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Research Article

Microbial Utilization of Ramie Gum: A Potential Avenue for Biotechnological Applications

SK Wasim Ahmed[I#], Espita Palwan[I#], Soumya Samal[I], Shaon Ray Chaudhuri*[I]

Microbial Technology Laboratory, Department of Microbiology, Tripura University, Suryamaninagar, Tripura West, Tripura 799022, India

[#]Equal contribution, [*]Corresponding author

Abstract

Ramie (*Boehmeria nivea*) contains approximately 30-35% gum, composed of cellulose, hemicellulose, lignin, pectin and waxes. Ramie gum is a rich source of carbon, which can be utilised as a media component for growing microorganisms. The current study was conducted to investigate the mode in which Ramie gum stimulates the growth of different bioremediating bacterial strains. The high concentration of total carbohydrate in the Ramie gum substituted Luria–Bertani (LB) broth enhanced the log phase of growth substantially for *Bacillus* sp. (MCC0008), *Micrococcus luteus* (SRCHD08), and *Pseudomonas* sp. (SRCOD5) being cellulase, amylase and pectinase producing strains. Bacterial strains *Brevundimonas diminuta* (SRCHD03), *Brucella pseudintermedia* (SRCHD05), and *Ochrobactrum* sp. (SRCHD06), which were non-producers of cellulase, amylase and pectinase, showed no impact of Ramie gum substitution in LB on their growth. These extracellular enzymes (cellulase, amylase and pectinase) can degrade cellulose, hemicellulose and pectin, producing bacteria-utilizable sugar compounds. The stimulation of biofilm formation might be attributed to the presence of pectin and xylan in the gum through the involvement of the Spo0A gene. The strains showing higher growth and biofilm stimulation demonstrated higher bioremedial ability. Hence, a waste (the gum) that needs to be removed for spinning of the fiber can be appropriately valorised through use as a bacterial feed.

Keywords

Ramie gum; microbial growth enhancement; natural carbon source; growth phase; cellulose; amylase; pectinase

Introduction

Microorganisms are the reservoir of biodiversity on earth, displaying diverse metabolic profiles while playing fundamental roles in biogeochemical cycles such as the carbon, sulfur, phosphorus, and nitrogen cycles for the circulation of the chemical elements within biotic and abiotic compartments in Earth's ecosystem [1,2,3]. Microorganisms also play a critical role in bioremediation through bioaugmentation, biostimulation, and natural attenuation. Beneficial microbes are involved in plant growth promotion and increasing productivity, either by supplying minerals and nutrients [4], secreting plant growth promoting phytohormones [5] for plant growth or by inhibiting the plant pathogens.

Six macronutrients, such as carbon, hydrogen, oxygen, nitrogen, sulfur, and phosphorus, are the main building blocks of life [6]. The growth of the microbe strongly depends on the cellular metabolic activity and synthesis of the biomolecules, including nucleosides, nucleotides, essential sugars, amino acids, lipids, and organic phosphates, which are the building blocks for DNA (deoxyribonucleic acid), RNA (ribonucleic acid), proteins, cell membranes, and bioenergetic compounds such as ATP (adenosine triphosphate). Microorganisms are highly

adaptive to changes in their surroundings, making them ubiquitous in the earth's ecosystems [4, 7]. Microbes can thrive under extreme environmental conditions, including nutritional and water scarcity [8]. The survival of the microbes in the extreme environments is supported by the functional or native conformation of the proteins or enzymes, genetic plasticity, and biofilm formation ability [8, 9].

Microbes can be cultivated in culture media containing nutrients like carbon, inorganic phosphate, nitrogen, sulfur, inorganic minerals, water, and vitamins [10]. Different enrichment media provide essential nutrients and growth factors for the microbial cells and play a regulatory role for microbial trade-off mechanisms and resource investment. Depending on the surrounding extracellular nutrient profiles, microbes adopt different strategies (resource acquisition, growth strategies, and maintenance) for the adaptation to the environment [11]. Plant-origin compounds have been continuously used in microbiological and biotechnological applications. Agar is one of the most commonly used plant-origin compounds in the life science field because of its clarity, stability, and non-interfering nature with the microbial metabolisms [12]. A study by Jain et al. (2005) on the potential application of guar gum as gelling agent in addition to agar on 33 microbial cultures (11 bacteria and 12 fungal cultures) revealed normal differentiation and growth in guar gum similar to agar [12, 13].

