ISOLATION AND CHARACTERIZATION OF EFFICIENT PLANT GROWTH PROMOTING, CELLULOLYTIC AND PHOSPHATE SOLUBILIZING MICROORGANISMS FOR THE PREPARATION OF BIOACTIVE PHOSPHOCOMPOST FROM THE AGROWASTES

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Abstract:

The waste crop residues can be utilized in agricultural fields to improve crop productivity and soil quality after undergoing composting before being used as manure. Rapid methods of composting makes use of recent advances in technology for expediting the process, like amendment with minerals, use of efficient microorganisms and so on. A wide range of microorganisms consisting of fungi, bacteria and actinomycetes decomposes readily degradable materials and polymers like carbohydrates, proteins, amino acids cellulose and lignin that can speed up the composting along with its enrichment. So, the investigations were conducted to isolate the efficient plant growth promoting microorganisms responsible for faster cellulose decomposition and phosphate solubilization which can be used for the preparation of bioactive phosphocompost from the agricultural waste. Total of 26 bacteria, 17 actinomycetes and 23 fungal isolates were screened and characterized from different samples such as municipal solid waste, mushroom compost, rural compost and soil collected from different sites of District Shimla and Solan of Himachal Pradesh. On the basis of their plant growth promoting properties, phosphate solubilization and cellulose decomposition efficiencies the best isolates were tested for their compatibilities. The isolates showing compatibilities were finally selected for the preparation of consortium to prepare bioactive phosphocompos

Keywords:

Isolation, microbial consortium, Plant growth promoting, cellulolytic, Phosphocompost.

Introduction:

Higher economic growth and rapid urbanization of human population are usually accompanied with generation of enormous amount wastes. Management of such an enormous amount of wastes has become an important environmental issue and economic necessity. India produces about 500 million tons (Mt) of crop residues annually, out of which 2.85 Mt yr-1 crop residues are produced in Himachal Pradesh (MNRE 2009). 0.41 Mt crop residues are burnt in Himachal Pradesh every year (Pathak et al. 2010). These crop residues are the valuable resources provided appropriate technologies are adopted to transform it in to a marketable product of some economic value (Mohan Singh, 2004). A diverse group of microorganisms transforms decomposable materials to a stable by-product that contains plant nutrients and humus. Rapid methods of composting makes use of recent advances in technology for expediting the process, like amendment with minerals, use of efficient microorganisms, cellulolytic organisms, frequent turnings and so on. These approaches expedite the decomposition process. The compost is usually deficient in nutrients such as phosphorus. But there is the possibility of improving the P availability through the inoculation of Phosphate solubilizing microorganisms and P source such as Rock phosphate. Enrichment with the cellulolytic fungi plays an important role in hastening the decomposition process. Gaur et al. (1982) reported that the inoculation with mesophilic fungi lowered the C: N ratio of agricultural wastes and recommended the use of microbial inoculums for accelerating the process of decomposing

Material And Method:

ISOLATION OF MICROORGANISMS Isolation of microorganisms was carried out from samples collected from dung heap, rural compost, municipal solid waste compost, mushroom compost and agricultural field soil collected from the sites namely Nauni, Salogra, Chambaghat, Chail, Barog of District Shimla and Solan of Himachal Pradesh. The population capable of growth on different

media was counted and reported as cfu/g of soil or compost. Various groups of cellulolytic and phosphate solubilizing microorganisms responsible for organic matter decomposition and P solubilization were isolated by the spread plate technique using different medium such as Nutrient agar medium for bacteria, Malt extract agar for fungus and Kennight medium for actinomycetes. MORPHOLOGICAL AND BIOCHEMICAL CHARACTERIZATION The colony morphology of the bacterial isolates was studied and biochemical tests such as simple staining, gram staining, catalase, oxidase, MRVP test, carbohydrate fermentation casein and gelatin hydrolysis, Hydrogen sulphide production were done by standard procedures. SCREENING OF THE MICROORGANISMS FOR THEIR PLANT GROWTH PROMOTING ACTIVITIES, ABILITY TO SOLUBILIZE PHOSPHORUS AND DEGRADE CELLULOSE The selected isolates were streaked on the Pikovskaya's agar plates and incubated at 35°C 2°C for 48 hrs. The isolates showing yellow coloration around the streak were further selected for analyzing their Phosphate solubilizing efficiency (PSE %) by Plate assay method (Pikovskaya's 1948). For testing the ability of isolates to degrade cellulose, Czapek-mineral salt medium was used (Aneja, 2009). The selected bacterial isolates were also screened for their abilities to perform multifarious plant growth promoting activities i.e. P solubilization, growth on N free media, siderophore production, antagonism against major soil borne pathogens.

