IOSR Journal of Environmental Science, Toxicology and Food Technology (IOSR-JESTFT)
e-ISSN: 2319-2402,p- ISSN: 2319-2399.Volume 9, Issue 6 Ver. III (Jun. 2015), PP 07-13
www.iosrjournals.org

Diversity of Actinomycetes in Parboiled Rice Mill Effluent

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Abstract: *A Study Was Conducted To Find The Diversity Of Actinomycetes Present In Parboiled Rice Mill Effluent Situated In Balod District Of Chhatisgarh State, India. Samples Were Collected From Two Sites, One Being Effluent Outlet Drain And The Other The Paddy Soaking Tank. Sixteen Isolates Of Actinomycetes Have Been Recovered From Parboiled Rice Mill Drain Effluent. No Actinomycetes Could Be Recovered From Paddy Soaking Tank. Streptomyces Was The Most Prominent Genus Recovered. Rest Of Them Belonged To The Genus Micromonospora, Nocardia, Kitaspora And Streptosporangium. Actinomycetes Show Diverse Colony Characteristics Which Changes With Different Cultural Conditions, While Morphological Characters Remain Same.*

Keywords : *Actinomycetes, Diversity, Effluent, Parboiled, Rice Mill.*

I. Introduction

Due To Huge Cultivation And Multiple Varieties Of Rice Grown In Chhattisgarh, It Is Commonly Called As 'Bowl Of Rice', And Rice Is One Of Staple Crop Here, For Which Parboiled Rice Is Processed In Parboiled Rice Mills. It Is A Popular Product Throughout The Country. It Is Consumed In A Sizable Quantity All Through The Year. Husk And Part Of The Bran From Paddy Are Removed To Produce Edible Rice By Rice Mill Processing. Parboiling Is A Pre Milling Process For Paddy Which Is Known To Originate In India [1] . A Large Amount Of Water For Soaking Of The Paddy Is Required For The Process Of Parboiling. Two Simple Steps Are Performed – Firstly Soaking In Hot Water At 60-70º C For 3 - 3.5 Hrs [2], And Then Secondly Water Is Drained Out. If Not Properly Treated, This Water Results In Water Pollution. It Also Causes Odour Nuisance To Nearby Residents. Water Pollution Is Caused By High Levels Of Organic Material Present In Effluent [3]. High Values Of Bod, Cod And Organic Contents Are Recorded In This Effluent. Organic Contents Are Mainly In The Form Of Starch And Pieces Of Husk. Thus, It Has The Potential To Deteriorate And Damage The Environment [4]. It Is A Rich Source Of Carbohydrates And Vitamins And Forms A Good Source Of Food For Microbes And To Flourish [4]. As Actinomycetes Are Widely Spread In Water Bodies, They Play An Important Role In The Natural Mineral Cycles Owing To Their Ability Of Growing At Low Concentrations Of Carbonaceous Substances As Well As To Degrade Recalcitrant Organic Substances[5]. They Are Well Known Also For The Production Of Primary And Secondary Metabolites Which Are Of Immense Economic Importance. Though Fungi Were Discovered As First Antibiotic Producers (Penicillin), Actinomycetes Are Now Known As A Major Group Of Microbes That Produce More Number Of Antibiotics. As Antibiotics Exhibit Life Saving Properties, They Are Of Immense Help To Mankind. After The Antibiotics Were Discovered As Medicines, A Revolution Has Occurred In Farming And Industries. Sometimes They Are Correctly Known As The "Medical Marvels". A Wide Range Of Important Enzymes Are Produced By Actinomycetes, Some Of Which Are Produced On An Industrial Scale. They Play A Major Role In The Pharmaceutical Industry. Some Enzymes, E.G. Lipase, Amylase And Cellulase From Actinomycetes Are Important In Textile, Fermentation, Food, And Paper Industries [6]. They Have The Ability To Degrade A Wide Range Of Hydrocarbons, Pesticides, And Aliphatic And Aromatic Compounds. They Perform Microbial Transformations Of Organic Compounds, A Field Of Great Commercial Value. Many Genera Of Actinomycetes Have The Potential For Use In The Bioconversion Of Wastes Into High-Value Chemical Products. Few Members Of This Group Of Microbes Have Been Found To Degrade Pesticides With Widely Different Chemical Structures. According To Some Scientists, The Fresh Water Habitats May Be Sources Of Actinomycetes That May Produce Useful Metabolic Products [7]. The Actinomycetes Participate In The Turnover Of The Soil Components And Transformation Of Organic Compounds [8,9,10]. In Soil Major Function Of Actinomycetes Is Decomposition And Mineralization Cycles With The Production Of Extracellular Enzymes, Such As Cellulases, Amylases .