Lignocellulosic biomass is the cheapest source of carbon containing glucose, xylose, and arabinose monomers [14]. Utilization of the plant-origin Ramie (*Boehmeria nivea*) gum as an additive to diluted LB broth leads to enhanced microbial growth when compared to recommended concentration of LB broth alone [15]. It was reported that Ramie gum is composed of cellulose, hemicellulose, lignin, and pectin [15]. Microbes producing cellulase and xylanase can degrade the most abundant components of Ramie gum cellulose and hemicellulose, respectively [16]. Lignin can be broken down through laccases, peroxidases [17], and oxidase enzymes [18]. Pectin can be degraded by pectinase-producing bacteria [16]. Microbes having these enzyme-producing ability can degrade lignocellulosic materials into smaller units, which further helps to stimulate their growth [19]. Microbial utilization of lignocellulosic waste is a widely studied topic nowadays. It was reported that agro-waste can be valorised through bioethanol production by using microbial consortia (fungal and bacterial) followed by yeast-mediated fermentation [20]. Ray Chaudhuri et al. (2025) reported that lignocellulosic biomass of oil-extracted lemongrass can be valorised through mushroom cultivation [21]. Pre-composed Ramie decorticated waste was reported to be used for handmade paper making [22]. Literature reveals that complex polysaccharides like cellulose and xylan when used as carbon source for bacterial growth shows less antagonism among microbes when compared to growth on simple sugars like glucose [19]. Moreover, enriched medium often used for growing parent cultures in case of biofilm reactor setup at pilot/industrial scale for bioremediation and biotechnology purposes often discharges waste medium with high chemical oxygen demand (COD). This high COD is due to unutilized growth nutrients along with end product of microbial metabolism. The other alternative to minimizing this pollution would be to optimize the growth medium composition in a manner that there would be stimulation of bacterial growth and attachment with minimum discharge of unutilized growth nutrients. Hence, this study was conducted (a) to understand how and at which phase of growth the Ramie gum-supplemented LB medium stimulates the growth of different bioremedially important bacterial strains, (b) to understand whether the supplementation of 25% Ramie gum with 75% LB can stimulate the biofilm-forming ability of the microbes, (c) to decipher the possible mechanism of biofilm enhancement by the strains in response to gum polysaccharides, and (d) to check the elevated performance of the strains in terms of bioremediation ability by supplementing 25% Ramie gum with 75% LB.

Materials and methods

Ramie gum extraction

Five millilitre (5 ml) of 1% Na_2CO_3 solution was added to 1 g of Ramie fiber and boiled at 100°C for 20 minutes. After 20 minutes of boiling, the liquid was collected from the fibers. The total volume of collected liquid was measured, and the same volume of chilled acetone was added to it. The gum present in the solution was precipitated after adding chilled acetone. Finally, the acetone was decanted, leaving the precipitate behind. The precipitated gum slurry was dried in a hot air oven.

Enzyme production by the isolates

The microbes selected for the study were potent bioremediants. While *Bacillus* sp. (MCC0008) removes nitrate [23], *Pseudomonas* sp. (SRCOD5) removes ammonia, and *Micrococcus luteus* (SRCHD08), *Brevundimonas diminuta* (SRCHD03), *Brucella pseudintermedia* (SRCHD05), as well as *Ochrobactrum* sp. (SRCHD06), remove hexamine [24]. The capacity of bacteria to produce extracellular enzymes is an essential criterion for its identification. These profiles aid in identifying the functional characteristics of bacteria, such as their ability to use or break down particular substrates. The extracellular enzyme-producing ability of the isolates (MCC0008,

SRCHD03, SRCHD05, SRCHD06, SRCOD5, and SRCHD08) were checked using standard protocols and reported (Gogoi et al., 2024; extracellular protease, amylase, catalase, oxidase, DNase, and lipase as per Nandy et al., 2007; cellulase according to Kasana et al., 2008; pectinase according to Yao et al., 2017; and gelatinase following Cruz et al., 2012) [25, 26, 27, 28, 29].

Microbial growth on different concentration of media solution

Media solutions were made in different concentrations: 25% Ramie gum (0.25 gm in 100 ml distilled water), 100% LB [1 gm LB {1% Tryptone, 0.5% Yeast Extract and 0.5% sodium chloride (NaCl)} mix in 100 ml distilled water at pH 7.0], and Ramie gum: LB (25%:75%, 25%:50%, 25%:25%) were prepared and sterilized at 121°C for 15 minutes. Each concentration of media (200 microliters) was separately inoculated with 1% (v/v) of the culture {which could be *Bacillus* sp. (MCC0008), *Brevundimonas diminuta* (SRCHD3), *Brucella pseudintermedia* (SRCHD05), *Ochrobactrum* sp. (SRCHD06), *Pseudomonas* sp. (SRCOD5), and *Micrococcus luteus* (SRCHD08)} in biological replicates of three within 96-well plates. Then the plate was placed inside the microplate reader (Bio Tek, EPOCH2TS) for 28 hours at 37°C and the growth in terms of optical density (OD) was measured during the entire growth period at 600 nanometer (nm).

Biochemical Assay

The COD and total carbohydrate content of the media (25% Ramie gum in water, 100% LB, 75% LB, 25% Ramie gum with 75% LB) were quantified to decipher the mode of action of gum on microbial growth. Raw Ramie gum is not totally soluble in water; however, 25% Ramie gum was completely soluble in LB broth. The total carbohydrate estimation was carried out using the phenol sulphuric acid method [30]. COD was assessed using the dichromate method [25]. All estimations were done in triplicate followed by statistical validation of the finding.

Biofilm-forming ability

In a sterile 24-well tissue culture plate (Tarsons, Cat. No. 980030), 2 ml sterile media (100% LB and 25% gum with 75% LB) were added and inoculated with 1% of actively growing cultures of isolates MCC0008, SRCOD5 and SRCHD08 individually. Uninoculated media were considered as negative controls for the experiment. The plate was incubated for 24 hours at 37°C under static condition. After 24 hours, biofilm-forming ability was quantified using Martin's method [31] involving crystal violet staining. All estimations were done in biological triplicates with statistical validation of the data.

