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Thermotolerant N_2-Fixing and P-Solubilizing Microbes from Partially Decomposed Municipal Solid Waste

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Abstract: A total of nineteen thermotolerant isolates comprising ten N_2-fixing and nine Phosphate solubilizing organisms were isolated from partially decomposed municipal solid waste. All the thermotolerant N_2 -fixers and Phosphate solubilizers performed better at an incubation temperature of 30±2°C. Out of several isolates tested, isolate TNF-10 fixed maximum Nitrogen and isolate TPSB -9I released maximum soluble P at 30±2°C and 50±2°C.

Key words: Municipal solid waste % N_2-fixers % P-solubilizer % Thermotolerant

INTRODUCTION

As a consequence of escalating fertilizer costs, fluctuating agricultural product prices and increasing awareness among farmers regarding myths of chemical inputs, chemical farming is replaced by counter revolution shifting to traditional system of crop production where use of organic material is most preferred. Though organic matter is avaialable in plenty, their application directly to soil leads to some deleterious effects such as immobilization of nutrients, phytotoxicity etc. [1]. An organic matter stabilization process, popularly called composting, seems to be the desirable treatment to overcome such problems. This is an organic matter decomposition process which involves aerobic respiration and passes through a thermophilic stage [2]. It yields a stable product called compost which can be used as nutrient source in crop production.

The composts are usually poor in nutrient content and generally contains about 0.5-0.8% N, 0.5-0.7% P and 0.5 to 0.8 K [3].The quality of final compost generated can be improved by a technique called enrichment [4]. Use of beneficial microbial inoculants such as N_2-fixers and Phosphate solubilizers (P-solubilizers) for enrichment of compost is receiving attention recently [4-6]. Most of the beneficial microbial inoculants available are mesophilic in nature and they are killed, if they are inoculated at the initial period of decomposition. Therefore, they should be inoculated only at or after cooling stage. So it is very difficult for a farmer to identify the stage where the beneficial inocula have to be used. This work was performed to isolate some thermotolerant N_2-fixers and P-solubilizers which survive even during thermophilic stage and can be inoculated during initial stages of composting.

MATERIALS AND METHODS

Isolation and Characterization: The urban solid waste which has attained active state of decomposition was used for isolation work. Fifty gram of decomposing organic waste was placed in 250 ml conical flask and incubated at 50°C for three days. The isolation of P-solubilizing and N_2-fixing microorganisms from urban solid waste sample was done by serial dilution and plating method using Sperber's medium [7] and Norris N-free medium [8] respectively. 10 g of compost sample was transferred into 90 ml of sterile water blank and serially diluted. 1ml aliquot from appropriate dilutions were transferred aseptically into sterile plates and appropriate volume of molten lukewarm medium was poured to respective plates. The plates were gently rotated to uniformly distribute the inoculum before the medium was solidified. The plates were then incubated at 28±2°C temperature for 3 days. The representative colonies on Norris N-free medium and representative colonies showing clear zone on Sperber's medium were purified and maintained on nutrient agar slants. These isolates were subjected to some morphological and biochemical tests [9].

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Determination of N2-fixing Ability of Selected Isolates: The selected N2-Fixing isolates were inoculated to Norris N-free liquid medium maintained in two sets.The one set was incubated at 30±2°C and another at 50±2°C. Both sets were incubated for 10 days and the total nitrogen content of liquid medium was analyzed by Microkjeldhal method [10].

Determination of Phosphate Solubilizing Efficiency of Selected Isolates: The selected P-solubilizing isolates were grown on Sperber's liquid medium containing different insoluble Phosphorus sources such as hydroxy apatite (0.1%), tricalcium phosphate (0.1%), Udaipur rock phosphate (0.1%), Iron phosphate (0.1%) and manganous phosphate (0.1%). Initial pH of all the media were set to 7. All these treatments were maintained in two sets. One set was incubated at 30±2°C and another at 50±2°C. Both the sets were incubated for 4 days The water soluble P content of culture supernatant was estimated by following stannous chloride method [10]. The pH of the supernatant was determined potentiometrically.

