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Actinomycetal Diversity of Western Region of Madhya Pradesh

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Abstract

Actinomycetes are peculiar group of microorganisms with numerous members having different kinds of features and extensive commercial importance. The strains present in the soil depend on the geographical area and climatic conditions. A collection of *Actinomycetes* was developed, predominating *Streptomyces*. Enzyme profiling for all the cultures was performed. The selected cultures were characterized biochemically. 16S rRNA sequencing was performed to identify the isolates to genus level.

Key words: Enzyme profiling, actinomycetal characterization, sequencing, diversity

Introduction

Actinomycetes, a heterogeneous group of microorganisms are present in abundance in soil. Their market share in antibiotic production is already well noticed and they are also proving their importance in the enzyme market. Since Waksman's discovery for streptomycin, hundreds of them have been explored by researchers for the production of antimicrobials against drug resistant pathogens¹. They are well known for the production of Amylase^{2,3}, Protease^{2,3}, Lipase⁴, Glucose isomerase⁵, Cellulase, Lignin peroxidase⁶. This unique group of microorganisms is responsible for the special fragrance of Geosmins (earthy smell) which can be felt after first monsoon shower⁷.

Actinomycetes have proved their importance as biocontrol agents, the antagonistic activity⁸ of *Streptomyces griseoviridis* towards a variety of plant pathogens including *Alternaria*

brassicola, *Botrytis cinerea*, *Fusarium avenaceum*, *F. culmorum*, *F. oxysporum* have been investigated⁹. Some of them have been reported as lignocellulose⁶ decomposers and also the source of antibiotics. The strains which exhibit the abilities to degrade lignocellulose and antagonize fungal root pathogens can be employed as efficient biocontrol agents. *Actinomycetes* are also known to produce agroactive compounds. Polyoxin B and D produced by *Streptomyces cacaoivar*, Mildiomycin by *Streptoverticillium rimofaciens* and Validamycin A were found to work as fungicides⁹. *Actinoplanes philippinensis*, *Actinoplanes missouriensis* and *Streptomyces clavuligerus* were reported to possess chitinolytic activity against *Drosophila melanogaster*¹⁰. *S. naganishii* and *S. michigansis*, the halotolerant bacteria produce antivirals against tobacco mosaic tobamovirus (TMV) and potato Y potyvirus (PVY)¹¹. *Streptomyces* are also found to have antidermatophytic activity against *Trychophyton rubrum*¹².

Streptomyces were also noticed to increase the yield of shrimps in the aquaculture when added as probiotic in the feed of *Penaeus monodo*¹³. They are known to degrade humic substances^{14,15} and also solubilise phosphates¹⁶ in soil which increases the soil fertility. *Actinomyces* are treasure for pigments also¹⁷.

Much of the work has also been done on *Actinomyces* for understanding their morphology and growth response on different constituents of media^{18,19}. The formation of aerial mycelium and sporulation has also been studied at genetic level²⁰⁻²².

Owing to the versatile characteristics of this group of bacteria their identification and characterization has become a matter of great concern. Researchers have not only attempted partial and whole genome sequencing but also analysis by restriction fragment patterns²³. Moreover metagenomic studies are primarily aimed at sequencing commercially harnessable actinobacterial genes²⁴.

This study aims at understanding the diversity of *Actinomyces* in compost pit samples and pointing out some isolates which are of commercial importance.

Experimental

The soil samples were collected from different places like compost pits, garden soil, construction sites and natural vegetation soil. The samples were enriched with calcium carbonate²⁵. Isolation was performed on Bennett's agar, *Actinomyces* isolation agar and Glucose asparagine agar plates. The purified isolates were preserved on Bennett's agar slants.

Selection of isolates was purely on the basis of colonial characteristics. Major criteria for picking up the isolates were the colony's spore mass colour, texture and pigmentation. The isolates were further studied for their spore arrangement pattern by slide culture technique²⁶.

