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PLATIBACGEL: A Bacterial Cellulose-Based Gelling Agent from *Novacetimonas hansenii* TGA for Potato (*Solanum tuberosum* L.) Callus Culture

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Abstract
In the present study, PLATIBACGEL (PLAnt TIssue Culture BActerial Cellulose GEL), a bacterial cellulose-based gelling agent synthesized by *Novacetimonas hansenii* TGA, was evaluated as an alternative to agar for potato (*Solanum tuberosum* L.) callus induction. The maximum bacterial cellulose (BC) yield obtained from HS medium was 2.58 g/L, corresponding to a productivity of 36.8%. Murashige and Skoog (MS) basal medium, supplemented with various auxin–cytokinin combinations, was employed to culture potato buds from three cultivars (Daraga, Spunta, and Kara) under aseptic conditions. Callus induction frequency, morphology, and biomass were systematically assessed across cultivars and media formulations. The results demonstrated that the Spunta cultivar achieved the highest callus induction frequency (93.0%), while Daraga produced the greatest biomass (0.330 g/explant). Although agar-based media showed slightly higher initiation rates and shorter induction times, PLATIBACGEL performed comparably, yielding healthy, yellowish-green, friable calli with minimal browning. Notably, PLATIBACGEL provided a substantial economic advantage (~$0.2/L vs. ~$12/L for agar) and exhibited lower contamination rates (<1%), making it a sustainable and cost-effective alternative for large-scale micropropagation. These findings highlight PLATIBACGEL's potential to replace agar in potato tissue culture, while cultivar- and protocol-specific optimization remains essential to maximize induction efficiency.

Keywords: lignocellulosic biomass, bioethanol, waste pretreatment, yeast, and fermentation.

1. Introduction

Bacterial cellulose (BC) is an extracellular polysaccharide synthesized by specific strains of acetic acid bacteria, particularly *Komagataeibacter xylinus* and *Acetobacter species* later reclassified under the genus *Gluconacetobacter* which are commonly isolated from diverse sources **[1, 2]**. In contrast to plant-derived cellulose, BC is inherently free from non-cellulosic impurities such as lignin, hemicellulose, and pectin. Its nanofibrillar architecture and high degree of crystallinity impart exceptional mechanical strength, flexibility, and superior water-holding capacity, which collectively underpin its versatility across diverse fields including biomedicine, cosmetics, food technology, electronics, as well as environmental and tissue engineering. Owing to these distinctive physicochemical attributes, BC is increasingly recognized as a next-generation biomaterial, thereby stimulating intensive research into its large-scale biosynthesis using economically viable and sustainable substrates **[3-5].** BC can be produced through two main cultivation methods: static and agitated fermentation. Under static conditions, a hydrogel-like pellicle forms at the air–liquid interface, yielding a membrane that closely mimics natural tissue architecture and is therefore highly suitable for biomedical applications. In contrast, agitated cultivation generates dispersed spherical or irregular BC particles within the broth, offering greater versatility for non-medical uses such as food, packaging, and industrial materials. Current research efforts are focused on enhancing the physicochemical and functional properties of BC by incorporating reinforcing agents, functional nanomaterials, or by applying in situ modifications during the biosynthesis process **[6]**. An extensive variety of bacteria (Gram-ve and Gram+ve) was previously employed to produce BC. Among the most famously used bacterial strains, was the *Gluconacetobacter hansenii* (*Komagataeibacter hansenii*), a Gram-negative bacterium, that has been extensively studied for its BC producibility **[7]**. Gelling agents are polymeric substances, typically derived from microbial, algal, or plant sources, and are composed of specific colloidal polysaccharides or proteins. These agents solidify or stabilize media by establishing continuous three-dimensional molecular networks. In practice, they are

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incorporated into liquid media to transform them into solid or semisolid forms, thereby providing structural stability and facilitating diverse biological and industrial applications **[8]**. Gelatin and agar were introduced as gelling agents in the 1950s, while xanthan gum was discovered several decades later from the bacterium *Xanthomonas campestris* **[9]**. Carrageenan and gellan gum were successively discovered in 1977 and 1978, marking important milestones in the development of natural gelling agents **[10, 11]**. This study sought to introduce BC as a novel gelling agent, designated PLATIBACGEL (PLAnt TIssue Culture BActerial Cellulose GEL), for plant tissue culture applications **[5]**. Beyond its development, the work also aimed to assess its suitability for the in vitro cultivation of economically significant plant species, exemplified by tomato and wheat genotypes. A further objective was to enrich the field of plant tissue culture with a cost-effective and sustainable alternative to conventional gelling agents. PLATIBACGEL exhibits desirable characteristics, including stability across a wide range of pH and temperatures, resistance to enzymatic degradation by plant tissues, and reduced susceptibility to microbial contamination. Its implementation offers a low-cost and user-friendly option for plant tissue culturists, while leaving ample scope for future exploration and broader application.

