoderma candidum* and develops effective production methodology which can be easily adopted.

Key-Words: *Trichoderma candidum*, Biocontrol, Mass production, Formulation

Introduction

Due to plant diseases every year nearly 10-20% of the total world food production was decreased and leads to loss of billions of dollars. Chemical control is offer nonspecific in its effect, killing beneficial organisms and it may have undesirable health and environmental pollution risk. Biological control of pathogens using *Trichoderma candidum* is very promising method against soil borne plant parasitic fungi. The fungal pathogens play a major role in the development of diseases on many important field and horticultural crops; resulting in sever plant yield losses. Intensified used of fungicides has resulted in accumulation of toxic compound potentially hazardous to human and environment an also in the buildup of resistance of the pathogens. In order to tackle these national and global problems, effective alternatives to chemical control are being employed. Biological control is nature friendly approach that uses specific micro-organisms, which interfere with plant protection. Biological control by an antagonism is a potential, no chemical and ecofriendly approach for managing plant diseases[1]. Among several groups of plant diseases, major amount of work has been done on the biological/integrated control of soil borne fungal plant pathogens by using fungal antagonist like, *Trichoderma* spp.

*** Corresponding Author**
E.mail: mrg.bio2008@gmail.com

Trichoderma is one of the common fungal biocontrol agent, is being used world wide for suitable management of various foliar and soil borne plant pathogens[2]. Biocontrol agents like *Trichoderma* spp. are acclaimed as effective, ecofriendly and cheap, nullifying the ill effects of chemicals. Therefore, of late, these biocontrol agents are identified to act against on array of important soil borne plant pathogens causing serious diseases of crops. Therefore considering the cost of chemical pesticides and hazardous involves, biological control of plant diseases appears to be an effective and ecofriendly approach being practice world over. Further biological control strategy is highly compatible with sustainable agriculture and has a major role to play as a component of integrated pest management (IPM) programme. Large scale production, along with shelf life and establishment of bioagents in targeted niche, determine the success of biological control. Therefore cost effective large scale production, shelf life of formulation, establishment of bioagent in to targeted niche and consistency in disease control are the primary concern with augmentative biological control. Adaptation of technology in the biocontrol arsenal needs to be investigated. Development of acceptable easily prepared and cost effective formulations for delivery should be major goal. For mass multiplication of bioagent through solid state fermentation technology an enormous quantity of spore biomass is needed. Various substrates like sugarcane baggase, fruit juice waste, vegetable waste, rotten wheat grains etc. are being used for mass multiplication of *Trichoderma candidum* with various degree of success[3]. Moreover a huge amount of solid waste like sugarcane baggase, fruit juice wastes, vegetable waste and rotten wheat grains increasing pollution and disposal problems. Therefore looking towards need for large scale cost effective production of ecofriendly biopesticide, present investigation is carried out to evaluate locally available cheaper substrates for mass multiplication of *Trichoderma candidum* for sustainable environment and sustainable agriculture. Solid state fermentation has advantages over submerged (liquid state) fermentation such as high volumetric productivity, low cost equipments involved, better yield of product, lesser waste generation and lesser time consuming process[4].

Material and Methods

Isolation and Identification of T. candidum

Fungal species *Trichoderma candidum* was isolated from soil samples by using potato dextrose agar (PDA) medium. Samples were inoculated over plates by multiple tube dilution technique (MTDT) and the plates were incubated at 30^{o}C for 4 days. The fungal colonies which were picked up and purified by streaking and incubated at 30^{o}C for 7-8 days. Green conidia forming fungal bodies were selected and microscopic observation was identified to be *Trichoderma candidum*. The culture was maintained on PDA slants[5]

Solid state fermentation (SSF)

Term solid state fermentation (SSF) is applied for the processes in which insoluble materials in water is used for the microbial growth. In fermentative processes of this type, the quantity of water should not exceed the capacity of saturation of the solid bed in which the micro-organisms (fungus) grow. Water essential for the microbial growth and in SSF and it is present in thin layers and in occasions absorbs inside the substrates. In the western world the SSF has been fewer studied that the SmF and SLF[4].

Processing of substrates

The substrates like vegetable waste, fruit juice waste, sugarcane baggase, rotten wheat grains, either alone or certain combination with or without additives such as gram flour and yeast extracts and having differential moistures levels were evaluated as substrates for mass multiplication of Trichoderma candidum.

Preparation of solid substrate media

The waste substrate cuts in the form of pieces and shade dried it. The dried substrate measure and add gram flour and yeast extracts for nutritional support. The moisture level of that mixture was maintained up to 40%. The media got sterilized by autoclaved. Non-synthetic solid media i.e. vegetable wastes, fruit juice waste, sugarcane baggase, rotten wheat were tested. 50 g of each solid material was taken in 500 ml conical flasks, inoculated with 5 mm mycelia mat incubated at 28^{o}C incubator for 7-10 days. The spore count was made as mentioned earlier.

Liquid state fermentation

Term liquid state fermentation (LSF) is applied for the processes in which soluble materials in water is used for the microbial growth. In fermentative processes of this type, the quantity of water should exceed. Water essential for the microbial growth and in LSF and it is present in thik layers and in occasions absorbs inside the substrates.