Selected cultures were picked up for biochemical characterization. These isolates were also simultaneously analyzed for producing hydrolytic enzymes like amylase, protease, lipase

and cellulase. Amylase production was checked by inoculating the cultures on Starch Agar plates. Protease production was checked on media containing gelatin for gelatinase and casein for caseinase and also on milk agar plates. Cellulose and CMC was incorporated in separate plates for checking cellulase production. Glucose isomerase was checked on xylose agar media²⁷. The sugar utilization test was performed in phenol red broth in tissue culture multiwell plates. These cultures were also checked for their ability to grow at different pH and temperature. The cultures producing good levels of glucose isomerase were selected for identification to genus level by 16s rRNA Sequencing.

All the cultures were also screened for their antimicrobial activity against six gram negative and three gram positive bacteria.

Results and Discussion

The samples yielded a wide variety of *Actinomyces* cultures which had diverse growth patterns. The collection had organisms growing at alkaline as well as acidic pH and a wide temperature range. They also produced varying degrees of enzymes.

Isolation

Actinomyces appear after 4 to 5 days on agar plates, while screening from soil and many a times are overgrown by typical bacterial colonies. The appearance of colonies between 2nd to 4th day seems to be like a typical bacterial colony. This is because of the formation of substrate and aerial mycelia but no sporulation. Confirmation of an actinomycetal colony can be done by observing the leathery texture of the colony. The colonies are tightly held on the agar surface like a plant on the soil surface.

Growth on solid Media

The isolates had typical features like velvety (Fig. 1a, 1d) powdery and chalky (Fig. 1c, 1e, 1f) growth. The characteristic feature of *Streptomyces*, the grey spore mass colour appeared with aging (Fig. 1a, 1c, 1e). Most of the antibiotic producers were pigmented (Fig. 1b, 1f). The appearance of cultures varied on different media. They differed in the time required for sporulation and attaining the spore colour on maturity. The isolates sporulated profusely on media containing wheat bran and the pigments

were produced to maximum on Bennet's agar media. Colonial patterns also varied in the collection as a single isolate responded differently in different situations. The media composition was found to influence the extent of sporulation and appearance of aerial mycelia which is in accordance with earlier researcher¹⁴. Some isolates were found to have web like thick fibrous appearance (Fig. 2a,2e) on their colony while some of them had water droplets on the colony surface (Fig. 2b). The colonies appear dome shaped with soft depressions on the top (Fig. 2e,2h). The colonies also developed concentric rings on aging (Fig. 2c,2d,2f). Production of melanoid pigments was widespread among the isolates (Fig. 2i). Our collection had some isolates which exhibited light coloured aerial spore mass and produced abundant soluble pigment (Fig 2i) contrasting the jet black colour spore mass possessing isolate which did not produce any pigment (Fig. 2g).

Growth in Liquid Media

In liquid, the cultures grew as small beads in clear transparent media like Bennett's broth. The morphology of beads was also peculiar for different isolates. (Fig. 3) Some produced smooth surface beads and others had fibrous outgrowths on the beads. The isolates having gray spore mass colour produced brown pigmentation with orange beads (Fig. 3c) and isolates with spore mass colour ranging from purple to orange had bright orange beads with orange soluble pigmentation in liquid media. The pigmentation in the liquid media also varies with the composition. The media containing soybean meal used for the production of antibiotics promoted varied pigmentation. Same isolate produced different pigmentation in presence of different carbon and nitrogen sources.

Slide Culture Technique

Pattern of spore arrangement was studied on Bennet's agar by slide culture technique. The spores were found to be arranged in monoverticillate with and without spirals and biverticillate with and without spirals. Some isolates had open spirals while others exhibited closed spirals.