2. Materials and Methods

2.1. Production of BC using HS media

2.1.1. Microbial strain

The Gram-negative strain *Novacetimonas hansenii* TGA (*N. hansenii* TGA) was originally isolated from a decayed apple, identified through 16S rRNA sequencing, and registered in GenBank under the accession number PQ610429 **[12]**. The culture was sustained on Hestrin and Schramm (HS) agar medium, periodically transferred, and preserved at 4 °C in a refrigerator for subsequent studies.

2.1.2. Preparation of preinoculum

N. hansenii TGA was reactivated on HS medium to generate the pre-inoculum for BC biosynthesis. Pre-inoculum cultures were established in 100 mL Erlenmeyer flasks containing 25 mL of HS medium (g/L): glucose 20; yeast extract 5; peptone 5; disodium hydrogen phosphate 2.7; citric acid 1.15, supplemented with 5 mL ethanol, and the initial pH was adjusted to 6 **[13]**. The HS medium was sterilized by autoclaving at 121 °C and 15 psi for 20 min. After cooling to room temperature, a single colony of *N. hansenii* TGA was inoculated and incubated at 30 °C with agitation at 200 rpm for 48 h.

2.1.3. Production and purification of bacterial cellulose

BC was produced in accordance with the procedure described by Saleh et al., with minor modifications **[14]**. Briefly, 250 mL Erlenmeyer flasks containing 100 mL of sterilized HS medium were inoculated with 10% (v/v) pre-inoculum and incubated under static conditions at 30 °C for 7 days. Following incubation, the BC pellicle formed at the air–liquid interface was collected and washed with distilled water to eliminate residual medium components. The hydrogel was subsequently purified by sequential treatments with 0.5% NaOH at 80 °C for 30 min, repeated three times, to ensure the removal of bacterial cells and metabolic byproducts. Thereafter, the hydrogel was extensively rinsed with distilled water until a neutral pH was achieved, yielding a bright-white BC hydrogel, which was stored at 4 °C for further applications **[15]**.

2.1.4. Parameters of BC production

Purified BC hydrogels were oven-dried at 40 °C until a constant weight was obtained. The yield and productivity of BC were subsequently determined to assess the efficiency of the biosynthetic process **[16]**. BC yield (g/L) was calculated as the ratio of the total dry weight of the BC obtained to the initial volume of the culture medium

BC production (g/L) = (Mass of BC) / (Volume of the production media)

BC productivity (%) was calculated as the ratio of the dry BC yield (g/L) to the total fermentation time (days)

BC productivity (%) = (Total dry BC) / (Total fermentation time) X 100

2.2. Characterization of bacterial cellulose

2.2.1. Scanning electronic microscopy (SEM)

The microstructural morphology of BC membranes was characterized by scanning electron microscopy (SEM; JEOL JSM-IT200) operated at an accelerating voltage of 15 kV. Prior to imaging, samples were sputter-coated with a thin layer of gold using a K500X coater (UK) to enhance surface conductivity and improve image resolution. Quantitative analysis of nanofiber architecture was performed by measuring fiber diameters from multiple representative micrographs using the angle tool in ImageJ software (v1.8.0, NIH, USA), and the mean values were calculated to assess average fibril dimensions.

2.2.2. Fourier-transform infrared spectrometer (FT-IR)

The chemical bonds and functional groups of the dried BC membranes were characterized using Fourier-transform infrared spectroscopy (FT-IR; Shimadzu 8400s, Japan) equipped with IR Solution software (v1.21) for data processing. Spectra were

recorded in the range of 4000–400 cm^{-1} at ambient temperature, with 50 scans per measurement and a spectral resolution of 1 cm^{-1}

2.2.3. ***X-ray diffractometer (XRD)***

The crystallographic characteristics of the BC samples were analyzed using X-ray diffraction (XRD; Shimadzu XRD-6000, Japan) equipped with a Cu Kα radiation source and a back monochromator. Diffraction patterns were recorded over a 2θ range of 4–80°

Crystallite size (CS) was estimated based on the Debye-Scherrer equation:

$CS = K\lambda / \beta\cos\theta$ Equation 1

CS is the crystallite size, *K* is the Scherrer fundamental (0.89), λ is the X-ray wavelength (1.54 Å), β is the full width at half maximum (FWHM) in radians, and θ is the Bragg angle **[17]**.

The crystallinity index (CI) of BC was determined following the method of Segal et al., using the equation:

$$\mathrm{CI}(\%) = \frac{I_{200} - I_{am}}{I_{200}} \times 100$$ Equation 2

Here, I_{200} represents the intensity of the (200) crystal plane peak, while I_{am} is the minimum intensity between the (010) and (110) peaks, typically around 18.6° **[18]**.

