

Preliminary and Biochemical Characterization of Bacterial Strains Isolated from Ramie Cane Soils

Darsi Phebe Sarah Koti Ratnam¹, Davala Simon²

¹Department of Botany & Microbiology, Andhra Christian College, Guntur, Andhra Pradesh, India

²Department of Botany and Microbiology, Acharya Nagarjuna University, Guntur, Andhra Pradesh, India

*Corresponding author email : phebesarah63@gmail.com

ABSTRACT

For the present study a total of 18 bacterial isolates were isolated from Ramie canes soils. Preliminary characteristics like colony characteristics and substrate concentrations were studied for the microorganisms. Physicochemical factors affecting the bacterial growth. The three seasons winter, summer and rainy seasons were also affected the growth of the bacteria. Various substrates like starch peptone, xylan peptone, Glycerol, Asparagine, glucose nitrate agar, carboxymethyl cellulose agar and pectin peptone agar media were studied. Maximum colony growth was recorded in pectin peptone agar media. Cream white, orange, red, yellow, lemon yellow and watery white colonies were recorded. The size of the bacterial strains was recorded in 1-55 mm in diameter. All the strains were entire lobed, convex having smooth and rough surface. of the 18 strains 11 were gram positive rods and the remaining strains were negative rods. Among the 18 strains, we were selected six strains for further studies like effect of seasons on growth and amino acid infiltration of bacteria. Maximum growth was recorded in winter season with 85.0 colony count in the presence of CCLS strain. There are 10 amino acids were introduced into the bacteria, with maximum growth was WCES bacterial strain. The results of this study reveal that the soil microflora associated with the ramie cane soils. These bacterial strains were used in the process of decortication and degumming.

Keywords : Ramie Canes, Isolation, Bacteria

I. INTRODUCTION

Ramie is a perennial herbaceous plant of the Utricaceae family, that provides a fibre of excellent quality. Harvest time and crop affected the agronomic performance and fibre properties of ramie. Ramie showed a good adaptability to the pedo-climatic conditions of the Mediterranean region, providing new perspectives for arable farming system. The crop persisted for upto 13 years giving stable off

take of bast fibre. The main change in the cell wall composition during the development concerns lignin and cellulose than hemicelluloses material.

Ramie has been grown in China for many centuries, and farmers in ancient China are known to have used the fiber to weave clothing. It may have been used in cloths for wrapping mummies in Egypt. Though ramie and flax are difficult to distinguish in ancient

cloth, ramie's resistance to bacteria and mildew would make it appropriate for mummy wrapping [1]. Ramie was used to produce an open-weave fabric called mechera, used for shirts and dressing gowns suitable for warm climates. The French painter Raoul Dufy designed in the early 20th century patterns for prints on mechera used by the French shirtmaker Charvet [2].

Brazil began production in the late 1930s with production peaking in 1971. Since then, production has steadily declined as a result of competition with alternative crops, such as soybeans and the important synthetic fibers [3].

Ramie (*Boehmeria nivea* L.) is one of the oldest crops in China and the second most important fiber crop in terms of area sown. Ramie root and leaf have therapeutic use in TCM. Thirteen genes that belong to the cellulose synthase gene family (four), the expansin gene family (three), and the xyloglucan [4]. Ramie is used to make such products as industrial sewing thread, packing materials, fishing nets, and filter cloths [5]. It is also made into fabrics for household furnishings (upholstery, canvas) and clothing, frequently in blends with other textile fibers (for instance when used in a mixture with wool, shrinkage is reported to be greatly reduced when compared with pure wool.) Shorter fibers and waste are used in paper manufacture. Ramie ribbon is used in fine bookbinding as a substitute for traditional linen tape [6].

Hence this work related to isolate the bacterial colonies from ramie cane soils. Various bacteria and fungi were present in the ramie cane soils. Therefore the present study mainly focussed on bacterial preliminary characterization, biochemical characterization and their optimization studies. These ramie canes were used as baits for obtaining effective decorticating and degumming microbes.