Slide culture technique was helpful in determining the species of the isolates. Fig. 4a and 4b shows spiral spore chains attached to the aerial mycelium which are the characteristics of *Streptomyces*. Fig. 4c is showing densely packed spores along the hyphae, a typical feature of

Saccharomonospora. Monoverticillate arrangement of spores can be seen with spirals in Fig. 4e and without spirals in Fig. 4d. The verticils of aerial mycelium arising at regular intervals giving rise to spiral spore pattern are shown in Fig. 4d This isolate belongs to category *Streptovercillium*. The isolate MR1 has pale yellow appearance on solid media and the spore pattern indicates this to be belonging to *Micromonospora* Fig. 4d.

Enzyme Production

All the isolates were subjected to enzyme profiling, they were found to be very good amylase, protease, cellulase, lipase and glucose isomerase producers. The zone of hydrolysis was observed on fifth day of inoculation (Table 1).

Some isolates exhibited wide antimicrobial spectrum. Fifteen out of seventy five isolates were active against Gram positive bacteria and six were active against Gram positive as well as Gram negative bacteria.

Biochemical Characterization

The cultures selected for biochemical characterization were good glucose isomerase producers. All of them were found to be Gram positive. They had typical mycelial pattern with spiral spore chains. Most of them were able to use glucose, xylose and raffinose. They were found to belong to *Streptomyces* sp. according to the result of biochemical analysis.

Conclusion

The above study reveals the existence of a wide variety of *Actinomycetes* in compost pit and garden soil samples of this area of Madhya Pradesh. The indigenous diversity is very rich in enzyme producers and bioactive compounds. The capacity of huge number of isolates to degrade cellulose can be well correlated to the presence of lots of plant residues present in composts pits. High efficiency for production of enzymes like amylase, caseinase,