RESULTS

Ten representative thermotolerant N_2-fixing and nine P-solubilizing microorganisms (Table 1) were isolated from the partially decomposed urban solid waste. The selection criteria in case of N_2-fixing bacteria was their ability to grow on N-free medium and that in case of P-solubilizer was of clear zone diameter. Out of this, only three isolates from each group were retained for further studies. Out of 19 isolates 15 isolates were gram positive and 4 isolates were gram negative.

All the thermotolerant N_2-fixers fixed more nitrogen at 30±2°C than at 50±2°C. The isolate TNF-10 fixed maximum nitrogen (20.37 mg/100 ml and 29.45 mg) both at 50±2°C and 30±2°C, respectively. Isolate TNF-9 was next best in fixing N_2 and it fixed 10.46 mg/100ml at 50±2°C and 17.27 mg/100ml at 30±2°C (Fig. 1).

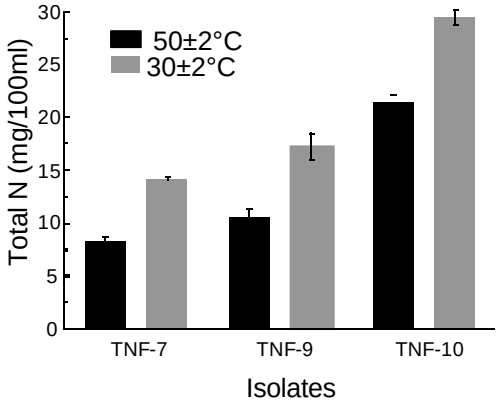

Fig. 1: The N2-fixing ability of Thermotolerant species at different incubation temperatures (n=3, mean±SD)

Table 1: characters of thermotolerant bacterial isolates from partially decomposed municipal solid waste

Isolate No.	Characters
TNF-3	Gram positive,creamy white shiny colony
TNF-4	Gram positive,creamy white shiny colony
TNF-6	Gram positive, pink coloured colony
TNF-7	Gram negative, small creamy colony
TNF-9	Gram negative, small shiny colony
TNF-10	Gram positive, shiny colony
TNF-17	Gram positive,creamy white colony
TNF-18	Gram positive, dull white colony
TNF-19	Gram positive, dull white colony
TNF-20	Gram negative, shiny colony
TPSB-1	Gram positive, dull white colony
TPSB-2	Gram positive,creamy white irregular colony
TPSB-3	Gram negative,reddish irregular colony
TPSB-4	Gram positive, white irregular colony
TPSB-5	Gram positive,creamy irregular colony
TPSB-6	Gram positive,creamy white irregular colony
TPSB-7	Gram positive, shiny colony
TPSB-8	Gram positive,creamy white irregular colony
TPSB-9	Gram positive,creamy white irregular colony

Table 2: Concentration of soluble phosphate released from different insoluble P sources at different incubation temperatures (n=3, mean±SD).

Insoluble P-Sources	Incubation temperature	P-Solubilized by thermotolerant isolates TTPSB7	TPSB8	TPSB9
HA	50±2°C	16.51±0.51	14.31±0.05	19.86±1.36
	30±2°C	29.57±1.24	36.16±1.05	35.32±0.95
ALP	50±2°C	11.42±0.42	12.36±0.64	16.38±1.37
	30±2°C	23.50±0.88	28.57±0.53	30.32±1.84
TCP	50±2°C	10.31±1.10	10.36±0.51	13.57±1.40
	30±2°C	26.31±3.20	18.56±1.59	27.58±0.75
IP	50±2°C	10.41±0.97	7.68±18.42	10.87±0.24
	30±2°C	21.51±1.40	18.42±0.32	28.46±1.29
RP	50±2°C	11.32±1.15	4.36±1.03	16.36±0.82
	30±2°C	30.36±2.81	19.37±0.96	31.41±1.85

Table 3: The pH of the culture supernatant of the eight selected rock phosphate solubilizing bacteria (n=3, mean±SD).