Result And Discussion:

ISOLATION AND ENUMERATION OF MICROBIAL POPULATION: The microbial count varied to great extent with locations. A summary of microorganisms isolated from the samples collected from different locations/sites/subsites of Solan and Shimla districts of Himachal Pradesh is presented in Table 1.

MORPHOLOGICAL, PHYSIOLOGICAL AND BIOCHEMICAL CHARACTERIZATION OF SELECTED BACTERIAL ISOLATES. A total of 26 bacteria, 17 actinomycetes and 23 fungal isolates were selected out of total 111 purified isolates. The data pertaining to morphological characteristics of these

isolates are presented in Table 2. The biochemical characteristics of the bacterial isolates are given in table 3. Almost all the isolates showed positive reaction for Voges Proskauer, oxidase, catalase and carbohydarate fermentation test whereas, negative reaction for methyl red, hydrogen sulphide production. 20 isolates were positive for grams reaction and gelatin hydrolysis, 15 were positive for casein hydrolysis. SCREENING OF BACTERIAL, ACTINOMYCELIAL and FUNGAL ISOLATES FOR CELLULOLYTIC AND MULTIFARIOUS PLANT GROWTH PROMOTING TRAITS The data in Table 4 revealed that out of 26 bacterial isolates, 18 isolates were P solubilizers, 17 were nitrogen fixers, 15 were HCN producers, 19 were siderophore producers and only 7 were cellulolytic. Only 12 isolates showed antagonism against Fusarium oxysporum and 7 isolates showed antagonism against Pythium ultimum. From table 5 and 6, it is concluded that out of 17 actinomycelial and 23 fungal isolates were 12 actinomycetes and 18 fungal isolates were P solubilizers, 10 actinomycetes and 14 fungal isolates were N fixers, 9 actinomycelial and 14 fungal isolates HCN producers, 13 actinomycelial and 17 fungal isolates were siderophore producers. Whereas, 3 actinomycelial and 8 fungal isolates were cellulolytic. IN VITRO COMPATIBILITY ASSESSMENT FOR THE PREPARATION OF MICROBIAL CONSORTIUM On the basis of screening, total 7 isolates i.e. two bacterial (DB1, DB22), two actinomycelial (DA7, DA17) and two fungal (DF12, DF14), were selected and checked for their synergistic activities and compatibilities by cross streaking method in different combinations. Table 7 revealed that combination of DB1+DA7+DF14 and DB22+DA17+DF14 showed synergism amongst them. Among these two combinations, isolates of DB1+DA7+DF14 were more efficient in PGP and cellulolytic activities and so, were selected for the further experiments. Phosphate solubilizing bacteria include fungi as well as bacteria. Many phosphate-solubilizing bacteria (PSB) belong to the genera Pseudomonas, Bacillus, Rhizobium, Agrobacterium, Micrococcus, Enterobacter, Erwinia,, Penicillium and Aspergillus (Gulati et al., 2007). Strongly cellulose degrading

fungi are represented by species of the genera Aspergillus chaetomium, Aspergillus fumigatus, Aspergillus flavus, Curvularia, Fusarium, Memoniella, Phomo, Thielavia and Trichoderma (Makeshkumar, 2011; Mohammad, 2010). Several fungi and bacteria have been screened out from the soil and decomposing plant residues and evaluated for their biodegradative activity on waste materials. Compost is rich in cellulolytic and other various types of microorganisms. Hameeda et al., (2006) isolated the plant growth promoting rhizobacteria from the compost. Makeshkumar (2011) isolated six potential cellulose degrading fungus (i.e., Fusarium sp., Aspergillus fumigatus., Cladosporium sp., Aspergillus flavus, Pyricularia sp. and Nigrospora sp.) from compost of agrowastes. Cellulolytic fungi viz. Aspergillus niger and
Trichoderma viridae along with rock phosphate was used for compost making in a study by Zayed and Motaal, (2005). Increase in the solubilization of rock phosphate added in the compost by the action of Aspergillus awamori was also reported by Mishra et al., 1982.

Conclusion:

The last decade has lead to an increasing awareness of the problems associated with the classical methods of waste treatment. It was realized that the elimination of waste materials by burning, or dumping in sanitary landfills is not the final solution to all waste problems. Composting is one of the more economical and environmentally safe methods of recycling waste generated by the society. Due to the complexity of substrates and intermediate products, microbial diversity and the succession of populations is a prerequisite to ensure complete biodegradation. However, the rural compost is not rich in nutrients. To enrich and speed up the composting the efficient microorganisms can be inoculated along with other nutrient sources. There is a need to explore the microbial world in search of the efficient microorganisms for the betterment of composting techniques.