Bioremedial Study

Bioremedial ability under immobilized condition was tested for the isolates MCC0008, SRCOD5 and SRCHD08. Actively grown cultures were inoculated in 50 ml Falcon tubes containing sterile Raschig ring with 15 ml 100% LB and 25% gum in 75% LB broth, respectively. The tubes were incubated at 37°C for 24 hours for biofilm establishment on the surface of the Raschig ring. After the incubation, the cultures were decanted from the respective 50 ml Falcon tubes. Tubes with MCC0008 and SRCOD5 biofilm were recharged with 25% Ramie gum in 75% LB broth. On the other hand, the tube with the SRCHD08 biofilm was recharged with 25% Ramie gum in 75% LB broth with 100 mg/L hexamine. Nitrate (after 5 hours), ammonia (after 10 hours) and hexamine (after 24 hours) were quantified using the Salicylic acid method [32], Nessler's reagent method [33] and the Hantzsch reaction method [34], respectively. All estimations were done in biological triplicates with statistical validation of the data.

In silico analysis

The contigs of the genome sequence of MCC0008 were submitted to the Rapid Annotation using Subsystem Technology (RAST) server. The number of contigs were 331. The system (SEED viewer) was searched for the presence of genes involved in biofilm formation.

Statistical analysis

For the statistical analysis, an F-test followed by a T-test was performed. The statistical analysis was done at a 95% confidence level.

Results

Ramie gum extraction

Ramie fiber grown at Tripura University contains 30-35% Ramie gum, of which only 2.52±0.78% Ramie gum can be easily extracted.

Enzyme assay of the isolates

The enzyme-producing ability of the six isolates is given in Table 1. The enzyme production of isolate MCC0008 is reported in Banerjee 2018 [35], while those for SRCHD03, SRCHD05, SRCHD06 and SRCHD08 are reported in Samal et al, 2024 [24].

Table 1: Extracellular enzyme producing ability of the bacterial isolates.

Enzymes	MCC0008 [35]	SRCHD03 [24]	SRCOD5 (Unpublished data)	SRCHD06 [24]	SRCHD08 [24]	SRCHD05 [24]
Catalase	+	-	+	+	+	+
Oxidase	+	+	+	+	-	+
Protease	+	-	-	-	+	-
Amylase	+	-	+	-	-	-
Lipase	+	-	-	-	-	-
DNase	+	-	-	-	-	-
Cellulase	+	-	+	-	-	-
Pectinase	+	-	+	-	+	-
Gelatinase		+	-	+	+	+

Microbial growth enhancement by Ramie gum

Based on the previous report by Banerjee et al. (2018) [35], the initial set of the experiment was carried out with the following media composition: only LB (100%) and gum: LB (25%:75%) (Figure 1). The result showed a significant growth enhancement (Table 2) in 25% Ramie gum with 75% LB broth as compared to 100% LB broth for *Bacillus* sp. (MCC0008) *Micrococcus luteus* (SRCHD08), and *Pseudomonas* sp. (SRCOD5) as shown in the Figure 1, Table 3. However, the *Brucella pseudintermedia* (SRCHD05), *Brevundimonas diminuta* (SRCHD03), and *Ochrobactrum* sp. (SRCHD06) showed no significant growth difference (Table 2) among 100% LB and gum to LB ratio of 25%:75%.

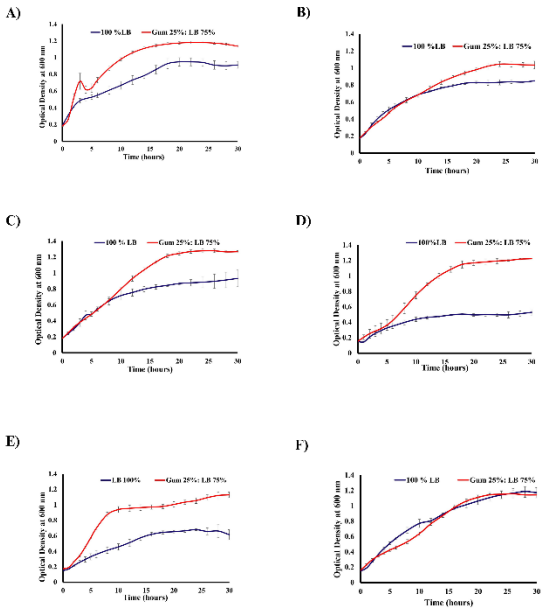

Figure 1: Growth curves of the isolates were done in biological triplicates using a 96-well microplate reader (Bio Tek, EPOCH2TS) for 28 hours at 37°C. In this study 100% LB broth and 25% gum:75% LB were used. (a) Growth curve for isolate MCC0008, (b) Growth curve for isolate SRCHD03, (c) Growth curve for isolate SRCHD06, (d) Growth curve for isolate SRCHD08, (e) Growth curve for isolate SRCOD5, (f) Growth curve for isolate SRCHD05.

Table 2: Statistical analysis of the growth difference of bacterial isolates in the presence of 25% gum with 75% LB and 100% LB alone.