Preparation of Liquid substrates media

Liquid media; vegetable wastes powder, fruit juice waste powder, sugarcane baggase powder, rotten wheat grains powder were evaluated for the growth and sporulation of *Trichoderma candidume*. 200 ml of each medium was poured in 500 ml capacity Erlenmeyer days at 30^{o}C[4].

Results and Discussion

In the present study, several naturally available substrates of both solid & liquid media were tested for mass multiplication of *Trichoderma candidum.* The success of biological control depends not only the isolation, characterization & pathogenicity, but also on the successful mass production of the fungal agent in laboratory. Large scale availability of the pathogen is a primary requirement in the biocontrol programmed. For a successful integrated pest management program me, the agents like the pathogenic fungi should be amenable to easy & cheap mass multiplication.

Rotten Grains

Grains are cheap, easily available & act as best nutritive media for the mass multiplication of many micro-organisms. In India the wheat Grains mostly used, that's because there are huge storage of wheat in godowns. This whole storage is in used therefore possibilities of rotten wheat. The Rotten wheat used of substrate for mass multiplication of *Trichderma candidum*[6]. The *CFU* count recorded on solid media of wheat was 71.9 x 10^{8}. While on liquid wheat water media 77.1 x 10^{8}.

Sugarcane baggase

The sugarcane baggase is easily or almost freely available substrate for mass multiplication of fungal agent. The sugarcane baggase contains much more amount nutrition for growth of fungal agent *Trichoderma candidum.* The highest CFU count was recorded on solid

Vegetative waste and fruit juice waste

Vegetable waste & fruit juice waste are recorded for the maximum spore production. Vegetable wastes are support nutrition for maximum spore production than fruit juice waste. CFU count recorded on solid media of vegetable waste is 41.2 x 10^8 & liquid media 55.5 x 10^8. While the CFU count recorded on solid medium of fruit juice waste 34.7 x 10^8 & on liquid medium 46 x 2 x 10^8. The vegetable waste & fruit juice waste was found to be the cheapest & best suitable media for the large-scale production of fungi.

Conclusion

From the study it was clear that all the evaluated substrates are able to production of *Trichoderma candidum*. The sugarcane baggase is the substrate that was yield high amount of mycelia, spore & higher CFU count was recorded on sugarcane baggase i.e. 101.3 x 10^8 on solid medium & on liquid medium 94.7 x 10^8. The both solid & liquid state can be confirm for the production *Trichoderma candidum.* There are high spore count was recorded on sugarcane baggase media. The formulated CFU count with inert carrier sodium silicate was 41.5x10^8.

References

1. Bailey, D.J., Kleczkowski, A., Gilligan, C.A., (2004). Epidemiological dynamics and the efficiency of biological control of soil-borne disease during consecutive epidemics in a controlled environment. *New Phytol.*, **161**: 569-576.
2. Dominguesa, F.C., Queiroza, J.A., Cabralb, J.M.S., Fonsecab, L.P., (2000). The influence of culture conditions on mycelial structure and cellulose production by *Trichoderma reesei* rut C-30. *Enz. Microbial Technol*. **26**: 394-401.
3. Esposito, E., da Silva M.,(1998). Systematics and environmental application of the genus *Trichoderma*. *Crit. Rev. Microbiol.,* **24**: 89-98.
4. Kocher, G.S., Kalra, K.L., Banta, G., (2008) Optimization of cellulase production by submerged fermentation of rice straw *by Trichoderma harzianum* Rut-C 8230. *Int. J. Microbiol.,* **5(2)**: 8230.
5. Gamal, M., Abdel-Fattah, Yasser, M., Shabana Adel Ismail E., Younes Mohamed Rashad (2007) *Trichoderma harzianum*: A biocontrol agent against *Bipolaris oryzae*. *Mycopathology*., **164**: 81-89.
6. Gupta, R., Saxena, R.K., Goel, S., (1997) Short communication: Photoinduced sporulation in *Trichoderma harzianum*-An experimental approach to primary events. *World J. Microbiol. Biotechnol.*, **13**: 249-250.

Table 1: Media formulations

Name of substrates	Type of yeast media	Weight of substrates (gm)	Volume of media (gm/ml)	Weight of Gram flour (%)	Weight of extracts(%)
Vegetable waste	Solid	70gm	-	30	3
Fruit juice waste	Solid	70gm	-	30	3
Sugarcane Baggase	Solid	10gm	-	40	43
Rotten wheat	Solid	85gm	-	5	5
Vegetable waste	Liquid	10gm	200	43	43
Fruit juice waste	Liquid	10gm	200	43	43
Sugarcane baggase	Liquid	10gm	200	43	43
Rotten wheat	Liquid	10gm	200	43	43

Table 2: Effect of different substrates on population of *T. candidum*

Substrate media (x108)	Spore count (x108 of Solid media	Spore count of liquid media
Vegetable waste	41.2	55.5
Fruit juice waste	34.7	46.2
Sugarcane baggase	101.3	94.7
Rotten wheat grains	71.9	77.1

Sugarcane baggase media i.e. 101.3×10^8 where as on liuide media of sugarcane baggase is 94.7×10^8.