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Table 1: Enumeration of Bacteria, Actinomycetes and Fugal population associated with different samples collected from various sites

LOCATION	SITES	SAMPLES	Microbial count (×10^4 cfu / g soil)			Actinomycetes count (×10^3 cfu / g soil)	Fugal count (×10^3 cfu / g soil)
			Nutrient Agar (NA)	Pikovaskaya's agar (PVK)	Soil extract media (SEM)		
SOLAN	NAUNI	Rural compost	121.00	83.00	106.13	67.11	12.13
		Mushroom compost	103.34	72.33	104.03	54.66	10.45
		Soil	126.34	84.33	116.01	55.12	17.11
	CHAIL	Rural compost	111.31	74.00	68.02	58.66	18.01
		Mushroom compost	90.64	53.67	62.00	33.15	13.21
		Soil	113.00	77.34	84.66	37.33	20.21
	CHAMBAGHAT	Rural compost	97.30	89.00	98.33	45.62	21.28
		Mushroom compost	76.33	54.00	72.03	28.12	15.11
		Soil	80.34	61.01	82.31	31.22	19.42
	SAPROON	Rural compost	78.03	59.10	100.13	51.00	22.31
		Soil	98.89	63.11	103.88	42.12	17.26
	BAROG	Mushroom compost	121.76	78.33	111.65	34.27	11.34
	SALOGRA	Municipal solid waste	87.12	34.12	45.13	21.21	8.34
	DHARAMPUR	Rural compost	132.44	67.88	167.43	56.18	24.81
		Soil	104.54	44.32	93.24	39.23	15.23
SHIMLA	SHOGI	Rural compost	144.15	66.11	102.44	51.22	19.44
		Soil	98.66	76.65	113.77	44.31	17.63
	LAALPANI	Municipal solid waste	77.22	34.62	55.53	19.34	9.45

Table 2: Morphological and Biochemical characterization of the bacterial isolates

Isolates	Morphology			Simple staining	Grams staining
	Form	Elevation	margin		
DB1	Irregular	Raised	Erose	Rods	+
DB2	circular	Raised	Entire	Rods	+
DB3	Irregular	Flat	Undulate	Rods	+
DB4	Irregular	Flat	Erose	Rods	+
DB5	circular	Flat	Entire	Rods	+
DB6	circular	Raised	Undulate	Rods	+
DB7	Irregular	Convex	Entire	Rods	+
DB8	circular	Convex	Entire	Rods	+
DB9	circular	Flat	Entire	Rods	+
DB10	circular	Flat	Undulate	Rods	+
DB11	Irregular	Raised	Undulate	Rods	-
DB12	circular	Convex	Entire	Rods	-
DB13	circular	Convex	Entire	Rods	-
DB14	Irregular	Flat	Erose	Rods	+
DB15	circular	Flat	Entire	Rods	+
DB16	Irregular	Raised	Entire	Rods	-
DB17	circular	Convex	Entire	Rods	+
DB18	circular	Flat	Undulate	Rods	+
DB19	circular	Flat	Undulate	Rods	-
DB20	Irregular	Flat	Erose	Rods	+
DB21	circular	Convex	Entire	Rods	+
DB22	circular	Raised	Undulate	Rods	-
DB23	circular	Convex	Entire	Rods	+
DB24	Irregular	Raised	Erose	Rods	+
DB25	Irregular	Flat	Undulate	Rods	+
DB26	Irregular	Flat	Undulate	Rods	+

Table 3: Biochemical characteristics of the bacterial isolates

Isolates	Catalase test	Oxidase test	MR test	VP test	Carbohydrate fermentation	Casein hydrolysis	Gelatin hydrolysis	Hydrogen sulphide production
DB1	+	-	-	+	+	+	+	-
DB2	+	-	-	+	+	-	+	-
DB3	+	-	-	+	+	-	+	-
DB4	+	-	-	+	+	-	+	-
DB5	+	-	-	+	+	+	+	-
DB6	+	-	-	+	+	+	-	-
DB7	+	-	-	+	+	+	+	-
DB8	+	-	-	+	+	+	+	-
DB9	+	-	-	+	+	-	+	-
DB10	+	-	-	+	+	-	+	-
DB11	-	-	-	+	+	-	-	-
DB12	-	-	-	+	+	+	+	-
DB13	+	-	-	+	+	+	+	-
DB14	+	-	-	+	+	+	-	-
DB15	+	-	-	+	+	+	+	-
DB16	+	-	-	+	+	-	+	-
DB17	+	-	-	+	-	+	-	-
DB18	+	-	-	+	+	+	+	-
DB19	+	-	-	+	+	+	+	-
DB20	+	-	-	+	+	-	+	-
DB21	+	-	-	+	+	+	+	-
DB22	+	-	-	+	-	-	-	-
DB23	+	-	-	+	+	+	-	-
DB24	+	-	-	+	+	+	+	-
DB25	+	-	-	+	+	-	+	-
DB26	+	-	-	+	+	-	+	-