Isolates	LB 100% vs 25% Gum:75% LB	P value
MCC0008	Growth enhanced	$7x\ 10^{-3}$
SRCHD03	No growth enhancement	2.13×10^{-1}
SRCHD05	No growth enhancement	5.7×10^{-2}
SRCHD06	No growth enhancement	4.7×10^{-1}
SRCOD5	Growth enhanced	$6x\ 10^{-6}$
SRCHD08	Growth enhanced	8.82×10^{-7}

Table 3: Analysis of fold change in bacterial growth enhancement in 25% gum with 75% LB compared to 100% LB alone.

Isolates	25% gum:75% LB	Fold increase
MCC0008	Enhanced log phase	1.44
SRCOD5	Enhanced log phase	2.16
SRCHD08	Enhanced log phase	1.88

Based on the above data, three isolates (MCC0008, SRCOD5, and SRCHD08), which showed visible growth differences, were selected for the growth curve assessment in the varying concentrations of the media (Figure 2): (1) only 25% Ramie gum, (2) Ramie gum: LB (25%:50%), (3) Ramie gum: LB (25%:25%), (4) Ramie gum: LB (25%:75%), (5) only 100% LB. At the time point of 8 hours, SRCOD5 showed an OD of 0.411 in 100% LB and 0.874 in gum:LB (25%:75%) media at 600 nm. In the case of SRCHD08, it showed an OD of 0.437 and 0.889 in 100% LB and in gum:LB (25%:75%) media; for MCC0008, the growth in 100% LB and gum:LB (25%:75%) media were 0.732 and 1.057, respectively, at 12 hours of incubation. *Bacillus* sp., *Pseudomonas* sp., and *Micrococcus luteus* showed the higher growth on 25% gum and 75% LB broth as compared to other concentrations. In case of *Micrococcus luteus,* highest growth was seen in 25% gum and 25% LB but it was not significantly different (p value 0.241) from 25% gum and 75% LB. Hence, the latter was considered for subsequent studies. The lowest growth of *Bacillus* sp. and *Pseudomonas* sp. were observed in 25% gum while that for *Micrococcus luteus* was in 100% LB.

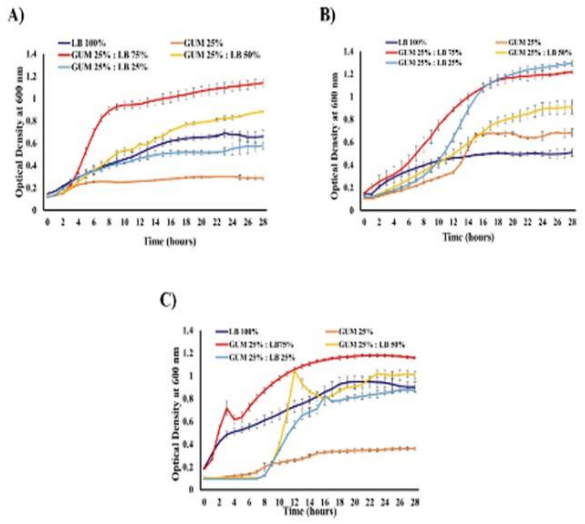

Figure 2: Growth curves of the isolates were assessed in biological triplicates using a 96-well microplate reader (Bio Tek, EPOCH2TS) for 28 hours at 37°C. In this study, 100% Luria Bertani broth and 25% gum:75% LB, 25% gum:25% LB, 25% gum:50% LB and 25% gum were used. (a) Growth curve for isolate SRCOD5, (b) Growth curve for isolate SRCHD08, (c) Growth curve for isolate MCC0008.

Further investigation was carried out to understand the mode of growth enhancement of the isolates using biochemical methods. The total carbohydrates estimation revealed that the addition of 25% gum in 75% LB media enhanced the carbohydrate content by 3.34-fold compared to 100% LB (Figure 3).

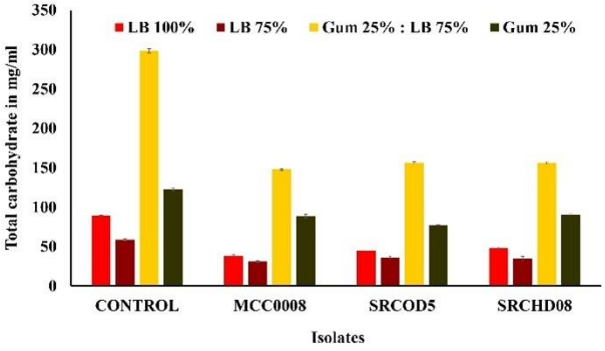

Figure 3: Total carbohydrate estimation in 100% LB (89.2 mg/L), 75% LB (58.8 mg/L), 25% gum with 75% LB (298.4 mg/L) and 25% gum (122.8 mg/L) before (control) and after inoculum with the isolates {MCC0008 (38.3, 31, 147.8, 88.6 mg/L), SRCOD5 (45, 35.8, 156.4, 76.8 mg/L), and SRCHD08 (47.9, 34.9, 156, 90.5 mg/L). All estimations were done in biological triplicates with statistical validation of the data.

