ISOLATION OF POTENTIAL MICROORGAMISMS TO APPLY AS A COAGULANT FOR RUBBER (*Hevea brasiliensis*) LATEX.

K. G. A. Maduhansika[1,2], A. G. B. Arugoda[1], T. H. P. S. Fernando[2*], A. H. M. N. R. Aberathne [2], H. R. G. N. Peiris[2] and K. A. L. Vidushika[2]

[1]Department of Agricultural and plantation Engineering,
The Open University of Sri Lanka, Sri Lanka
[2]Department of Plant Pathology and Microbiology,
Rubber Research *Institute, Sri Lanka*

Rubber plant (*Hevea brasiliensis*) which belongs to the family Euphorbiaceae is a most economically important crop as natural rubber plays a significant role in the Sri Lankan economy. Rubber latex coagulation process is an essential step in the rubber production chain as the coagulation greatly influences the qualities of the final product. Though there are many chemical coagulants such as formic acid, acetic acid etc. used in the rubber industry, they have negative impacts on large amount of residual sludge formed at the end of the process. Hence the development of an eco-friendly coagulation method is critical for a sustainable rubber industry. The current study mainly focuses on isolating a potential microorganism for the coagulation of rubber latex and thereby developing a good coagulant product. Cup lump samples were collected from 05 locations of Dartonfield estate, Agalawatta, Sri Lanka. They were inoculated onto Potato Dextrose Agar (PDA) medium and incubated for five days. Five bacteria cultures were selected and sub-cultured on PDA medium. Morphological characters were observed on the PDA medium after 03 days of incubation. Gram staining was done for each bacterium using a standard protocol. Mass cultures were prepared using Potato Dextrose Broth (PDB). pH values of each isolate were measured for two weeks at a day interval. The time required for the coagulation of rubber latex by each bacterium was measured by adding bacteria solution to the fresh rubber latex and a control was maintained using formic acid. Ribbed Smoke Sheets (RSS) were prepared and dry weights were measured. All the bacterial samples showed approximately similar morphological characters with slight differences. Sample 02, 03 and 04 were Gram positive while sample 01 and 05 were Gram negative. All the samples showed different pH values. Sample 3 showed the least dry weight while sample 2 showed the highest value. Sample 03 can be used as a good natural coagulant to coagulate rubber latex as it coagulates rubber latex within a short time period (2 hours). Considering the above facts, it was concluded that sample 3 showed similar or comparable activity to the control. The use of biological agents for the latex coagulation provides an added advance as it is critical for the development of a sustainable rubber industry.

Key words – Bacteria, coagulant, formic acid, rubber latex

*Corresponding Author: *sarojinifernando787@gmail.com*

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Rubber Research *Institute, Sri Lanka*

INTRODUCTION

The rubber plantation industry plays a significant role in the Sri Lankan economy as rubber production is essential in human day-to-day life. Coagulation of latex is a crucial step in the rubber processing chain, as it significantly influences the properties of the final rubber product. In the commercial production of rubber, formic acid, acetic acid, and sulfuric acid are frequently used as latex coagulants (Othman and Chan, 1980; Chukwu *et al*., 2010). Modern advancements have introduced innovative techniques like Assisted Biological Coagulation, where specific bacterial strains or substrates are introduced to enhance carbohydrate fermentation and expedite the coagulation process (Krishnaswami and Yadav, 1976; Jayachandran and Chandrasekaran, 1998). In recent years, microwave-induced coagulation has emerged as a sustainable and green solution, potentially revolutionizing the industry.

However, excessive usage of the chemicals used in the latex coagulation process is harmful to the human as well as to the environment. Hence, the reduction of chemical usage is critical for sustainable production in the rubber industry. Microorganisms such as bacteria, which produce enzymes or acids for the acceleration of the latex coagulation process, can be used as an eco-friendly method. The current study focuses on the development of a microbial product through the isolation of naturally available potential microorganisms with specific enzymatic properties conducive to efficient and sustainable latex coagulation.

This study intends to address this problem by exploring the possibility of using native bacteria isolated from rubber cup lumps as a cost-effective and environmentally friendly replacement for latex coagulation. Furthermore, the research aims to provide a sustainable alternative by isolating the potential microorganisms, which not only lowers production costs but also complies with the industry's expanding environmental concerns. By seeking eco-friendly alternatives, the rubber industry can contribute to reducing its environmental footprint. However, the presenting study has the potential to provide novel, economical, and ecologically friendly solutions, helping both the rubber sector and the wider community by isolating and analyzing promising microorganisms for latex coagulation.

METHODOLOGY

Sample Collection

Five rubber cup lump samples were collected from different locations of Dartonfield Estate. The samples were collected into sterilized cups and sealed separately until transported to the laboratory.