Table 4: Screening of selected bacterial isolates for multifarious plant growth promoting traits

Isolates	P solubilization	Growth on N free medium	HCN production	Siderophore production	Cellulolytic	Antagonism against	
						Fusarium oxysporum	*Pythium ultimum*
DB1	+++	++	+++	++	+	+	+
DB2	++	++	-	++	-	-	-
DB3	+	+	+	-	+	+	-
DB4	++	-	+++	+	-	-	-
DB5	++	+++	-	+	+	+	-
DB6	-	-	++	+++	-	+	-
DB7	+++	+	+	++	-	-	-
DB8	-	++	++	-	-	-	-
DB9	-	-	+	+	+	+	-
DB10	++	+	-	+++	-	+	-
DB11	++	+	+	-	+	-	-
DB12	-	++	++	+	-	+	-
DB13	+	+	-	+	-	+	-
DB14	+++	+++	+	+++	-	-	-
DB15	-	-	-	++	-	+	-
DB16	-	+++	-	++	+	-	+
DB17	++	-	-	+	-	-	-
DB18	++	+++	+	-	-	-	+
DB19	-	-	++	-	-	+	-
DB20	+++	-	-	++	+	-	+
DB21	-	-	+++	+++	-	+	-
DB22	++	+++	+	+	-	-	+
DB23	++	-	-	-	-	-	+
DB24	+	++	-	-	-	-	+
DB25	+++	++	-	++	-	-	-
DB26	+	++	++	+++	-	+	-

*values ranging from 55-75 % (++), ≤ 55% (+), ≥ 75 % (+++), no activity (-) ; ** values ranging from 20-25 (++), ≤ 20 (+), ≥ 25 (+++), no activity (-) ; ***values ranging from 50-80 % (++), ≤ 50% (+), ≥ 80 % (+++), (-) no activity ; # Values ranging from 20-35 (++), ≤ 20 (+), ≥ 35 (+++), no activity (-)

Table 5: Screening of selected actinomycelial isolates for multifarious plant growth promoting traits

Isolates	P solubilization	Growth on N free medium	HCN production	Siderophore production	Cellulolytic
DA1	+++	-	-	++	+
DA 2	++	+++	-	+	-
DA 3	+++	-	-	-	-
DA 4	++	+	++	+	-
DA 5	++	+++	+	-	-
DA 6	+	-	++	+++	-
DA 7	+++	++	++	+++	+
DA 8	-	++	++	-	-
DA 9	+++	-	+	+	-
DA 10	++	+	-	+++	-
DA 11	+++	++	-	-	-
DA 12	-	-	+++	+++	-
DA 13	+++	++	-	+++	-
DA 14	-	-	+	++	-
DA 15	-	-	-	++	+
DA 16	-	++	-	++	-
DA 17	+++	++	+++	++	-

Table 6: Screening of selected fungal isolates for multifarious plant growth promoting traits

Isolates	P solubilization	Growth on N free medium	HCN production	Siderophore production	Cellulolytic
DF1	++	++	+	++	-
DF2	+++	+	-	++	-
DF3	+	+	+	-	+
DF4	+++	-	+++	+	-
DF5	++	+++	+	-	-
DF6	+	-	++	+++	-
DF7	++	-	+	++	+

Isolates	P solubilization	Growth on N free medium	HCN production	Siderophore production	Cellulolytic
DF9	+++	-	+	+	+
DF10	++	+	-	+++	-
DF11	+++	++	-	-	-
DF12	++	++	+	++	+
DF13	-	++	++	+	+
DF14	+++	++	++	+++	+
DF15	++	-	-	++	-
DF16	-	++	-	++	+
DF17	+++	++	-	++	-
DF18	++	++	+	+	-
DF19	-	-	++	-	-
DF20	+++	-	-	++	+
DF21	-	-	+++	+++	-
DF22	++	+++	-	+	-
DF23	+++	-	-	-	-

Table 7: Dual culture compatibility assessment amongst eleven bacterial isolates of tomato seedlings

Bacterial Isolates	Synergism with actinomycetes isolates	Synergism with fungal isolates	Compatibility
DB1	DA7	DF14	+
DB1	DA17	DF12	-
DB1	DA7	DF12	-
DB1	DA17	DF14	-
DB 22	DA7	DF14	-
DB 22	DA17	DF12	-
DB 22	DA7	DF12	-
DB 22	DA17	DF14	+