II. Materials And Methods

The Materials Used And Methodology Followed Was As Described As Under :

2.1. Collection Of Samples

Samples Were Collected In Air Tight Plastic Bottles From Two Sites At Parboiled Rice Mill.Viz- From Paddy Treatment Tank And From Effluent Out Let Drain And Brought To The Laboratory Was Further Experiments

2.2. Isolation Of Actinomycetes

Isolation Of Actinomycetes Was Done Using Selective Medium – Asparagine Glycerol Agar. Dilution And Pour Plate Method Was Followed. The Plates Were Incubated At 28˚C For One Week. Thereafter Colonies Were Picked And Streaked On Aga Medium Plates To Check Purity . They Were Incubated. For One Week At 28˚C Temperature. Then Pure Colonies Were Picked And Transferred To Slants.

2.3. Characterization Of Actinomycetes

A. Gram Staining

B. Study Of Morphological Characters

25 Ml Of Sterilized A.G.A. Medium (Hi-Media, Difco) Was Poured As Aseptically In Sterilized Petri Plates. For Each Actinomycete Isolate The Plates Were Spot Inoculated In Triplicates. The Inoculated Were Kept At Room Temperature For 24 Hours To Promote Moderate Drying And To Check Sterility Before Incubation. The Next Day Sterilized Cover Slips Were Placed Aseptically At An Angle Of 45 º In The Solidified Medium Near The Place Of Inoculation. Inoculum From The Slope Culture Spread Along The Line Where The Upper Surface Of The Cover Slip Touches The Agar Surface. The Plates Were Incubated At 28 º C For 21 Days. The Cover Slip Was Removed From The Culture Plates, Stained In Cotton Blue And Observed Under The Microscope [11]. This Work Is A Portion Of My Thesis Which Had Involved Study Of Effect Of Pesticides On Actinomycetes. Hence Two Isolates Were Identified By 16s Rrna Sequencing Method Viz. S.Lilaceus And S. Luridus Which Were Selected For Further Experiments. This Data And Data For Biochemical Properties Are Not Given Here.

III. Results

After Gram Staining, The Isolates Which Were Gram Negative Were Discarded. Further Experiments Were Done By The Rest Sixteen Isolates Which Were Found To Be Gram Positive. The Gross Morphology Of The Actinomycetes Was Studied By Simple Coverslip Technique Devised By [11]. Temperature Of Incubation Was 28˚C. No Actinomycetes Could Be Recovered From Paddy Treatment Tank Probably Due To High Temperature. Principal Genera Identified According To Morphological Descriptions, Size Of Conidia And Mycelium Given In Bergy's Manual [12] Were Streptomyces, Micromonospora, Nocardia And Streptosporangium**.**

Isolate No. Cp – 1
Micromonospora Genus Recovered From Rice Mill Effluent
Colony Characteristics: Colony White Tough And Leathery, Smooth, With Flat Margins. 0.9 Cm In Size.

Morphology - Branched, Slender, Coenocytic Mycelium, 1.0 µm In Diameter, Aerial Mycelium At Maturity Forms Straight Conidiophores On Which Single Spherical Conidia Were Present. Size Of Conidia Was 1µm.