Inoculation of samples

Each sample was inoculated to the separate Petri dishes with Potato Dextrose Agar (PDA) medium using a sterilized inoculation loop. Inoculated Petri plates were incubated at room temperature for five days.

Isolation of microorganisms

Five major bacteria colonies were selected from the resulting mixed cultures of microorganisms grown on the media using the literature knowledge. Pure cultures of each colony were prepared by sub-culturing them using the streak plate method.

Identification of microorganisms

Morphological characters

Morphological characters such as shape, size, color, surface appearance, and texture of isolated bacterial colonies were observed on PDA medium after 03 days of incubation at room temperature.

Gram staining

A drop of bacteria suspension was placed on a glass slide. It was spread on the glass surface and a thin smear was prepared. It was heat-fixed using the flame. A few drops of 12.1 mg/ml of Crystal Violet were added onto the slide and allowed to stand for 0.5 min. Later, a few drops of Gram's iodine were added directly onto the Crystal Violet on the slide for 0.5 min. The mixture of Gram's iodine and Crystal Violet was drained from the slide and washed rapidly with running tap water followed by a gentle flow of decolorizing fluid for 20 sec. The slide was washed rapidly with tap water and Safranin was added to the specimen for 20 seconds. The slide was rinsed with tap water and dried with a blotting paper. Prepared slides for each bacteria sample were observed under a light microscope.

Preparation of Potato Dextrose Broth (PDB)

Potatoes were peeled and cut into small pieces. They were boiled and crushed. The resulting potato mixture was filtered using a muslin cloth and 1 Liter of potato solution was prepared. A volume of 200 ml of potato solution was divided into five flasks. Later, 200 g of sugar was added into each flask and each 200 ml solution was made up to 1 Liter.

Mass culture preparation

Mass cultures were prepared by inoculating five isolated bacteria samples into previously prepared 150 ml of PDB medium in each flask. The inoculated flasks were incubated at room temperature for 2 weeks.

Comparison of pH

pH values of each isolate were measured for two weeks at one-day intervals.

Comparison of the coagulation time

One litre of fresh rubber latex was poured into each plastic tray. Five replicates of each bacterial sample were mixed to form a homogenized mixture. A volume of 300 ml of each homogenized bacteria mixture was poured into previously prepared trays. A volume of 200 ml of Formic acid was added to the control tray. Time taken for coagulation was recorded.

Ribbed Smoke Sheet (RSS) preparation

Coagulated rubber samples in each tray were subjected to prepare RSS in the factory of the Rubber Research Institute of Sri Lanka using a standard method. Milled sheets were cleaned and put in a shade to drip dry before being smoke-dried. The sheets were hung at a smokehouse for three to five days for smoke-drying. The dried sheets were subjected to weight.

Data Analysis

Data analysis was carried out using Mini tab software

RESULTS AND DISCUSSION

Identification and characterization of five isolated bacterial cultures to a certain extent were performed based on their colony morphology and results of gram staining. According to the morphological characterization, all the bacterial samples showed a smooth surface appearance. Furthermore, all the samples were white except sample 03 which showed transparent nature. All the colonies were irregular and had a flat elevation. Sample 02, 04 and 05 had an entire margin while sample 01 had an undulate margin and sample 03 had a lobate margin. According to the results of the Gram staining test, samples 02, 03, and 04 were gram-positive while samples 01 and 05 were gram-negative.

In the pH test, all the samples have changed their pH values more or less similarly with the time of 02 weeks. While there are no significant differences between samples 2 and 1, all the other pair-wise comparisons among the sample treatments showed statistically significant differences. Samples 3, 4, and 5 exhibited slightly higher pH levels compared to sample 1, with differences ranging from approximately 0.31 to 0.43 units. All the testing results were significant with adjusted p-values ($p < 0.05$). However, there are no significant differences observed between samples 4 and 2, nor between samples 5 and 2, indicating similar pH levels among these pairs of samples. The differences between samples 3 and 4, as well as between samples 3 and 5, are not statistically significant. In both biological and chemical contexts, the pH of a solution directly influences the rate of reactions, the stability of compounds, and the behavior of molecules. In this study, pH has directly influenced the coagulation process.

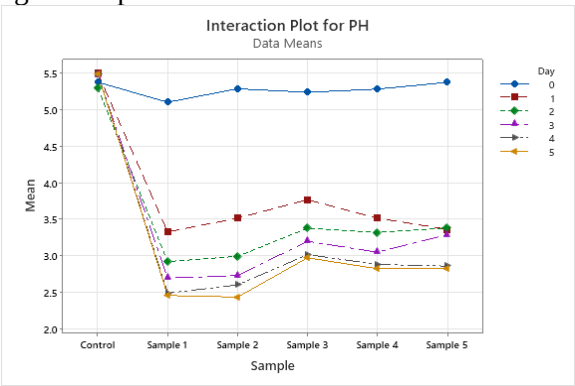

Figure 01: changing the pH of each bacteria sample with the time

Time taken for the coagulation is also a critical parameter in the rubber latex coagulation process as it can lead to improvements in product formulations and industrial processes. According to the results of the experiment, sample 3 was coagulated within a shorter period while sample 1 and sample 5 required more time compared to the control.