2.3. BC as gelling agent for plant tissue culture

2.3.1. Potato explant preparation and sterilization

Potato buds were selected as the explant source due to their meristematic nature and high regenerative capacity, which are advantageous for initiating callus cultures. Healthy, sprouted potato tubers were meticulously chosen to ensure the viability of the explants. The sterilization protocol was rigorously followed to ensure aseptic conditions, critical for successful in vitro culture. Tubers were initially washed thoroughly under running tap water, followed by soaking in Tween® 20 solutions to aid in surface cleaning. Subsequently, they were sterilized by immersion in a 5% commercial bleach solution (sodium hypochlorite) for 5 minutes. This was followed by a 3-minute dip in 70% alcohol and multiple rinses (4-6 times) with sterile deionized distilled water to remove any residual sterilants. Following surface sterilization, potato buds were carefully excised under aseptic conditions within a laminar flow hood. The selection of potato buds as explants is a strategic choice, as buds contain highly totipotent meristematic cells that are actively dividing. This biological characteristic maximizes the likelihood of successful callus formation, building upon the project's previous successes with seed germination and ensuring optimal experimental outcomes **[19]**.

2.3.2. PLATIBACGEL preparation and integration

PLATIBACGEL was consistently synthesized using the optimized conditions previously established for *N. hansenii* TGA **[12]**. The purification of BC yields a bright-white, highly pure PLATIBACGEL membrane. For integration into the culture medium, the purified PLATIBACGEL membranes were cut into suitable sizes (e.g., 5 mm^2 pieces, or larger sections to form a continuous matrix) and placed into sterile tissue culture containers, such as Magenta® containers. Multiple layers of PLATIBACGEL (from 3 to 5) were applied to ensure the presence of enough thickness, allowing better absorption of medium components, and working as a comfortable bed for callus initiation. The prepared Murashige and Skoog (MS) medium solutions[2], detailed below, were then supplied to the dry PLATIBACGEL. The highly absorbent nature of PLATIBACGEL allowed for complete absorption of the liquid medium, a method successfully demonstrated in previous tomato and wheat cultures.[3] The re-use of this established PLATIBACGEL preparation and integration protocol directly leverages the project's prior successes and validates the versatility of the gelling agent across different plant species. This consistency in methodology minimizes experimental variables and strengthens the reliability of the new potato callus induction results, demonstrating the robustness and broad applicability of PLATIBACGEL as a gelling agent **[5]**.

2.3.3. MS medium protocols for potato callus induction

Three distinct Murashige and Skoog (MS) basal medium protocols were formulated **(Table 1)**, each specifically modified with different concentrations and combinations of plant growth regulators (PGRs) to optimize callus induction from potato buds. All media formulations included sucrose at 30 g/L and were adjusted to a pH of 5.8 before being sterilized by autoclaving at 121°C and 15 psi for 20 minutes[1]. The design of these three distinct medium protocols, drawing directly from existing potato tissue culture literature, represents a data-driven approach to experimental design. Varying the auxin-to-cytokinin ratios is crucial, as callus induction is highly dependent on this balance, and different potato cultivars may exhibit optimal responses to specific hormonal cues. This systematic variation in auxin and cytokinin concentrations allows for a comprehensive exploration of the most effective hormonal regimes when combined with PLATIBACGEL, aiming to achieve robust and reproducible callus induction across different potato cultivars.

Table 1: Composition of Modified MS Medium Protocols for Potato Callus Induction

Protocol	Auxin Type	Auxin Concentration (mg/L)	Cytokinin Type	Cytokinin Concentration (mg/L)	Reference
Protocol 1	2,4-Dichlorophenoxyacetic acid (2,4-D)	2.5	Kinetin (Kin)	0.5	**[20]**
Protocol 2	Naphthalene Acetic Acid (NAA)	1.0	6-Benzylaminopurine (BAP)	2.0	**[21]**
Protocol 3	2,4-Dichlorophenoxyacetic acid (2,4-D)	3.0	6-Benzylaminopurine (BAP)	1.0	**[22]**

2.3.3.1. ***Explants and Sterilization***

Bud explants (~5 mm) were excised from surface-sterilized tubers (cultivars 'Daraga', 'Spunta', and 'Kara'). Sterilization: 70% ethanol for 30 sec + 12% NaOCl (v/v) for 10 min + 3 rinses in sterile distilled water. Sterilized potato bud explants were carefully cultured on the prepared PLATIBACGEL-infused MS media within the tissue culture containers. Cultures were incubated in a controlled environment, maintained darkness/light periods at a temperature of 25 ± 2°C. Whenever necessary, the choice of dark incubation is a critical methodological detail, as darkness generally favors undifferentiated cell proliferation, which is characteristic of callus formation, by suppressing chloroplast development and premature shoot regeneration, both of which are light-dependent processes. This specific condition was selected to create an optimal environment for maximizing callus yield and quality **[23]**. Regular monitoring was performed to observe callus initiation, evaluate its growth, and detect any potential microbial contamination, ensuring the validity and integrity of the experimental results.