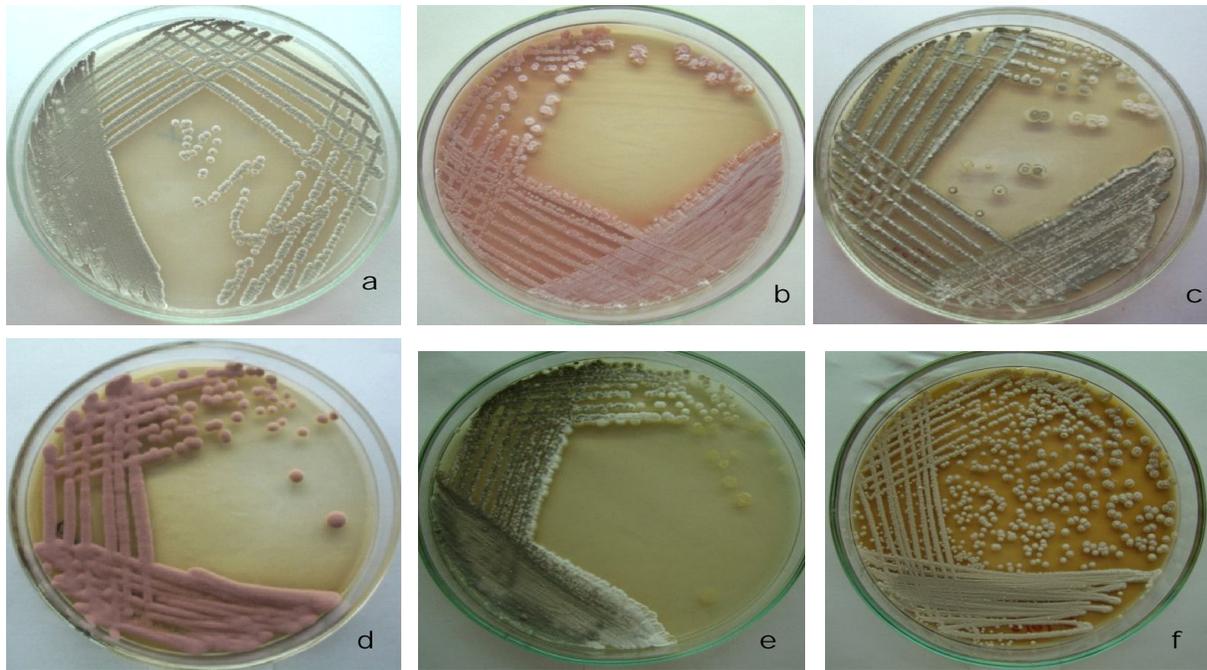


Fig.1. Typical growth pattern of Actinomycetes

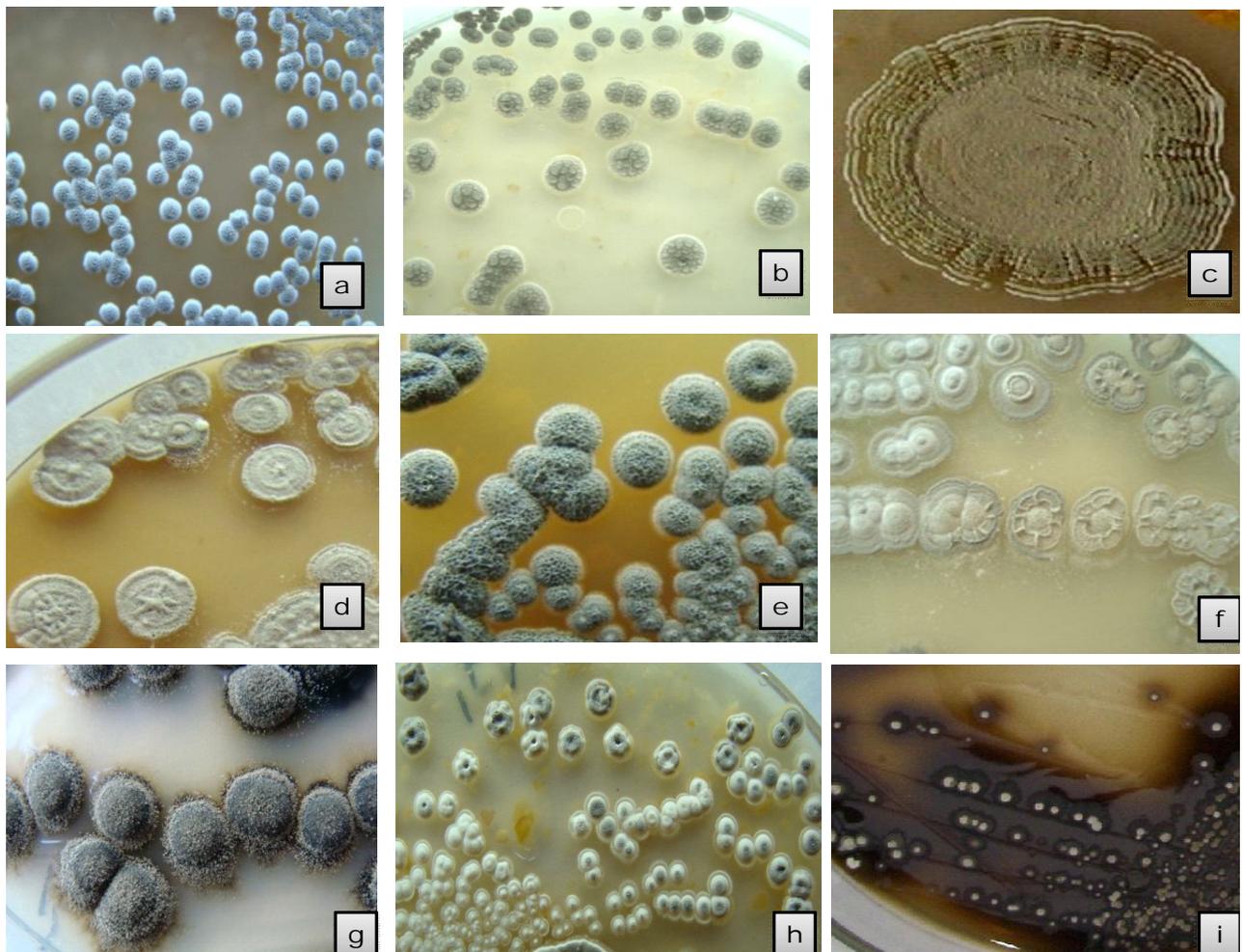
