SELECTION OF PROCYMIDONE DEGRADING MICROORGANISMS FROM COMPOSTING OF PLANT WASTES

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ABSTRACT

A screening and selection program for procymidone degrading microorganisms was performed. As a result, a total of 65 strains from plant waste composting heaps were isolated. Most of them (80%), were able to grow in media with the pesticide as sole carbon and energy source and 4 strains showed an increase of growth higher than 35-40%, the limit considered as adequate for microorganisms useful in bioremediation. A subsequent analysis of three of these strains showed a procymidone biodegradation level over 35-40%, similar to those described in bibliography for other pesticides. Considering this as a preliminary work, results presented here are very promising, since both genetic and environmental modifications, can be made to improve the bioremediation capacity.
Keywords: *procymidone, microbial biodegradation.*

INTRODUCTION

The agricultural model prevailing in South-East of Spain is characterized by an intensive production, which is supported by the use of fertilizers and the application of pesticides to prevent the action of phytopathogen microorganisms. Some practices, as well as the huge area dedicated to these labours, favour the accumulation of this kind of compounds. Certainly, this is a harmful situation, both for environment and human beings. Despite of it, and taking into account economic reasons, at the present it seems rather unlikely to develop an intensive agriculture lacking pesticides. Nevertheless, some other approaches to solve this problem are feasible, being bioremediation one of the most interesting proposals, not only from a safety point of view but also economically (Pieper and Reineke, 2000; Semple et al., 2001).

Bioremediation, the use of microbial species to remove pollutant substances (Atlas and Pramer, 1990), is a consequence of the vast metabolic capacity of microorganisms. This capacity derives from the ancient coexistence between substrates and microorganisms, and the numerous evolutionary possibilities that it creates (Glazer and Nikaido, 2001). Thus, although pesticides are relatively recent compounds, bioremediation has been successfully used in their treatment and microorganisms like *Pseudomonas* (Karpouzas et al., 2000) or *Phanerochaete* (Lee et al., 1991) have shown good results in the degradation of this kind of chemicals.

Procymidone is a dicarboximidic fungicide used in vegetables and fruits against *Botrytis*, *Monilia* or *Sclerotinia*. When the pesticide is correctly applied, higher levels than those legally admitted are not reached; however, a frequent use can favour its accumulation, both in plants and soils (Vanni et al., 2000; Pang and Close, 2001). Besides the correct application, the microbial degradation of the pesticide appears as a useful treatment to avoid problems associated to accumulation. Thus, the aim of this work was directed to the isolation and selection of microorganisms able to metabolise procymidone.

MATERIAL AND METHODS

Screening and isolation: Samples (5 g) obtained from plant waste composting heaps at different stages, were placed in 250 ml erlenmeyer flasks containing the selection media: 95 ml of basal salt solution according to Janshekar et al., 1982, added of 1 ppm procymidone (Kenolex® 50 WG, Kenogard, Barcelona, Spain). Flasks were incubated for 7 days at 30°C. After incubation, 5 ml from these flasks were inoculated in a fresh medium of similar characteristics and incubated for 14 days at 30°C. This enrichment process was repeated once more. For microbial isolation, plates with solid selection media (added of 2% bacteriological agar) were inoculated with a loop with samples from enrichment media. After incubation for 5 days at 30°C, the five more abundant morphotypes were selected and freeze-dried.

Growing selection: The isolated microorganisms were assayed in relation to their capacity to grow on media supplemented with procymidone as sole carbon source. Flasks of 250 ml containing 50 ml of selection media were inoculated with 0.5 ml of bacterial suspensions of $O.D._{550}$=0.5-0.6 (Shimadzu UV-A, Shimadzu, Tokyo, Japan) and incubated at 30°C for 4 days (bacteria), or 7 days (fungi). Samples for bacterial counts were extracted at 0, 48 and 96 hours; fungal counts were extended to 168 hours. All assays were achieved in triplicate and growth was compared with that obtained in control flasks, in which procymidone was replaced by glucose (0.5% w/v).

Degradation assay: Best strains according to results from growing selection were tested for their capacity to metabolise the pesticide. The identification of the strains, performed by molecular methods, was based on the analysis of the sequence obtained from the direct amplification of the 16S rDNA gen. Culture, incubation and sampling conditions were similar to those described previously, except in that procymidone concentration was increased up to 2 ppm. The analysis of the samples for the quantitative determination of the fungicide was performed by HPLC equipped with a HP Zorbax SB-C-846 column and coupled with a MS detector.

RESULTS AND DISCUSSION

The screening for pesticide-degrading microorganisms in compost samples led to the isolation of 65 strains. Most of the strains isolated were bacteria (55), while the remainder were at 50% actinomycetes and fungi. Despite of the main role these microbial groups play in the biotransformation process that takes part in composting, it might not be surprising their limited presence. Some studies have shown actinomycetes affect in a qualitative way, more than quantitatively, thus their biodiversity in composting heaps use to be poor (Michel et al., 2002). Moreover, most of them are difficult to grow on lab media (Epstein, 1997).