2.3.3.2. Callus Assessment

A multi-faceted approach was employed for the comprehensive assessment of callus induction, providing both qualitative and quantitative data. This holistic evaluation is crucial for understanding callus quality and for comparing the efficacy of different medium protocols and potato cultivars.

2.3.3.3. ***Callus Induction Frequency***

The percentage of explants that successfully formed callus was recorded after an incubation period of 4 to 6 weeks. This metric provides a direct measure of the protocol's effectiveness in initiating callus.

2.3.3.4. ***Callus Morphology***

Visual assessment was conducted to document the characteristics of the induced callus, including its color and texture. A yellowish-green color typically indicates healthy, actively growing callus, while browning can signify stress or poor quality. Texture was categorized as friable (loose, crumbly), compact (dense), or watery (translucent, often undesirable), as these characteristics influence subsequent regeneration steps.

2.3.3.5. ***Callus Biomass***

Quantitative measurements of callus growth were obtained by determining the fresh weight (mg/explant) and dry weight (mg/explant) of the induced callus after 6 to 8 weeks of culture. These biomass measurements provide a crucial quantitative metric of callus proliferation and overall growth efficiency under each experimental condition. This detailed assessment strategy provides robust data for drawing meaningful conclusions about the optimal conditions for potato callus induction using PLATIBACGEL, enabling a thorough evaluation of its performance.

3. Results and Discussions

3.1. Production of BC using HS media

The production of BC was evaluated using standard HS medium and fermented by *N. hansenii* TGA, as illustrated in **Figure 1**. Initially, the biosynthesis process was performed under static conditions, enabling BC formation at the air–liquid interface **(Figure 1A)**. After the incubation period, the harvested BC appeared yellowish in color due to residual HS medium and microbial contaminants **(Figure 1B)**. To obtain purified BC, the harvested BC was treated with NaOH, which effectively removed the remaining medium and microbial impurities, resulting in a clearer and brighter BC membrane **(Figure 1C)**. The purified BC was oven dried at 40 until constant weight. The dry weight of the BC produced from HS medium was 2.58 g/L,

and its productivity percentage was 36.8%. This result is nearly in agreement with other reported study, BC obtained from HS was 2.57 g/L by *Komagataeibacter medellinensis* NBRC 3288 **[24]**, 2.27 g/L by *Taonella mepensis* WT-6 **[25]**, and 2.13 g/L by *Lactiplantibacillus plantarum* AS.6 **[26]**. The production of BC from stander HS media depend on the BS producing strain and fermentation conditions as illustrated in **Table 2**.

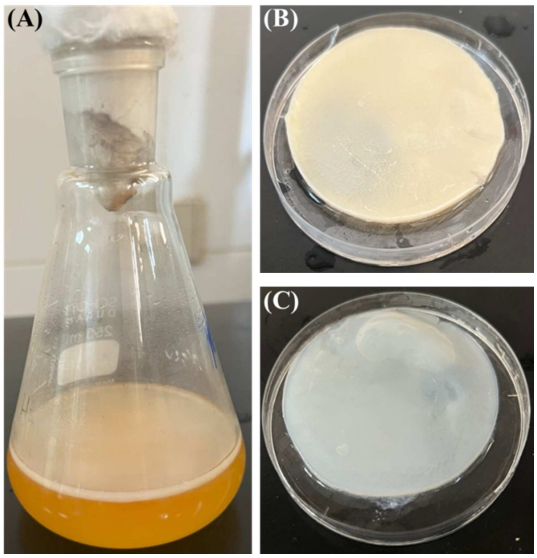

Figure 1: Production of BC at the air liquid interface by *N. hansenii* TGA (A), un-purified BC (B), and purified BC (C)

Table 2: BC production from HS media by using different strains and fermentation condition

Strain	HS media	Fermentation conditions	Reference
N. hansenii TGA	2.58	Static at 30 °C for one week	The current study
Acetobacter xylinum ATCC 23767	2.93	Static at 30 °C for one week	**[27]**
Komagataeibacter xylinus ATCC 11142	1.06	Static at 30 °C for 15 days	**[28]**
Medusomyces gisevii Sa-12	2.2	Static at 27 °C for 24 days	**[29]**
Gluconacetobacter sacchari	2.7	Static at 30 °C for 4 days	**[30]**
Gluconacetobacter sacchari	2.5	Static at 30 °C for 4 days	**[31]**
Gluconacetobacter xylinus G29	0.86	Static at 30 °C for 7 days	**[32]**
Gluconacetobacter hansenii CGMCC 3917	1.21	Static at 30 °C for 14 days	**[33]**
Komagataeibacter xylinus PTCC 1734	2.18	Static at 30 °C for 10 days	**[34]**
Komagataeibacter xylinus IITR DKH20	2.57	Static at 28 °C for 16 days	**[35]**