Isolate No Cp – 2
Streptomyces Genus Recovered From Parboiled Rice Mill Effluent

Colony Characteristics: Colour Of Colony White, Thick But Not Leathery, Flat Margins, Central Portion Raise . Size 1.2 Cm In Diameter.

Morphology : Slender, Coenocytic Mycelium, 0.5 – 0.7µm In Diameter, Spiral Sporangia, Spherical Spores Are Present, 1.2 µm In Diameter. (Plate No.8)

2. Isolate No. Cp – 3.
Streptomyces Genus Recovered From Parboiled Rice Mill Effluent
Colony Characters : Colony Colour White, Leathery. With Flat Margins 1.3 Cms Diameter.

Morphology - Slender, Coenocytic, Branched Mycelium, 1.0 µm In Diameter, Aerial Mycelium At Maturity Forms Straight Conidiophores On Which 10- 15 Spherical Conidia Are Present In Straight , Unbranched Chains. Size Of Conidia Was 1.2 µm . (Plate No.3)

Isolate No. Cp – 4.
Streptomyces Genus Recovered From Parboiled Rice Mill Effluent
Colony Characteristics : - Colony Colour White, White Initially, Concentric Alternating Zones Of Black Conidia And White Mycelium Appeared After 6 Days Of Incubation . With Flat Margins 1.7 Cm Diameter.

Morphology - Branched, Slender, Coenocytic Mycelium, 0.8 µm In Diameter, Aerial Mycelium At Maturity Forms Straight Conidiophores On Which 10- 15 Spherical Conidia Are Present In Straight , Unbranched Chains. Size Of Conidia Was 1.2 µm. Arthospores Present.(Plate No. 5)

Isolate No. Cp – 5.
Streptomyces Liliaceus Recovered Form Parboiled Rice Mill Effluent

Colony Characteristics: Colony Colour White, Rough, Concentric Rings Observed. With Flat Margins.

Morphology - Branched, Slender, Coenocytic Mycelium, 1.0 µm In Diameter, Aerial Mycelium At Maturity Forms Straight Conidiophores On Which 10 - 25 Spherical Conidia Are Present In Straight , Unbranched Chains. Size Of Conidia Was 0.8 - 1µm. (Plate No. 1).

Isolate No. Cp – 7
Nocardia Genus Recovered From Rice Mill Effluent
Colony Characteristics: Colony Pink , Smooth And Shiny With Flat Margins. 1.9 Cm In Size.

Morphology - Initially Slender, Coenocytic Mycelium Which Was 1.0 µm In Diameter Was Observed. Later Mycelium Fragmented Into Small Bacillary Forms.

Isolate No. Cp – 10
Streptomyces Genus Recovered Form Parboiled Rice Mill Effluent
Colony Characters – Colony Colour White, Smooth. With Raised Margins 1 Cm In Diameter.

Morphology - Branched, Slender, Coenocytic Mycelium, 1.2 µm In Diameter, Aerial Mycelium At Maturity Forms Straight Conidiophores On Which 10 - 15 Spherical Conidia Are Present In Straight , Unbranched Chains. Size Of Conidia Was 1µm.

Isolate No. Cp – 12.
Streptomyces Luridus Recovered Form Parboiled Rice Mill Effluent.
Colony Characteristics : Colony Colour White, Smooth. With Raised Margins, 1.4 Cm In Diameter.

Morphology - Branched, Slender, Coenocytic Mycelium, 1.0 µm In Diameter, Aerial Mycelium At Maturity Forms Straight Conidiophores On Which 10- 25 Spherical Conidia Are Present In Straight , Unbranched Chains. Size Of Conidia Was 1µm.

Isolate No Cp – 14.
Streptomyces Genus Recovered Form Parboiled Rice Mill Effluent
Colony Characteristics: Colony Is Pale White Irregular In Shape. Maximum Length Measured Was 2.3 Cms. Smooth Textured And Margins Irregular And Raised.