Before implementing this modified medium (25% gum with 75%) which enhanced the growth of the isolates, for pilot/industrial scale application, it was necessary to understand their impact on the environment. Therefore, COD of the medium before and after growth of the isolates were assessed. There was 22.28% lower COD in 25% gum with 75% LB as compared to 100% LB (figure 4). So, it was enhanced carbohydrate with reduced COD which would stimulate bacterial growth with

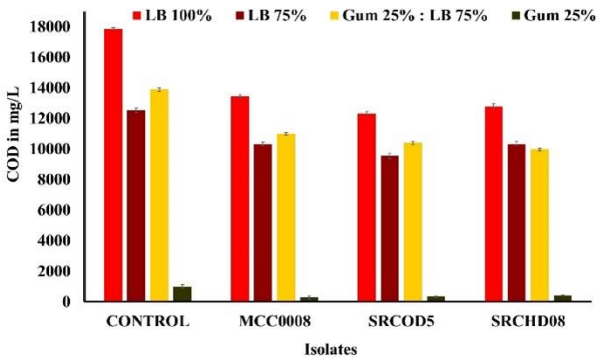

Figure 4: COD estimation in 100% LB (17875.8 mg/L), 75% LB (12519.4 mg/L), 25% gum with 75% LB (13891.3 mg/L) and 25% gum (1012.05 mg/L) before (control) and after inoculum with the isolates {MCC0008 (13437.7, 10330.4, 10997.6, 296.1 mg/L), SRCOD5 (12328.3, 9562, 10405.4, 359.8 mg/L), SRCHD08 (12789.3, 10341.7, 9981.8, 412.3 mg/L). All estimations were done in biological triplicates with statistical validation of the data.

reduced impact on the environment due to lower COD of the discharged media.Based on the COD reduction ability, MCC0008, SRCOD5, and SRCHD08 were selected for further experimental validation. The biofilm-forming ability of the isolates MCC0008,

SRCOD5 and SRCHD08 showed 1.46 (p-value 1.3x 10^{-10}), 1.97 (p-value 1.9x 10^{-17}) and 1.13 (p-value 6x 10^{-5}) fold higher enhancement in biofilm-forming ability (Figure 5).

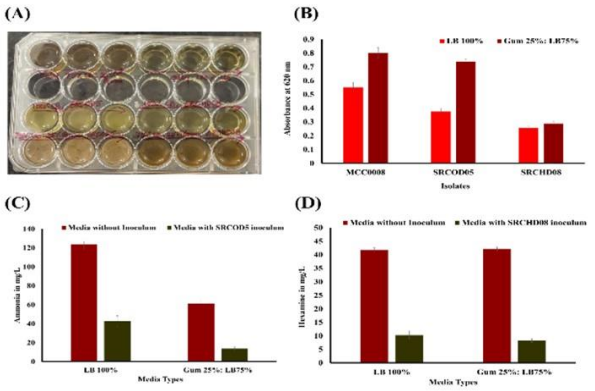

Figure 5: (A) Biofilm formation ability in a 24 well tissue culture plate; (B) Biofilm quantification using Martin's method, in which the absorbance of isolate MCC0008 at 620 nm in 100% LB and Gum 25%: LB75% were 0.551 and 0.802, for isolate SRCOD5 were 0.37 and 0.74, respectively. For isolate SRCHD08 it was 0.25 and 0.29, respectively. (C) SRCOD5 showed 65.42% ammonia removal in 100% LB (initial and final concentration was 123.5 and 42.77 mg/L) and 77.7% ammonia removal in Gum 25%: LB 75 % (initial and final concentrations were 61.1 and 13.67 mg/L); (D) SRCHD08 showed 75.3% hexamine removal in 100% LB (initial and final concentration was 41.8 and 10.3 mg/L) and 80.28% hexamine removal in Gum 25%: LB 75 % (initial and final concentrations were 42.1 and 8.3 mg/L). All estimations were done in biological triplicates with statistical validation of the data.

The isolates MCC0008, SRCOD5, and SRCHD08 were tested for their biofilm-based bioremediation ability to remove organic pollutants (nitrate, ammonia, and hexamine), where it was found that the developed biofilm bioreactor in 25% Ramie gum in 75% LB showed a significant increase of 12.1% (p value 6.4x10^{-6}) of ammonia and 4.8% (p value 8.9x10^{-6}) of hexamine removal compared to the biofilm system in 100% LB. On the other hand, MCC0008 could completely remove nitrate from an initial concentration of 4.3 mg/L in both systems.

In silico analysis

RAST data of the draft genome sequence of *Bacillus* sp. MCC0008 [23] revealed presence of genes for Spo0A (matrix-producing and sporulation gene), SinI, SinR, Eps C and Eps D (for exopolysaccharide biosynthesis), as well as Tas A protein (matrix protein component); pointing towards the possible pathway of biofilm induction. From the RAST analysis, it was found that the closest neighbors of strain MCC0008 were *Bacillus anthracis*, *Bacillus cereus*, and *Bacillus thuringiensis*. All the species were reported to contain the Spo0A gene for biofilm stimulation. In *Bacillus thuringiensis*, Spo0A is very important for biofilm formation [36]. Another study reported that the Spo0A mutant *Bacillus thuringiensis* is incapable of air-liquid interface biofilm formation, and Spo0A-P directly induces biofilm formation. Spo0A controls expression of sinI, which represses the biofilm repressor SinR [37]. Spo0A, SinI, and SinR are reported to be present in *Bacillus cereus* and involved in the biofilm formation [38]. It was also reported that *Bacillus anthracis* contains the Spo0A gene [39].