II. METHODS AND MATERIAL

Isolation of bacteria

Ramie canes were cut into small pieces with appropriate lengths (2"- 4") and were surface sterilized. The pieces were buried at a depth of 3" in the soil of coconut in holes bored with sterilized troves. The pieces were removed after desired interval and washed in gentle stream of water to remove excess soil. The canes were then placed on Glucosenitrate Agar, cellulose peptone Agar and incubated 96 hours and at 35⁰ C temperatures. After incubation the pure colonies were picked and maintained for further studies. 18 species of bacteria were isolated from soil surrounding Ramie canes [7].

Source of cultures

A collection of bacteria isolated from hot water spring environments is available in Botany research laboratory. Some of these bacteria were used in this study cultures of *Agrobacterium* and *corynebacterium* were obtained from national collection of industrial microorganisms, national chemical lab pune. In addition the cultures isolated through ramie baits were used in various experiments [8].

Culture incubation and colony morphology

Cultures were incubated at 26 to 28⁰ C in the laboratory and variation in temperature was recorded during the entire year study. In all the experiments the cultures were incubated for 8 days and the colony characteristics were studied. The colony characters like incubation period, grams test, margin, elevation and colour of the colonies were observed [9].

Substrates

Different substrates were used in the study for the maximum bacterial growth. The substrates were starch peptone, xylan peptone, Glycerol, Asparagine, glucose nitrate agar, carboxymethyl cellulose agar

and pectin peptone agar media used for the bacterial growth [10].

Amino acid infiltration

There are 10 amino acids were used in the study for the bacterial strains isolated in the ramie canes. These amino acids were used 10mg/litre was used. Major effects and the results were recorded. Infiltration was measured by the use of spectrophotometer at 480 nm [11].

III. RESULTS AND DISCUSSION

From the present study reveals that, a total bacterium of 18 bacterial strains were isolated from ramie canes.

Soil samples were air dried and by using 3 to 4 serial dilutions for the successful isolation of bacteria. The pure colonies were separated and maintained in slants. Freshly prepared pure colonies were spot inoculated on the plates. These plates were incubated for 72 hours on room temperature. Colony growth and morphology was observed in (Table-1). Among the 18 strains 4 strains LYCES-13, CCES-11, WCESP-2 and WCES-5 showed the white colour colonies. Maximum no of strains showed the cream in colour. The two strains CCERD-10 and CCERD-12 showed orange colour colonies.

Table -1. Colony Characteristics

Isolate no.	Colour of the colony	Size (mm)	Margin	Elevation	Surface	Grams test
CSIR-1	Cream	20	Lobed	Flat	Rough	Positive
LYCES-13	White	1	Entire	Convex	Smooth	Positive
CCES-11	White	3	Lobed	Convex	Smooth	Positive
WCESP-2	White	5	Wavy	Flat	Rough	Positive
WCES-5	White	4	Wavy	Convex	Smooth	Negative
WCRS	Cream	4	Lobed	Convex	Smooth	Positive
WSIR	Cream	4	Entire	Convex	Smooth	Negative
CCWSD	Cream	3	Entire	Flat	Smooth	Negative
CCLS-18	Cream	2	Entire	Convex	Smooth	Negative
<i>Pseudomonas</i>	Cream	5	Entire	Flat	Smooth	Positive
OCESC-16	Cream	4	Entire	Flat	Smooth	Positive
WCWPS-3	Cream	5	Entire	Convex	Smooth	Positive
WCLS	Lemon yellow	2	Entire	Convex	Smooth	Negative
WWCES-19	Yellow	3	Lobed	Convex	Smooth	Negative
CCESD-12	Orange	5	Entire	Flat	Smooth	Negative
CCERD-10	Orange	3	Entire	Convex	Smooth	Positive
RCES-17	Cream	2	Lobed	Convex	Smooth	Negative
CCRS	Watery white	3	Entire	Low Convex	Smooth	Positive

Effect of substrates on isolation of bacteria

The bacteria from ramie canes grew well in the substrates like starch, peptone, xylan peptone, glycerol Asparagine, Glucose nitrate agar, CMC and peptone agar. Among the 18 bacteria the strain CSIR-

1, WCES-5 and CCRS showed the maximum growth by utilising the substrates. The three strains *Pseudomonas*, CCWSD and WCCS showed minimum growth on by using these substrates. The two strains CCESD-12 and RCES-17 showed negative growth by means of there was no growth (Table-2).