Morphology - Branched, Slender, Coenocytic Mycelium, 1.0 – 1.5 µm In Diameter, Aerial Mycelium At Maturity Forms Straight Conidiophores On Which 10- 25 Spherical Conidia Are Present In Straight , Unbranched Chains. Size Of Conidia Was 1µm.

Isolate No Cp – 15.
Streptomyces Genus Recovered Form Parboiled Rice Mill Effluent
Colony Characteristics: Colony Pale White, Greenish In Centre. 1.6 Cm In Diameter.

Morphology - Branched, Slender, Coenocytic Mycelium, 1.0 µm In Diameter, Aerial Mycelium At Maturity Forms Straight Conidiophores On Which 10- 15 Spherical Conidia Are Present In Straight , Unbranched Chains. Size Of Conidia Was 1µm.

Isolate No Cp – 16.
Streptomyces Genus Recovered Form Parboiled Rice Mill Effluent
Colony Characteristics: White Tough And Leathery, With Flat Margins, 1.9 Cm X1.4 Cm

Morphology - Branched ,Slender, Coenocytic Mycelium, 1.0 µm In Diameter, Aerial Mycelium At Maturity Forms Straight Conidiophores On Which 10- 15 Oval Conidia Are Present In Straight, Unbranched Chains. Size Of Conidia Was 1µm X 0.6 µm.

Isolate No. Cp – 17
Streptosporangium Genus Recovered From Parboiled Rice Mill Effluent.
Colony Characteristics: Colour Of Colony White Cottony Rough Appearance 2.1 Cm In Diameter.

Morphology - Branched, Slender, Coenocytic Mycelium, 1.5 µm In Diameter, Aerial Mycelium At Maturity Forms Spherical Sporangia Which Bears Spores. Very Short Sporangiophore Were Observed Bearing Single Sporangia. Size Of Sporangia Was Found To Be 1.7 µm.

Isolate No. Cp – 18.
Streptomyces Genus Recovered Form Parboiled Rice Mill Effluent
Colony Characteristics: Colony White Tough And Leathery, Smooth ,With Flat Margins. 1.7 Cm In Size.

Morphology - Branched, Slender, Coenocytic Mycelium, 1.0 µm In Diameter, Aerial Mycelium At Maturity Forms Straight Conidiophores On Which 10-15 Spherical Conidia Are Present In Straight , Unbranched Chains. Size Of Conidia Was 1µm.

Isolate No. Cp – 19.
Streptomyces Genus Recovered Form Parboiled Rice Mill Effluent.
Colony Characteristics: Colony White Tough And Leathery, Smooth, With Flat Margins. 0.9 Cm In Size.

Morphology - Branched, Slender, Coenocytic Mycelium, 1.0 µm In Diameter, Aerial Mycelium At Maturity Forms Straight Conidiophores On Which 10- 15 Spherical Conidia Are Present In Straight , Unbranched Chains. Size Of Conidia Was 1µm

Isolate No. Cp – 20.
Streptomyces Genus Recovered From Parboiled Rice Mill Effluent
Colony Characteristics: Colony Buff Coloured Tough And Leathery, Rough , With Flat Margins. 1.3 Cm In Size.

Morphology - Branched, Slender, Coenocytic Mycelium, 1.0 µm In Diameter, Aerial Mycelium At Maturity Forms Straight Conidiophores On Which 8-30 Spherical Conidia Are Present In Straight , Unbranched Chains. Size Of Conidia Was 1µm.

Morphological Properties Of Isolates Studied.