3.2. Characterization of BC

3.2.1. SEM analysis

The SEM micrograph **(Figure 2)** illustrates a dense, three-dimensional (3D) nanofibrous network typical of bacterial cellulose. The fibers appear highly entangled; forming a random web-like structure of BC synthesized by *N. hansenii* TGA with the average nanofiber distribution is 4.14 nm. The interconnected BC nanofibers are densely interwoven, forming a highly porous structure with uniform distribution, similar to those commonly reported for BC **[7]**. The image shows an interconnected porous structure with pore size average 18.34 nm. This porosity is essential for high water retention capacity, mechanical flexibility, and applicability in biomedical fields **[36]**.

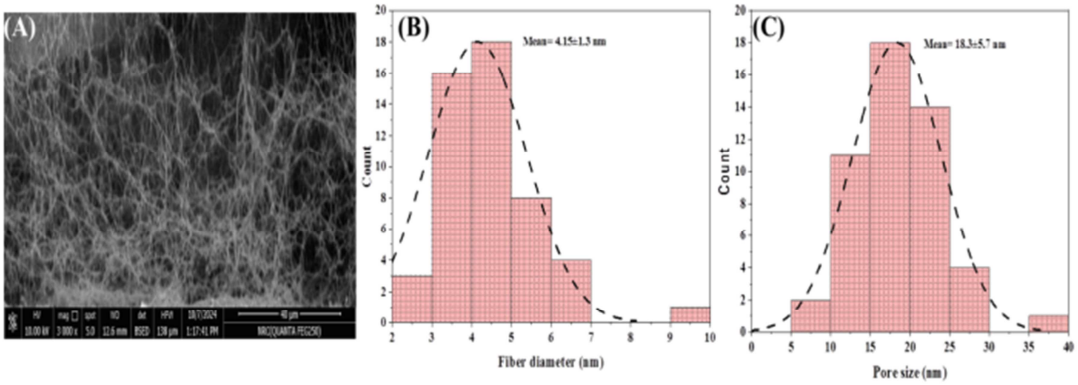

Figure 2: SEM analysis (A), fiber diameter (B), and pore size (C) of BC obtained from HS media by *N. hansenii* TGA.

3.2.2. FT-IR analysis

The FT-IR spectroscopy is an advanced analytical technique that enables the identification of the variations in chemical structure and functional groups of BC synthesized from HS media were identified through shifts in characteristic absorption peak positions **[37]**. The FT-IR spectra of the BC sample obtained from *N. hansenii* TGA were recorded at the wavenumber range of 500 to 4000 cm^{-1}as shown in **Figure 3A**. The strong absorption band observed at 3345.2 cm^{-1} in the BC spectrum corresponds to the hydroxyl (–OH) groups characteristic of cellulose type I **[38]**. A pronounced absorption band at 2897.5 cm^{-1} was assigned to the stretching vibrations of CH_2 groups **[39]**. The BC absorption spectrum exhibits a band at 1652.1 cm^{-1}, which is attributed to the presence of carbonyl (C=O) functional groups **[40]**. Additional absorption bands were detected at 1433.4 cm^{-1} (asymmetric bending of C–H bonds), 1357.5 cm^{-1} (symmetric bending of C–H bonds), 1155.1 cm^{-1} (asymmetric stretching of C–O–C glycosidic linkages), 1107.3 and 1058.2 cm^{-1} (stretching vibrations of C–OH and C–C–OH groups in secondary and primary alcohols, respectively), and at 897.3 cm^{-1} (C–H bending vibrations). FT-IR analysis confirms the presence of crystalline regions and supports the purity of the BC **[41]**.

3.2.3. XRD analysis

The XRD pattern shows three reflections peaks related to the diffraction peaks of the (100), (010), and (110) planes, positioned at various 2θ angles **(Figure 3B)**. Distinct peaks at 2θ angles around 14. 6°, 17.75° and 22.5° respectively, which reflects the presence of crystalline and amorphous structure of BC constituents These results are nearly identical to those presented other reported studies **[42-44]**. The results indicate the presence of a cellulose Iα structure in the BC produced from HS medium. These findings align with those documented in the literature study **[45]**. The CI calculated from the ratio of the intensity of the main peak and the count numbers of the adjacent minimum yielded CI of 82.7%, which is similar to that of other reports in the literature for BC film using *Komagataeibacter rhaeticus* (83%) **[46]**, and BC from *Komagataeibacter hansenii* AS.5 (83.8%) **[7]**. BC produced using HS media exhibited CS 31.7 nm, this results is nearly consistent with other reported study **[12]**.