Literature reveals biofilm formation by both *Pseudomonas* sp and *Micrococcus luteus*. *P. aeruginosa* develops biofilms in various environments. The biofilm structure consists of exopolysaccharides alginate, Pel, and Psl. Alginate is a polymer essential for biofilm protection and stability, while Psl is crucial for biofilm formation and stability. Pel, a glucose-rich polysaccharide, is also present in the biofilm. Surface proteins like PpgL protein and PA4204 gene play crucial roles in biofilm formation [40]. Genes involved in biofilm formation in case of *Micrococcus luteus* were phnA, phnB, cyaB, vfr, vps, glgC, wecB, wecC, and cysE [41].

Discussion

It has been reported that tryptone in LB media provides amino acids, and yeast extract provides nitrogen sources and a low concentration of organic carbon, while NaCl maintains osmotic balance [42] to support the growth of many culturable bacteria in the laboratory. On the other hand, Ramie gum contains cellulose (68.6–76.2%), hemicellulose (13.1–16.7%), lignin (0.6–0.7%), and pectin (1.9%), which are rich in carbon sources [16]. In this study, the data of total carbohydrate content revealed that 25% gum with 75% LB has 3.34-fold higher carbohydrate content as compared to 100% LB (Figure 3). The addition of a natural carbon source through 25% Ramie gum in 75% LB (with protein and limited carbohydrates) enables extracellular enzyme-producing bacterial isolates (cellulase, amylase, and pectinase) to thrive better, reaching higher cell densities and forming structured biofilms (except in the case of SRCH08). The isolates MCC0008, SRCOD5, and SRCHD08 in 25% Ramie gum with 75% LB, could utilize 150.6, 140, and 140.2 mg/L total carbohydrate after 10 hours of incubation at 37°C to boost their cell growth in log phase. The carbohydrate utilization in 100% LB was 50.94, 44.19 and 41.29 mg/L for isolates MCC0008, SRCOD5, and SRCHD08, respectively. On the other hand, isolates that did not have the ability to produce these extracellular enzymes were unable to utilize the additional polysaccharides present in the 25% Ramie gum and showed insignificant growth difference between

100% LB and 25% gum with 75% LB. COD data revealed that 25% gum with 75% LB had a lower COD load compared to 100% LB. The total carbohydrate utilization, COD load, and growth enhancement, emphasized the potential application of the Ramie gum as microbiological growth enhancer. These selected isolates (MCC0008, SRCOD5 and SRCHD08) are potential bioremediants; therefore, further investigation was carried out towards their bioremedial ability. The presence of 25% Ramie gum in 75% LB not only enhanced the cell growth but ensured strong biofilm formation except in case of SRCHD08 (showing structured biofilm formation). Literature reported an increase in the biofilm-forming ability in the presence of plant phytochemicals in many bacterial species. Ghosh et al. (2016) reported that the presence of phytochemicals in the leaf extract of *Nyctanthes arbour-tristis* stimulates the biofilm-forming ability of the epiphytic bacteria significantly showing 1.4 to 8.38 times enhancement; for the *Azadirachta indica* extracts, it was 1.2 to 3.6 times, and for *Ocimum sanctum* and *Mentha spicata*, it was 1 to 7.5 times and 2 to 6 times higher biofilm stimulation [43]. Complex carbohydrate from plant origin has been found to work as bacterial growth component often limiting antagonistic affect demonstrated by simple carbon like glucose during community interaction [19]. *Bacillus subtilis* also used these plant polysaccharides by converting them to UDP-galactose, which gets incorporated into the Extracellular Polymeric Substances of the matrix. This response of *Bacillus* sp. to plant polysaccharide was observed among different strains of the grnus [44]. Biofilm quantification data of this study revealed that the presence of 25% Ramie gum with 75% LB significantly enhanced the biofilm-forming ability of MCC0008, SRCOD5 and SRCHD08 when compared to 100% LB. Literature suggests that the extracellular matrix is made up of two major components, such as EPS and TasA protein [44]. The expression of enzymes responsible for EPS production and the TasA protein depends on the Spo0A gene, which is the main regulatory gene for biofilm formation [44] in *Bacillus* sp. Activation of Spo0A occurs when it phosphorylated through the kinases (kinase A to kinase E). The kinases phosphorylate the Spo0A after receiving the environmental signal. Though the low level of Spo0A stimulates the matrix formation, higher levels of Spo0A induces sporulation. The Spo0A-activated cells secrete toxins during food scarcity, which kills their sensitive neighbor cells and delays sporulation [45]. RAST data of the draft genome sequence of MCC0008 [23] revealed that it has Spo0A (matrix-producing and sporulation gene), SinI, SinR, Eps C and Eps D (for exopolysaccharide biosynthesis), and Tas A protein (matrix protein component). SinI is the repressor of SinR (transcriptional repressor of matrix genes). Activated Spo0A accumulation results in SinI synthesis. It was reported that a mutation in the SinI or Spo0A gene leads to inhibition of plant polysaccharides induced biofilm stimulation [44]. Ramie gum contains polysaccharides like pectin and xylan, which can act as environmental cues for biofilm stimulation using 25% gum and 75% LB (Figure 6).