Isolate No	Breadth Of Mycelium	Reproductive Structure	Conidia/Spore
Cp- 1 Micromonospora	0.5 -.07µm	Short Conidiophore	Size: 1.2 µm, Shape : Spherical, Number: 1, Arrangement: Single Spore
Cp-2 Streptomyces	1.2 µm	Spiral Sporangia	Size: 1.2 µm, Shape : Spherical, Number: 10-15, Arrangement: In Chains
Cp-3 Streptomyces	1.0µm	Straight Sporangia	Size: 1.2 µm, Shape : Spherical, Number :10-15, Arrangement: In Chains, Arthospores Present
Cp-4 Streptomyces	0.8µm	Straight Sporangia	Size: 1.2 µm, Shape : Spherical, Number: 10-15 Arrangement: In Chains
Cp-5 Streptomyces	1.0µm	Straight Conidiophore	Size :0.8-1µm, Shape : Spherical Number: 10-25 Arrangement: In Chains
Cp-7 Nocardia	0.8-1 µm	--	Mycelium Fragmenting Into Bacillary Forms
Cp-10 Streptomyces	1.0µm	Straight Conidiophore	Size: 1.2 µm Shape : Spherical Number: 15-20 Arrangement: In Chains
Cp-12 Streptomyces	1.0µm	Straight Conidiophore	Size: 1 µm, Shape : Spherical , Number: 10-25 Arrangement: In Chains
Cp-14 Streptomyces	1.0µm	Straight Conidiophore	Size: 1 µm, Shape : Oval Number: 10-25 Arrangement: In Chains
Cp-15 Streptomyces	1.0µm	Straight Conidiophore	Size: 1.2 µm Shape : Spherical, Number: 10-15 Arrangement: In Chains
Cp-16 Streptomyces	1.0µm	Straight Conidiophore	Size: 1.2 µm X0.5 µm, Shape : Oval, Short Constrictions Between Each Spore, Number: 10-15 Arrangement: In Chains
Cp-17 Streptosporangium	1.5µm	Spherical Sporangia	Size: 1.5-1.7 µm, Number: 1 Sporangium, Arrangement: On Short Sporangiophore, Branched.
Cp-18 Streptomyces	1.0µm	Flexuous Sporangia	Size: 1.2 µm, Number: 8-20, Arrangement: In Chains
Cp-19 Streptomyces	1.0µm	Straight Conidiophore	Size: 1.2 µm, Number :15-20, Arrangement: In Chains
Cp-20 Streptomyces	1.0µm	Straight Sporangia	Size: 1.2 µm, Number: 8-30,Arrangement: In Chains

Photographs of the Slides Following Morphological Structures Were Observed
Genus: Streptomyces
Chain Of Conidia As Found In Streptomyces Sp

