SUITABILITY OF THE HIGH TEMPERATURE PRE-INCUBATION METHOD FOR ISOLATION OF PECTINOLYTIC ACTINOMYCETES

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Abstract

A comparison of pectinolytic properties of actinomycetes isolated from soils by the use of the High Temperature Pre-Incubation method and the conventional method has revealed the advantage of using the former method for the isolation of pectinolytic species. This method thus holds promise of its exploitation for the isolation of the pectinolytic actinomycetes from any desired ecosystem.

Introduction

In a previous paper a plate-method designated High Temperature Pre-Incubation (HTPI) method suitable for the preferential isolation of actinomycetes from soils was described.[1] However, when the counts obtained by this method were compared with the counts recorded by the conventional method, an apparent drawback was observed in the decrease of the actinomycetal population. In as much as the method was intended for isolating actinomycetes attacking pectin rather than for the isolation of the organisms themselves, it was interesting to ascertain if the actinomycetes isolated by this method, in comparison to the conventional, yielded pectinolytic species. The results of this comparative study are presented in this paper.

Materials, Methods and Results

Six of the 73 soil samples examined earlier[4] were used as inocula. The samples differed from each other with respect to pH and actinomycetal population. The counts were established by the HTPI method as well as the conventional method using McBeth and Scale's medium[5].

From the results presented in Table I, it is clear that actinomycetal count recorded per gm of air-dried soil was higher by the conventional method than HTPI method, thus confirming the previous observations.

All the actinomycetal cultures encountered as a result of using both these methods were then isolated as pure cultures; 178 from the plates used in conventional methods and 115 from HTPI method. All the isolates were classified upto generic level, using for this purpose, Bergey's Manual[3].

TABLE I

Actinomycetal population of soils

Soil No.	pH	Actinomycetal counts × 10^3 (per g of air-dried soil)	
		Conventional method	HTPI method
A_{12}	3.7	12	9
A_{11}	6.3	23	21
C_9	8.7	190	85
B_{13}	5.9	310	230
A_7	7.0	435	280
A_{19}	6.8	4850	2600

It may be noted from Table II, that members of the genus *Mycococcus* were obtained only by conventional method; also, that the conventional method was preferable for the isolation of *Nocardia*. In the HTPI method, on the other hand, *Streptomyces* dominated. The population of *Micromonspora* isolated by the two methods was nearly the same.

TABLE II

Identity of Actinomycetes isolated by HTPI (115 cultures and conventional method (178 cultures)

Genera	HTPI Method	Conventional method
Mycococcus	0	48
Nocardia	17	51
Streptomyces	66	36
Micromonospora	32	43

When the pectinolytic attributes of the isolates (Table III) were studied by the method of Bilimoria and Bhat[9], it was recorded that whereas *Mycococcus* species were not pectinolytic, *Streptomyces* species were clearly pectinolytic. The pectinolytic attributes were evidenced in a greater number of the cultures made of *Streptomyces*, *Micromonspora* and *Nocardia* isolated by HTPI method than those made from the conventional plates, suggesting thereby the superiority of HPTI method over the conventional one for the isolation of pectinolytic actinomycetes.

TABLE III

Pectinolytic activity of actinomycetes

Genera isolated by	No. of isolates attacking pectin by			% positive
	Deesterification	Glycosidic action	Deesterification and glycosidic action	
Conventional method				
Mycococcus sp (48)*	0	0	0	0
Nocardia sp (51)	7	3	3	13.72
Streptomyces sp (36)	27	28	27	77.77
Micromonospora sp (43)	11	21	11	48.37
HTPI method				
Nocardia sp (17)	4	7	4	41.17
Streptomyces sp (56)	56	65	56	98.48
Micromonospora sp (32)	20	24	20	75.00

*No. in parenthesis indicates total number of isolates screened

DISCUSSION

The decrease in the total counts of actinomycetes observed in the HTPI plates is attributable to the destruction by heat (110°C for 10 min) of the non-sporulating type of actinomycetes as represented by the genera *Nocardia* and *Mycococcus* in contradistinction to the sporulating *Streptomyces* which resist the temperature for the given period.

The results, however, have left no doubts about the superiority of the HTPI method over the conventional method. The method thus appears to be suitable for easy and rapid isolation of pectinolytic actinomycetes.

Since the HTPI method has been found successful for isolation of pectinolytic actinomycetes from various ecosystems such as lake sediments, vegetable litters and ret water effluents of various fibre-yielding plant straws and leaves, it is reasonable to assume that it would prove equally handy for isolating this class of microorganisms from other ecosystems as well.

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