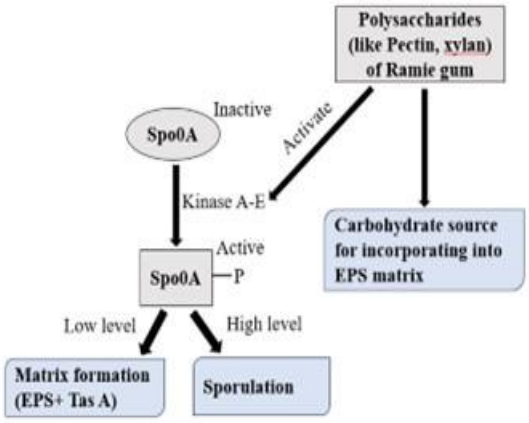

Figure 6: Possible mechanism for biofilm stimulation using 25% Ramie gum and 75% LB. Plant polysaccharide can induce the biofilm formation either by acting as an environmental cue for the activation of kinases, which in turn activate the Spo0A gene for EPS production, or by acting as a carbohydrate source for incorporation into the EPS matrix [44, 45].

It was reported that cellulose is made up of glucose molecules and can be easily degraded by cellulase [46]. Cellulolytic bacteria use cellulose as a substrate to produce glucose molecules [47]. Hemicellulose can be degraded by many enzymes, yielding hexose sugars like mannose, glucose, and galactose and pentose sugars like arabinose and xylose [46]. Lignin can be degraded by ligninolytic enzymes like oxidases and peroxidases and produce coniferyl alcohol, sinapyl alcohol, and p-coumaryl alcohol [18,46]. Pectin can be degraded into galacturonic acid, rhamnose, arabinose, glucuronic acid, galactose, xylose, and fucose [48].

Literature suggests that bacterial growth in LB broth is restricted due to a limited availability of utilizable carbon sources, and the addition of glucose can support bacterial growth [49] post exhaustion of utilizable carbon in LB. Hence, cultivation of six bacterial strains were checked with 75% LB medium along with 25% Ramie gum (as a carbon source). They extracellular enzyme producing isolates degraded the Ramie gum and led to higher cell growth feeding on the degraded materials.

Bacillus sp. was reported for its wax-utilizing ability [50]. The degraded wax is used as the carbon source. Rapid Annotation using Subsystem Technology (RAST) based data analysis of the strain MCC0008 has shown that the

strain has gene cluster for glucose, fructose, and mannose utilization. The enzyme assay showed that this strain has cellulase, amylase, and pectinase producing ability which can degrade cellulose, hemicellulose and pectin present in Ramie gum into glucose, fructose, and mannose. It was reported that *Bacillus subtilis* and other Bacillus prefer glucose as a carbon and energy source [51]. The most probable reason for the sudden drop of growth in 25% gum and 75% LB at the 4th hour was because of preferential utilization of glucose first and then switching to other carbon source. This isolate MCC0008 also showed higher growth on 25% gum and 75% LB as compared to other growth conditions. *Brevundimonas* sp., *Ochrobactrum* sp., and *Brucella pseudintermedia* were known to produce oxidase [52, 53, 54]. SRCHD03 (*Brevundimonas diminuta*), SRCHD06 (*Ochrobactrum* sp.), and SRCHD05 (*Brucella pseudintermedia*) used in this study have the ability to produce oxidase, as shown in Table 1, and thereby could degrade lignin and feed on it. However, the pathway for Lignin metabolism in bacterial system has higher degree of intricacy. Bacteria can degrade lignin to produce small aromatics, which are then consumed by them [55]. As per literature, *Pseudomonas* species are known to produce cellulase enzyme [56]. The cellulose present in Ramie gum can be degraded into glucose by cellulase-producing bacteria, which further can sustain their growth. The *Pseudomonas* sp. (SRCOD5) used in this study has cellulase- and pectinase-producing ability, as shown in Table 1. When they were grown on 25% gum along with 75% LB broth, they utilized the additional carbon source from the 25% gum along with the amino acids and organic compounds present in the 75% LB broth showing much higher growth as compared to 100% LB media. When 50% LB was provided along with 25% gum component, the growth was decreased as compared to 75% LB with 25% gum but still higher than 100% LB broth. However, when 25% gum was provided with 25% LB broth, the growth of the isolate SRCOD5 decreased compared to 100% LB broth, indicating that the additional carbon source was not solely responsible for the enhanced growth and a higher amount of catabolizable amino acids was also required for the growth of the isolate.