Table -2. Effect of substrate on isolation of bacteria

Strain no.	Starch peptone	Xylan peptone	Glycerol asparginase	Glucose nitrate agar	Carboxy methyl cellulose peptone agar	Pectin peptone agar
CSIR-1	0	+	0	+	+	+
LYCES-13	0	0	0	+	0	+
CCES-11	+	+	+	+	0	+
WCESP-2	0	0	0	+	0	+
WCES-5	+	+	+	+	0	+
WCRS	0	0	0	+	0	+
WSIR	+	+	+	0	0	0
CCWSD	+	0	0	0	+	0
CCLS-18	+	0	0	+	0	0
<i>Pseudomonas</i>	+	+	0	0	+	0
OCESC-16	0	+	0	0	0	+
WCWPS-3	0	0	+	0	0	0
WCLS	0	+	0	+	0	0
WWCES-19	+	0	0	0	+	0
CCESD-12	0	0	0	0	0	0
CCERD-10	0	0	+	0	0	+
RCES-17	0	0	0	0	0	0
CCRS	0	+	0	+	+	0

Only one strain WWCES-19 which showed yellow in colour. The strain CCRS showed the watery white colony. The size of the colonies ranges from 1-20 mm in diameter. Maximum colony diameter was showed by CSIR-1 with 20 mm in diameter. Of these 18 bacterial strains margin appear on entire with lobed. The elevation of the bacterial colonies are maximum convex and flat with smooth and rough surface.

Finally the 10 strains are gram positive and the remaining strains showed gram negative rods. *Pseudomonas* strain which showed the best colony characters among the strains. Therefore we selected 6 strains for further studies like amino acid infiltration and seasonal effects also.

Seasonal effect of bacteria associated with Ramie canes

Table -3 represents the 6 bacterial strains were introduced into winter, summer and rainy seasons. The study as conducted in 24, 48 and 72 hours of intervals. Maximum colony growth was observed in 72 h of incubation with 85.00 average of colony

count. The strain CSIR-1 which showed the maximum colony growth in rainy season with 30.0 colony count at 48 h of incubation. The other strains CCES, *Pseudomonas* sp. and WCESP showed minimum colony count. Only one strain CILS showed least colony count on three seasons.

Table 3. Seasonal effect of bacteria associated with Ramie canes

Strain no	Winter			Summer			Rains		
	24	48	72	24	48	72	24	48	72
CSIR-1	17000	21000	19000	13000	10000	14000	18000	30000	23000
CILS	500	-	-	-	500	-	-	500	-
CCLS	1000	2000	85000	-	2000	-	-	12000	-
CCES	10000	3000	12000	10000	-	-	-	6000	-
P.SP	17000	-	7000	2000	7000	6000	5000	6000	6000
WCESP	1000	-	2000	2000	-	2000	3000	4000	1000

Effect of amino acid infiltration of bacteria

Table-4 represents that there are 10 amino acids were studied for this study. Three strains CSIR-1, WCRS and CCLS showed positively reacted on this amino acid infiltration. Majority of the strains showed negative results. These six strains selected for further studies like characterization.

Table -4. Effect of amino acid infiltration of bacteria

Strain no	CMPCA PLATES									
	1	2	3	4	5	6	7	8	9	10
CSIR-1	+	+	+	-	-	-	-	+	+	-
WCES-5	-	-	-	+	-	-	-	-	-	-
CCES-11	+	-	+	-	-	+	-	-	-	-
WCRS	-	-	-	+	-	+	-	-	-	-
CCLS	+	-	-	-	+	-	-	+	-	+
WCESP	-	-	-	+	-	-	-	-	+	-

IV. CONCLUSION

Ramie canes were used in the process of decorticating and degumming. The bacterial colonies obtained

from ramie cane soils showed the best colony characters with maximum colony count on winter season. All the strains showed the maximum positive characteristics on preliminary and biochemical characterizations.

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