Results from the growing selection assay showed the capacity of most of these microorganisms to increase the number of colony former units (CFU). Thus, 78.9% of bacteria, 80% of actinomycetes and 100% of fungi were able to grow in media with procymidone as sole carbon source. Nevertheless, the number of isolates which showed an increase in growth higher than 10% with respect to initial levels was substantially lower. Thus, only 30.9% of bacteria and 20% of fungi grew above this limit. On the contrary, 60% of actinomycetes passed this frontier (Table 1).

Although a high metabolic rate of pesticide is not always correlated to a good growth level, a strong increase in the number of CFU is usually associated to a higher pesticide biodegradation (Struthers et al., 1998). In most of studies, microbial growth is not tested and concentration of pollutants is determined instead, so there is a very little information about the growing ability of microorganisms on pesticides. Nevertheless, the available data point out microorganisms

able to grow over 40% as the most adequate for pollutant biodegradation (Struthers et al., 1998).

Table 1. *Increase of growth (as percentage) for strains isolated in media supplemented with procymidone as sole carbon source.*

Bacteria						**Fungi**		
Strain	*Growth (%)*		*Strain*	*Growth (%)*		*Strain*	*Growth (%)*	
	Pesticide	Glucose		Pesticide	Glucose		Pesticide	Glucose
P_b-002	11.14	34.96	P_b-932	18.79	-0.03	P_f-515	20.46	33.39
P_b-545	11.57	-2.96	P_b-003	22.65	2.13			
P_b-561	12.96	21.37	P_b-413	23.62	25.16	**Actinomycetes**		
P_b-1032	13.03	19.25	P_b-432	24.16	65.07	*Strain*	*Growth (%)*	
P_b-941	14.68	1.86	P_b-945	27.37	57.73		Pesticide	Glucose
P_b-1011	15.35	3.53	P_b-543	37.34	-29.38	P_a-512	12.73	46.61
P_b-214	15.39	-9.03	P_b-541	41.11	38.74	P_a-1012	18.08	-4.01
P_b-241	16.59	-13.50	P_b-263	55.51	70.99	P_a-415	87.97	-5.62
P_b-112	16.60	15.85						

In our study, 4 strains showed the capacity to increase the number of CFU to this level or above: P_b-543, P_b-541, P_b-263 and P_a-415. Three of them (P_b-543, P_b-263 and P_a-415, respectively identified as *Brevibacillus parabrevis*, *Bacillus licheniformis* and *Streptomyces thermocarboxydus*) were used in the degradation assay to determine their capacity for bioremediation and pesticide elimination. P_b-541 was rejected because of difficulties on manipulation.

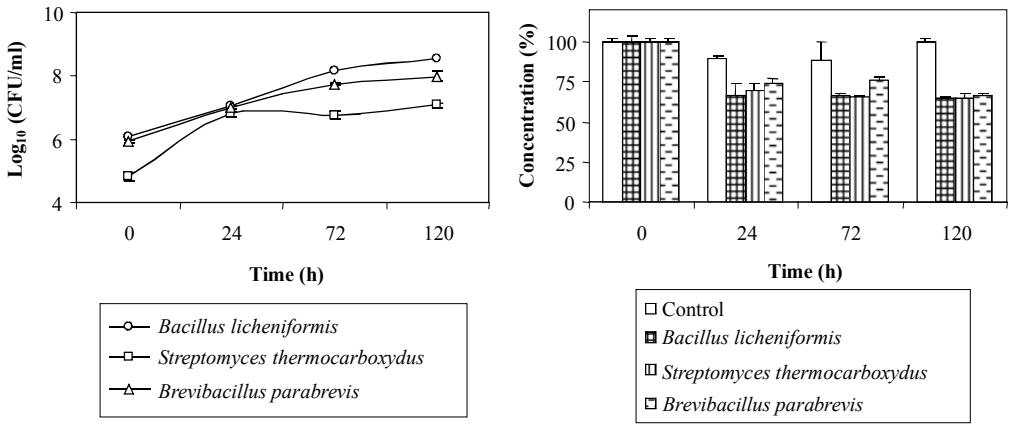

Figure 1. *Growth and procymidone degradation of selected microorganisms.*

Similar results regarding to procymidone degradation were obtained for all the three microorganisms (Figure 1). In all cases, a decrease in concentration between 35 and 40% was observed. Although it has been described a complete degradation of pesticide (Struthers et al., 1998; El-Fantroussi, 2000), this is no an usual event. On the contrary, variation limits for bioremediation effectiveness on literature are quite extensive, probably as a consequence of numerous factors that can affect the metabolism (Zablotowicz et al., 1998; Awasthi et al., 2000). Thus, variations between 10 and 40% for organophosphorus pesticides (Karpouzas et al., 2000) and between 30 and 70% for organochlorine compounds (Awasthi et al., 2000) have been described. Our results are promising, not only by their similarity to those observed in other studies, but because they are consequence of a preliminary work and, therefore, they can be improved, both gene-

tically and/or environmentally. In some cases, modifications in nutritional and environmental factors have succeed in duplicate the metabolised concentration of pesticide (Awasthi et al., 2000; Zanardini et al., 2002)

In conclusion, the three isolates, *Brevibacillus parabrevis*, *Bacillus licheniformis* and *Streptomyces thermocarboxydus*, show capacity to degrade procymidone and good perspectives for future development, and, therefore, they can contribute to reduce the negative impact caused by the use of chemicals highly recalcitrant.

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