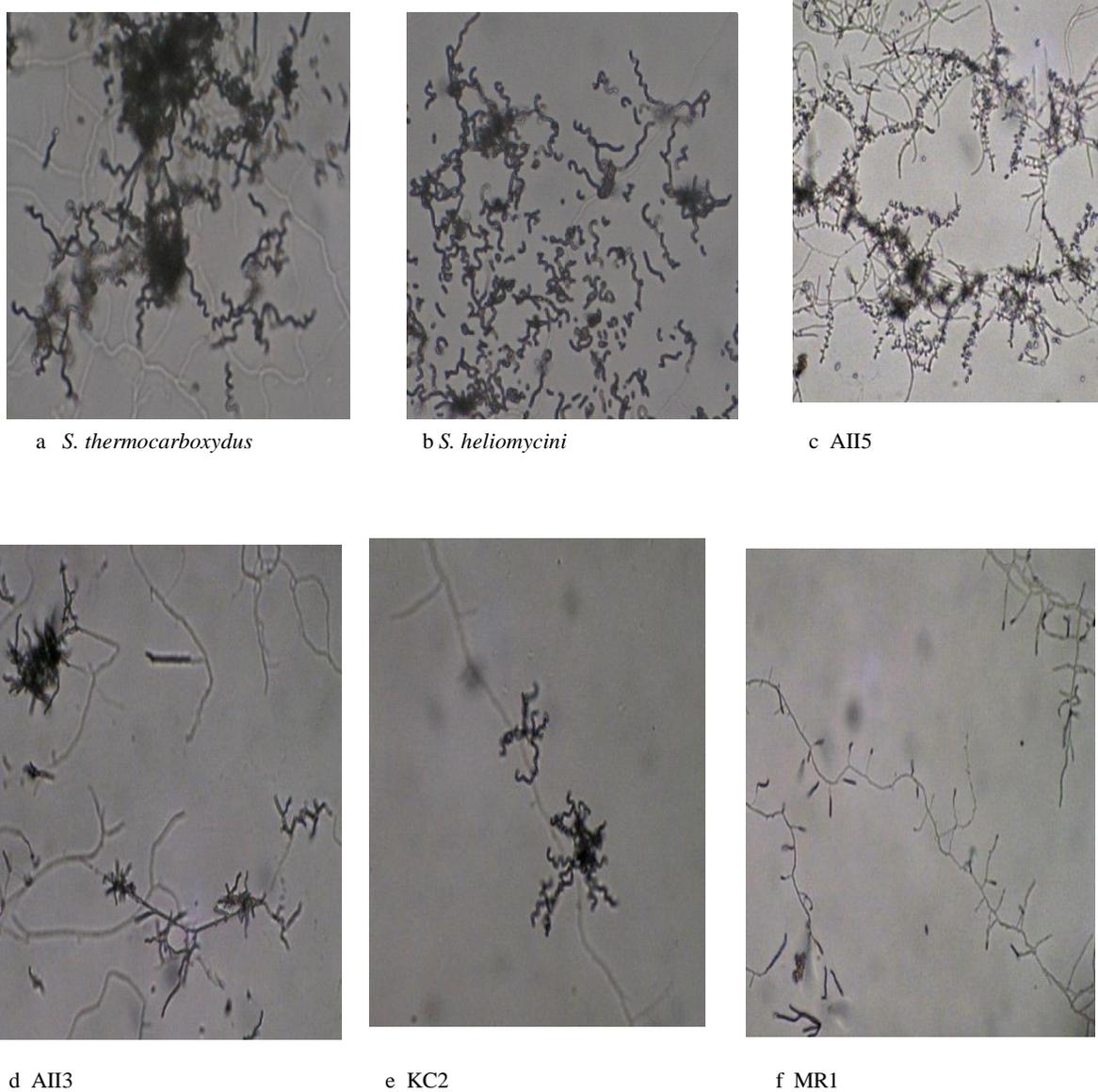


Fig. 2. Varied colonial patterns of isolates on solid media.