The carbon source used by the bacteria from Ramie gum can be cellulose-derived glucose or pectin-derived galacturonic acid/rhamnose/arabinose/glucuronic acid/galactose/xylose/fucose. It was reported that bacteria can utilize carbon sources sequentially (one carbon source after another) or simultaneously (co-utilization) [57]. Co-utilization of a carbon source is required for achieving good growth of bacteria. As LB broth contains catabolizable amino acids as a carbon source, the isolate SRCOD5 may co-utilize the carbon source of LB along with other carbon sources present in Ramie gum, resulting in higher cell growth as compared to 100% LB broth. It was reported that some bacteria including Bacillus subtilis and *Escherichia coli* prefer glucose over any other sugars [58]. They use a carbon catabolite repression (CCR) mechanism for inhibiting other secondary carbon sources, as shown in Figure 7 [59]. Bacteria tend to utilize other carbon sources when glucose is exhausted [60]. However, strategies for carbon utilization by microbes are still a complex process. It was also reported that under low concentrations of glucose, co-utilization of glucose and other pentoses can exist [59]. The isolate may utilize glucose as a carbon source or co-utilize glucose and other sugars by reducing the CCR mechanism.

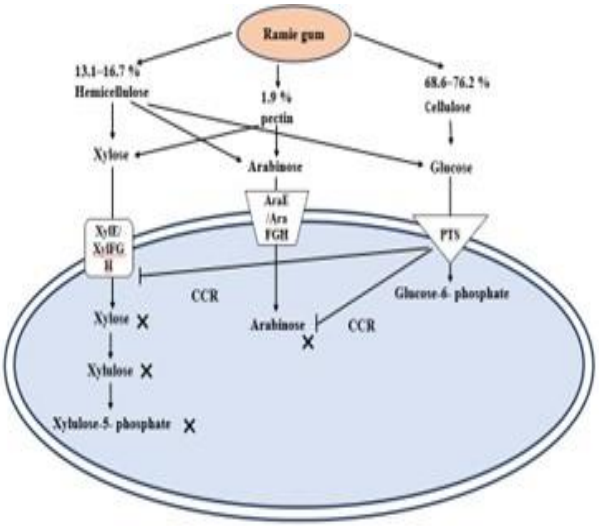

Figure 7: Mechanism of CCR by phosphotransferase system (PTS) in the presence of glucose, and other sugars. When glucose is present, the PTS induce CCR, which inhibits the transportation of xylose and arabinose. XylE (xylose-proton symporter); XylFGH (xylose ABC transporters); AraE (arabinose-proton symporter); AraFGH (arabinose ABC transporter) [59, 60].

The other isolate, SRCHD08, has pectinase-producing ability and thereby degrades pectin into galacturonic acid, rhamnose, arabinose, glucuronic acid, galactose, xylose, and fucose. The isolate utilized these carbon sources from 25% Ramie gum along with amino acids and organic compounds from 75% LB broth for achieving higher cell numbers. Literature suggests that bacteria utilize arabinose first and then xylose when glucose is absent [59].

Through this approach, bacterial growth can be enhanced with stable biofilm formation that could result in setting up of bacterial biofilm system with enhanced performance (bioremediation/enzyme production) and reduced COD of the released spend medium, ensuring economical eco-friendly pilot scale implementation of bacteria processes.

Conclusions

Through this approach, the waste from the Ramie fiber extraction process could be converted into a value added product for sustaining microbial growth of organisms with extracellular enzyme producing ability that could degrade the Ramie gum into simple carbohydrates that could be utilized for cell growth. Such bacterial species (having polysaccharides degrading extracellular enzymes producing ability) can utilize the amino acids and organic compounds from LB broth (75%) and additional sugars from Ramie gum for achieving higher growth and biofilm stimulation. This supplementation ensures reduced requirement of commercial growth medium for the purpose. On one hand the cost for microbial cultivation goes down due to less commercial medium requirement; on the other hand the extent of microbial growth increases, enhancing the microbial activity (be it enzyme production or bioremediation). In addition, the environmental concern due to discharge of spent medium is reduced to a major extent due to reduction of COD of the spend medium. Hence, a waste from plant origin is being valorized into microbial growth medium supplement with environmental protection. The Ramie gum after extraction was converted to Ramie flacks to store it for a longer time (Figure 8).

Figure 8: Flacks prepared from Ramie gum.

Hence, sustainable utilisation of Ramie gum is possible by utilising the gum as a bacterial feed.

List of abbreviations

LB- Luria Bertani, ATP-Adenosine triphosphate, DNA-Deoxyribonucleic acid, RNA- Ribonucleic acid. OD- optical density, COD-chemical oxygen demand, RAST-Rapid Annotation using Subsystem Technology, CCR-Carbon catabolite repression, PTS- Phosphotransferase system, XylE- xylose-proton symporter, XylFGH- xylose ABC transporters, AraE- arabinose-proton symporter, AraFGH-arabinose ABC transporter.

Author Contributions

Formal analysis, data curation, original draft preparation, validation, visualization/figures, software: EP and SWA; formal analysis: SS; Supervision, Investigation, resources, writing—review and editing, Conceptualization, methodology, funding acquisition, project administration: SRC. All authors have read and agreed to the published version of the manuscript.

Ethical Approval:

Ramie (Boehmeria nivea) fiber used in this study was obtained from our own experimental field (cultivated source) at Tripura University. No permissions or special approvals were required for the use of this plant material.

Availability of Data and Materials

The data supporting the findings of this study are included within the manuscript.

Conflicts of Interest

The authors declare that they have no conflicts of interest to this work.

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