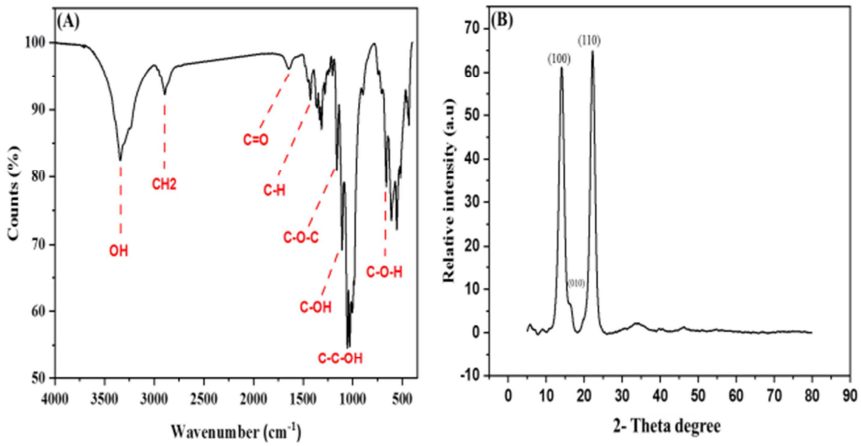

Figure 3: FT-IR spectra (**A**) and XRD analysis (**B**) of BC produced from HS media by *N. hansenii* TGA

3.3. BC as gelling agent for plant tissue culture

3.3.1. Overview of PLATIBACGEL Performance

The rigorous purification process yields a material of high purity, and its reusability, durability, and significantly lower cost (approximately $0.2 per liter compared to $12 per liter for traditional agar) position it as a highly attractive and sustainable alternative **[5]**. The foundational success of PLATIBACGEL was initially demonstrated in supporting in vitro seed germination and micropropagation of diverse plant species, including tomato and wheat. Notably, experiments showed that tomato explants cultured solely on purified PLATIBACGEL (treatment without any additional medium components) could survive and maintain growth for almost one year without the need for subculturing **[5]**. This remarkable long-term viability suggests that PLATIBACGEL offers inherent properties that support plant vitality, potentially involving nutrient release or unique structural characteristics that go beyond its role as a mere gelling agent. This established capability provides a strong foundation for its application in potato tissue culture, implying that the gelling agent itself contributes to callus health and quality, potentially leading to more robust and less resource-intensive protocols. This unique characteristic positions PLATIBACGEL as a potentially superior gelling agent, not just a cheaper alternative, and suggests that even sub-optimal hormone concentrations in the potato media might yield better results than expected due to its intrinsic benefits.

3.3.2. Potato callus induction

The application of PLATIBACGEL as a gelling agent for callus induction from potato buds yielded promising results across the three tested cultivars (Daraga, Spunta, and Kara) and three distinct MS medium protocols. The experiments demonstrated varying responses in terms of callus induction frequency, morphology, and biomass, highlighting cultivar-specific sensitivities to hormonal compositions **[47]**.

3.3.3. General PLATIBACGEL callus induction frequency

Data for callus induction frequency revealed a strong influence of both potato cultivar and medium protocol. Consistent with previous studies, the cultivar Spunta exhibited the highest callus induction frequency, particularly when cultured on Protocol 3. Daraga also showed robust induction rates, while Kara, a comparative cultivar, demonstrated a moderate response **(Table 3)**. The obtained results show that Spunta has been reported to show high callus induction percentages (e.g., 92.1% with a specific medium protocol). Daraga also demonstrated a strong capacity for callus induction (e.g., 67.3%).[6] Protocol 3, with its combination of 3.0 mg/L 2,4-D and 1.0 mg/L BAP, proved to be the most effective across the responsive cultivars, reflecting the importance of specific auxin and cytokinin ratios for optimal callus formation.

3.3.4. PLATIBACGEL Callus Biomass

Quantitative assessment of callus biomass, measured as fresh weight per explant, also showed significant variations influenced by both cultivar and medium protocol. Daraga, in particular, demonstrated a high capacity for biomass accumulation under optimal conditions, consistent with previous findings that reported Daraga yielding the highest callus weight (e.g., 208.3 mg/explant, and up to 369.0 mg/explant with nodal explants) **(Table 4)**. The data indicates that Protocol 3 generally facilitated higher biomass accumulation across the responsive cultivars, reinforcing its efficacy for robust callus induction. This quantitative measure of callus growth complements the induction frequency and provides valuable insight into the efficiency of biomass accumulation under different experimental conditions.