Fig. 3. Formation of different bead patterns of growth of isolates in liquid media.



a *S. thermocarboxydus*

b *S. heliomycini*

c AII5

d AII3

e KC2

f MR1

Fig.4. Spore arrangement pattern by Slide culture technique

Table 1 Enzyme profile of isolates [in terms of zone of hydrolysis (mm)]

Sr. No.	Isolate Name	Cellulase	Amylase	Lipase	Caseinase	Pectinase
1.	P1	40	30	-	-	32
2.	P2	37	35	-	-	40
3.	V1	58	41	-	-	26
4.	V2	22	26	-	-	-
5.	V3	25	27	-	44	-
6.	V4	45	45	-	-	22
7.	V5	44	-	47	-	28
8.	V6	41	48	-	-	-
9.	V7	44	35	-	-	37
10.	Ab	34	55	-	65	-
11.	KB1	44	40	31	-	23
12.	KB2	34	32	-	35	31
13.	KB3	40	32	-	-	25
14.	KB4	34	-	29	-	40
15.	M2	40	34	-	-	27
16.	M3	42	29	51	-	25
17.	M4	42	35	-	-	32
18.	Ga1	68	45	-	-	-
19.	Ga2	-	-	-	40	13
20.	Ga3	19	-	40	47	15
21.	Ga4	19	-	50	40	15
22.	Gu	-	-	26	-	-
23.	Gy1	45	-	-	36	30
24.	Gy2	36	-	-	-	22
25.	N1	18	-	-	-	-
26.	N2	44	-	-	45	-
27.	R1	-	-	-	-	34
28.	R2	34	-	38	30	19
29.	KC1	25	-	-	-	-
30.	KC2	38	-	-	-	39
31.	KC3	31	60	-	60	-
32.	KC4	33	33	-	-	-
33.	KC6	38	-	30	33	27
34.	KC5	-	-	-	-	10
35.	KC7	49	-	-	-	26
36.	KC8	38	-	39	-	-
37.	NPI1	-	-	-	62	-
38.	NPI2	-	26	35	-	18
39.	NPI3	31	-	-	36	18
40.	NPI5	33	-	-	-	15
41.	NPI6	41	29	-	33	24
42.	NPIII	35	-	-	-	32
43.	NPII2	47	-	-	-	-
44.	NPII4	35	-	-	-	-
45.	NPII5	42	24	-	-	-
46.	NPII6	-	-	50	-	-
47.	KNI1	45	-	39	48	27
48.	KNII1	27	20	-	-	-
49.	KNII2	40	-	21	30	26
50.	KNII3	50	-	-	-	-
51.	KNII4	44	-	42	-	-
52.	AII2	37	47	48	47	18
53.	AII3	40	47	43	40	29
54.	AII4	25	-	22	-	-
55.	AII5	35	-	37	61	-
56.	BII1	25	-	30	38	-
57.	MJ1	28	-	25	-	-
58.	MJ2	30	-	34	35	26
59.	VJ1	32	-	30	-	-
60.	VJ2	38	-	-	-	-
61.	KV	34	-	-	-	-
62.	S1	33	24	-	-	-
63.	S2	-	26	-	-	41

and lipase was also widespread among the isolates which signify their active involvement in organic matter degradation and biomanuring. The efficient producers of antimicrobials and enzymes can be put to use for commercial production. This ensures once again, that the nature still has hidden treasures, which can be of help in bioremediation, mineralization and production of health care products.

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