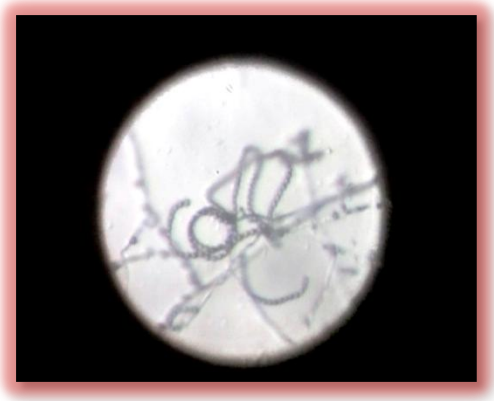

Plate No. 1

Plate No. 2

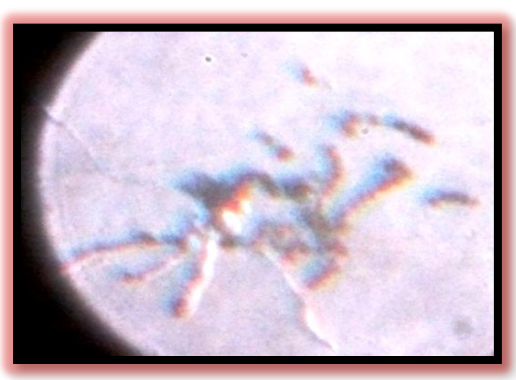

Plate No. 3

Plate No. 4

Orthospores Found In Streptomyces Sp

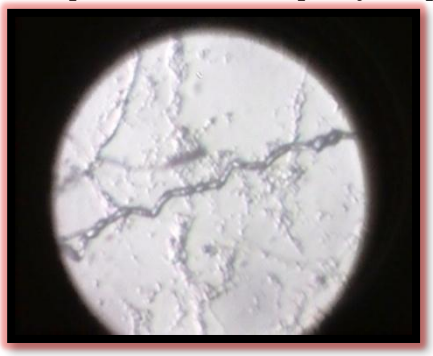

Plate No. 5

Flexuous Hyphae As Observed Of Streptomyces Sp

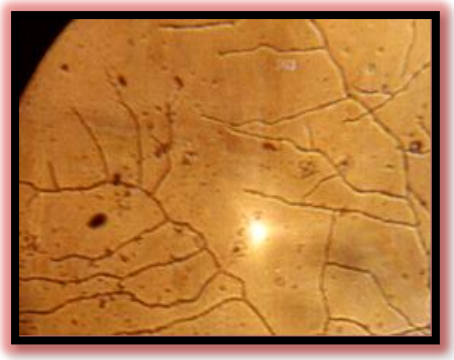

Plate No. 6

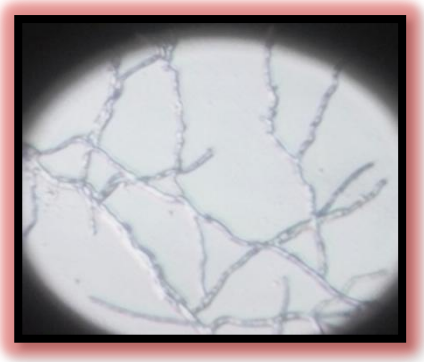

Plate No. 7

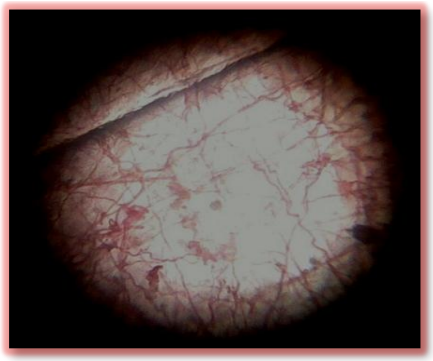

Plate No. 8 (Spiral Sporangium)

Plate No. 9 (Streptosporangium).

IV. Conclusion

Actinomycetes Show Remarkable Diversity In Their Colour Of Colony Which Changes With Different Cultural Conditions, While Morphological Characters Remained Same. They Show Colonies Of Various Colours Such As Pink, White, Grey, Brown, Green All In Different Shades. Some Of Them Also Showed Subsurface Growth. Colony Characters Vary As Smooth White To Tough And Leathery Structures Ray Like. Their Margins May Range From Smooth, Flat And Irregular To Margins With Raised Folded And Irregular Edges. Though Different Sporulating Structures Were Observed, Having One Thing In Common That The Spores And Conidia Are Borne In Chains In Genus Sterptomyces. No Spherical And Oval Sporangia Were Observed In Members Of Family Streptomycetaceae. The Sporangial Shape Was Straight, Spiral, Flexuous. It Was Observed That Straight Sporangia Were Always Present At Tips Of Hyphae. Structures Very Similar To Fungi Like Conidial Head And Broom Shaped Conidiophores Were Seen. But These Were Smaller In Size As Compared To Than Fungi. Also The Spore Size And Mycelium Width Did Not Exceed 2.0 Mm. Some Species Of Streptomyces Showed Formation Of Arthospores. Another Member Of Family Streptomycetaceae Was Isolated – Micromonospora Which Bears Single Conidia On Very Short Conidiophores. Only One Isolate Was Found To Be Micromonospora. One Isolate Each Of Nocardia Showing Fragmented Mycelium And Spherical Sporangium Bearing Streptosprangium Were Also Recovered From Parboiled Rice Mill Effluent.

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