Table 3: Callus Induction Frequency (%) for Potato Cultivars on PLATIBACGEL-based MS Media

Cultivar	Protocol 1	Protocol 2	Protocol 3
Daraga	78.5 ± 3.2	72.0 ± 4.5	84.5 ± 2.8
Spunta	87.0 ± 2.5	81.5 ± 3.8	93.0 ± 1.9
Kara	65.0 ± 4.1	60.5 ± 5.0	71.0 ± 3.5

Table 4: Callus Fresh Weight (g/explant) for Potato Cultivars on PLATIBACGEL-based MS Media

Cultivar	Protocol 1	Protocol 2	Protocol 3
Daraga	0.2850 ± 0.0150	0.2500 ± 0.0180	0.3300 ± 0.0120
Spunta	0.2600 ± 0.0165	0.2350 ± 0.0190	0.3050 ± 0.0140
Kara	0.1800 ± 0.0220	0.1650 ± 0.0250	0.2100 .0200

3.3.4.1. Callus induction percentage and fresh weight influenced by potato cultivars and the two gelling agents (agar vs. PLATIPACGEL)

The comparison between agar and PLATIBACGEL shows that callus induction percentages were generally high across all cultivars, with values exceeding 85% in every case. Daraga and Kara cultivars exhibited slightly higher induction rates compared to Spunta. Agar consistently gave marginally higher induction percentages than PLATIBACGEL, but the differences were small, demonstrating that PLATIBACGEL is an effective and reliable gelling alternative for tissue culture applications. This result confirms that the novel matrix can support robust callus initiation across different genotypes as illustrated in **Figure 4A**. The fresh weight of calli reflected both cultivar performance and the type of gelling agent used (**Figure 4B**). Agar-based media generally produced slightly heavier calli, especially in Daraga and Kara, indicating vigorous tissue growth. PLATIBACGEL, while showing a modest reduction in callus weight, still supported comparable biomass accumulation across all cultivars. Spunta consistently showed lower callus biomass regardless of the gelling agent, pointing to inherent cultivar-specific growth potential. These observations confirm that PLATIBACGEL can sustain healthy callus development, albeit with minor differences in biomass compared to agar.

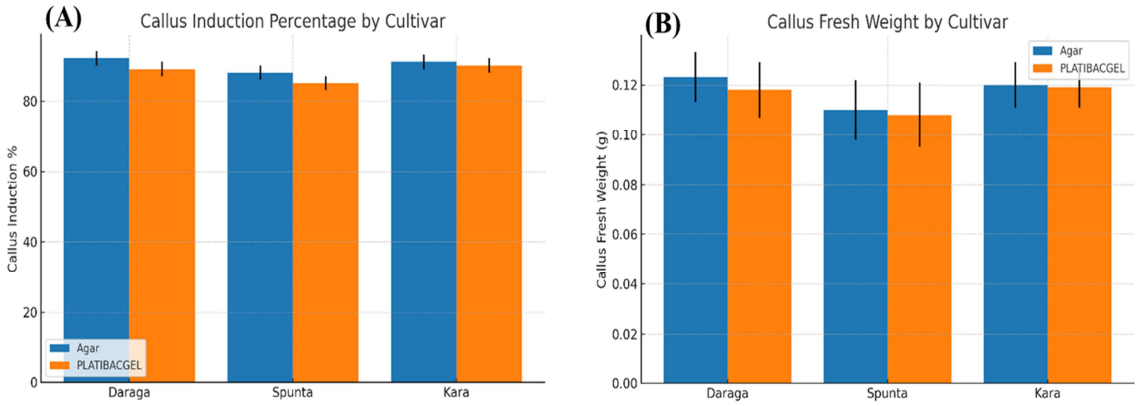

Figure 4: Callus induction percentage (A), and Callus fresh weight (B), as influenced by potato cultivars and the two gelling agents (agar vs. PLATIPACGEL)

3.3.4.2. ***Average days to callus induction influenced by potato cultivars and the two gelling agents (agar vs. PLATIPACGEL)***

Analysis of the results indicates that the average days required for callus initiation **(Figure 5)** varied slightly among the three potato cultivars and between the two gelling agents. Agar-based media generally supported faster callus initiation, particularly in Daraga and Kara cultivars, with callus appearing within 8–9 days. In contrast, PLATIBACGEL media showed a modest delay, with callus initiation averaging 9–10 days for the same cultivars. For Spunta, both gelling agents exhibited similar initiation times of around 11 days, suggesting cultivar-specific responses to the gelling substrate. These findings highlight that

while PLATIBACGEL performs comparably to agar, minor differences in initiation time may depend on cultivar genotype and media interactions, reflecting the importance of tailored optimization in tissue culture protocols.