Insoluble P-Sources	Incubation temperature	pH of the culture supernatant of thermotolerant isolates TPSB7	TPSB8	TPSB9
HA	50±2°C	4.31±0.31	4.58±0.37	4.06±0.97
	30±2°C	4.07±0.89	4.63±2.47	3.98±1.79
ALP	50±2°C	4.56±1.15	4.71±1.71	4.50±1.32
	30±2°C	4.58±4.58	4.56±1.39	4.31±0.87
TCP	50±2°C	5.01±0.90	5.21±0.05	4.69±0.58
	30±2°C	5.00±0.87	5.06±1.27	4.46±0.69
IP	50±2°C	4.97±0.86	4.90±0.58	4.76±0.66
	30±2°C	4.63±0.86	4.58±0.52	4.50±0.58
RP	50±2°C	4.56±0.45	4.75±0.75	4.63±0.65
	30±2°C	4.65±1.49	4.53±1.20	4.19±0.69

The P-solubilizing microbes also performed better at 30±2°C than at 50±2°C. Isolate TPSB -9 released maxim soluble P from all sources of insoluble P at both the incubation temperatures. Out of all the insoluble Phosphorus sources tested, maximum P should released from hydroxyapatite at 30±2°C (Table 2). Compared to other isolates, TPSB-9 showed maximum reduction in pH of the medium with different insoluble Phosphorus sources, at 30±2°C than at 50±2°C. But, the same isolate did not give better result in a medium containing Rock phosphate at 50±2°C. Under this condition, isolate TPSB-7 showed maximum pH reduction (Table 3).

DISCUSSION

The composts are usually poor in nutrient content and generally contain about 0.5-0.8% N, 0.5-0.7% P and 0.5 to 0.8 K [5]. The quality of final compost generated can be improved by a technique called enrichment [4]. Many reports show that enrichment of compost with beneficial microorganisms such as P-solubilizers and N_2-fixers increases nutrient content and manurial value [4-6]. The composting process involves the aerobic exothermic microbial decomposition of the initial substrate, which results in dynamic changes in temperature, moisture content, oxygen concentration and nutrient availability. These factors, in their turn, strongly affect the structure and diversity of the microbial community, microbial activities and the physical and chemical characteristics of the substrate [11]. Gazi *et al*. [12] reported that, during thermophilic phase of composting temperatures as high as 45 to 63°C have been recorded. If the mesophilic cultures which are non native to compost are inoculated, it may lead to their death. Under this condition, it is very difficult for a farmer to decide the time of inoculation of microbial inoculants. With that intention, in the present study, 19 thermotolerant beneficial isolates were isolated (Table 1) and selected isolates were characterized for their efficiency under mesophilic and thermophilic conditions.

Out of the 19 isolates, 15 isolates were gram positive and 4 isolates were gram negative. This result is in accordance with the work by Ntougias [13] who observed that spent mushroom compost is known to contain more number of gram positive bacteria than gram negative bacteria.

Most of the cultures available for compost enrichment are mesophilic in nature and their application should be done only during cooling stage [3]. The N_2-fixers and P-solubilizers isolated in this study showed maximum activity at 30±2°C than at 50±2°C.

This result clearly indicates that if these isolates are inoculated in the initial stage of compost, they survive at thermophilic stage and perform better at or after cooling stage.

The isolate TNF-10 fixed maximum nitrogen both at 30±2°C and 50±2°C. Isolate TNF-9 was next best in fixing N_2 (Fig. 1). The isolate TPSB-9I released maxim P from all sources of insoluble P at both the incubation temperatures. Out of all the insoluble P sources tested, TPSB-9I released maximum P from hydroxy apatite at 30±2°C (Table 2).

All the P-solubilizing isolates tested in the present study reduced the pH of the medium. Compared to other isolates, TPSB-9I showed maximum reduction in pH of medium at all insoluble P-sources and at both the temperatures except in medium containing Rock phosphate at 50±2°C. Under this condition, isolate TPSB-7 showed maximum pH reduction (Table 3). Reports in the literature suggest that microbial solubilization of mineral phosphate is mostly due to the excretion of organic acids causing acidification of the external medium [14, 15]. The pH reduction observed in the present study may be due to excretion of organic acid.

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