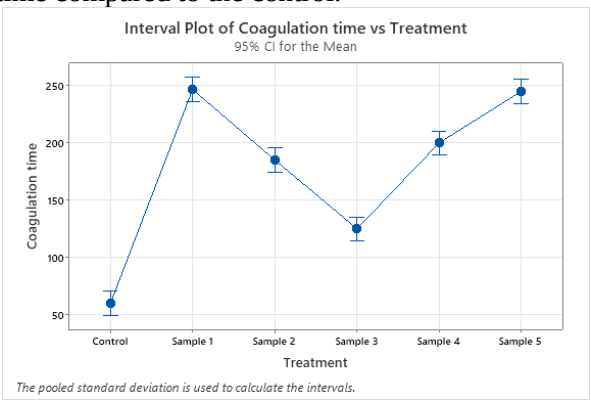

Figure 02: coagulation time(hours) required for each treatment

The dry weight of rubber sheets provides information about the material composition and density after the removal of all the moisture. This measure is critical in settings where precise material specifications are required for consistent production and application. Variations in dry weight can reflect changes in the process conditions, the quality of raw materials, or the efficiency of drying techniques. According to the experimental results, sample 3 showed a less dry weight while samples 2 and 5 showed the highest dry weight compared with the control.

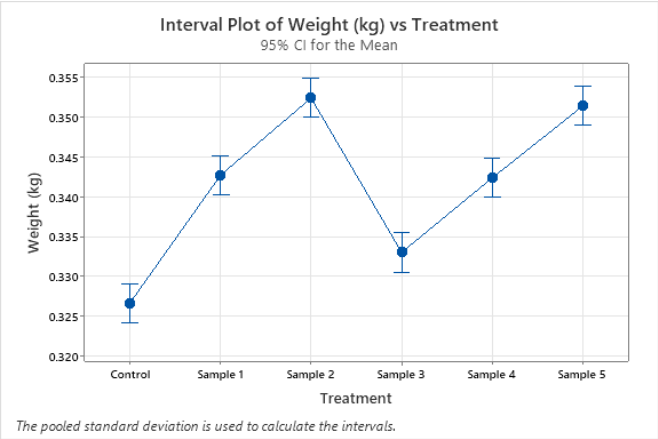

Figure 03: dry weight of each sheet

By considering the overall result of the study, sample 3 can be used as a good coagulant by further developing and testing their suitability to apply to the environment as mass production. The study may be useful to society as the rubber industry which produces a vast range of products plays a major role in the world. The use of biological agents for latex coagulation provides an added advance as it is critical for the development of a sustainable rubber industry.

CONCLUSIONS/RECOMMENDATIONS

According to the results of the study, all 5 latex samples with bacteria solutions were coagulated well. Among them, sample 3 required a shorter period for the rubber latex coagulation. Hence, it can be used as a good natural coagulant to coagulate rubber latex within a short period than using formic acid. However, the usage of formic acid is not eco-friendly as it can enter the natural ecosystem causing huge damage to humans as well as to the environment. Considering the above facts, it was concluded that sample 3 showed similar or comparable activity to formic acid; the control sample. However, the study should be expanded by increasing sample numbers, locations, and types of bacteria. Isolated bacterial cultures should be identified and characterized further for a good understanding. Molecular level identification and study of the properties of the final coagulated sheets are recommended.

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REFERENCES

Chukwu, M.N., Idiagha, J.A. and Ihuezor, M.O. (2010). Effect of acid coagulation level on the plasticity retention index (PRI) of natural rubber. *Multi-disciplinary Journal of Research Development*, 15(3): 1-4.

Jayachandran, K. and Chandrasekaran, M. (1998). Biological coagulation of skim latex using Acinetobacter sp. isolated from natural rubber latex centrifugation effluent. *Biotechnology Letters, 20*: 161-164.

Krishnaswami, P. and Yadav, D. P. (1976). Effect of four neighboring groups in kinetics of polymer reactions. *Journal of Applied Polymer Science volume*, 20(5): 1175-1185.

Othman, A. B., and Lye, C. B. (1980). Effect of pH Coagulation and Sulphuric Acid as a Coagulant on Natural Rubber Properties. *Journal of the Rubber Research Institute of Malaysia*, *28*(3).