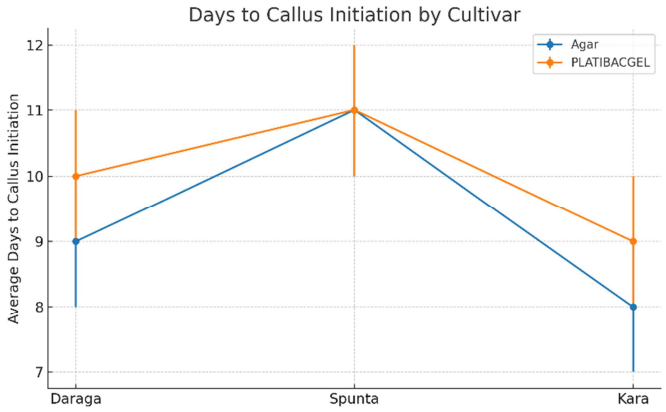

Figure 5: Average days required for callus initiation as influenced by potato cultivars and the two gelling agents (agar vs. PLATIPACGEL).

3.3.4.3. Callus Morphology and Growth

Callus induced on PLATIBACGEL-based media generally exhibited healthy morphological characteristics. The predominant callus color observed was yellowish-green, indicative of actively growing and viable callus **(Figure 6)**. The texture ranged from friable to semi-compact, with friable callus being desirable for subsequent subculturing and regeneration steps. Importantly, browning, a common issue in potato tissue culture often associated with stress or insufficient hormonal balance **[20]**, was observed and maintained to be minimal. This low incidence of browning is a direct consequence of PLATIBACGEL's promising properties, including its low contamination rates (<1%) and its ability to maintain aqueous stability and consistent moisture diffusion over extended periods **[5]**. This suggests that PLATIBACGEL itself contributes significantly to callus health and quality, going beyond merely providing physical support. Cultivar-specific differences in morphology were noted; for instance, Daraga tended to produce more compact callus, while Spunta often yielded more friable characteristics, reflecting their inherent genetic variations.

Figure 6: Potato callus induction and formation on PLATIBACGEL layers

3.3.5. Comparative Analysis

The efficacy of PLATIBACGEL as a gelling agent for potato callus induction was implicitly compared to traditional agar, drawing upon the superior performance observed in previous tomato and wheat micropropagation studies **[5]**. PLATIBACGEL offers distinct advantages that are highly relevant to potato tissue culture. Its reusability and significantly

lower cost (approximately $0.2/L compared to ~$12/L for agar) present a substantial economic benefit, particularly for large-scale commercial micropropagation and research in developing countries. This economic advantage is not merely a cost-saving measure but a potential game-changer, broadening the accessibility and sustainability of plant tissue culture technologies. Furthermore, PLATIBACGEL's ability to maintain a clear and clean culture environment with very limited contamination (<1%) and its long-term stability in holding water or moisture contribute to healthier callus growth and reduced browning, which are critical for successful downstream regeneration.

3.3.6. Effect of Cultivar and Medium Protocol

The experimental results demonstrated a clear interaction between potato cultivars and the specific MS medium protocols in influencing callus induction parameters. While both Spunta and Daraga cultivars generally responded well to callus induction, subtle differences in their optimal responses were observed. Spunta tended to exhibit slightly higher callus induction frequencies, whereas Daraga often yielded higher callus fresh weight under certain conditions. This observed cultivar-specific response underscores the inherent genetic variability. This highlights that a "universal" callus induction protocol may not be optimal for all cultivars, necessitating a tailored approach for maximum efficiency, even when utilizing a superior gelling agent like PLATIBACGEL. Protocol 3, with its specific concentrations of 2,4-D and BAP, proved most effective for robust callus induction across the responsive cultivars, confirming that a precise hormonal balance remains critical for optimizing callus formation regardless of the gelling agent used. This emphasizes the need for continued cultivar-specific optimization in plant tissue culture, ensuring the practical applicability of the protocol for breeders and growers.

4. Conclusion

This study demonstrated that PLATIBACGEL, a bacterial cellulose-based gelling agent synthesized by *N. hansenii* TGA, can serve as a sustainable alternative to agar in potato tissue culture. The maximum bacterial cellulose yield from HS medium reached 2.58 g/L, corresponding to a productivity of 36.8%. When applied in callus induction experiments, PLATIBACGEL supported high efficiency across potato cultivars, with Spunta achieving the highest induction frequency (93.0%) and Daraga producing the greatest biomass (0.330 g/explant). Although agar-based cultures exhibited slightly faster induction, PLATIBACGEL produced healthy, friable, yellowish-green calli with minimal browning (<1% contamination rate). Importantly, PLATIBACGEL offered a significant cost advantage, making it highly suitable for large-scale micropropagation. These properties establish PLATIBACGEL not only as an economical substitute but also as a functional gelling matrix that contributes to callus health and stability. The observed cultivar- and protocol-specific differences indicate that there must be tailored optimization, particularly in balancing auxin and cytokinin concentrations, to maximize callus induction efficiency. Overall, PLATIBACGEL provides a sustainable, scalable, and cost-effective platform for potato tissue culture, with promising implications for broader application in plant micropropagation and biotechnology.

5. Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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