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Pectin-Based Films for Applications in the Horticultural Sector: a Preliminary Characterization

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Plastic containers in the horticulture sector largely rely on plastics of fossil origin. Although these plastics have excellent mechanical properties, resilience towards chemical/microbiological degradation, durability, and affordable price, they have a high environmental impact due to their inherent non-biodegradability. In line with the most recent EU strategies on a trans-sectorial transition to sustainable systems, the horticultural sector is seeking for new materials to produce plant nursery plugs as an alternative to conventional plastics. The present work is a part of the project "BBPlug", which aims to add value to agri-food industry wastes, reducing plastics and fertilizers in horticulture. Here, we propose a new material made of pectin extracted from citrus peel as a green and biodegradable substrate to produce plant nursery plugs. To this purpose, pectin-based films were fabricated by solvent-casting from film-forming solutions with increasing amounts of glycerol as plasticizer (6.7 – 33.3 $g_{Glycerol}/g_{Pectin}$), microfibrillated cellulose (MFC) as reinforcing agent (2.7 – 8.1 mg_{MFC}/g_{Pectin}), and at two different pH values (3.5 and 7.0). Puncture resistance, water solubility, and oxygen-barrier properties of the films were then investigated. Films from formulations at pH = 3.5 exhibited an overall better mechanical behavior over their counterpart at pH = 7. The best puncture resistance and water solubility were displayed by films from the least glycerol-loaded formulations. The addition of MFC to the film-forming solution improved the oxygen-barrier properties of the films but led to a reduction in their water solubility. In other tests, a selection of different plant growth-promoting (PGP) bacteria was demonstrated to have a boosting effect on the development of a model vegetable (i.e., lettuce), thus offering creative opportunities for the advancement of the “BBPlug” project.

1. Introduction

Agri-food losses are considered one of the most generated bio-wastes around the globe, which may potentially be accompanied by dramatic economic losses, as well as strong environmental impact if remain unutilized. Therefore, their exploitation through the recovery of natural reusable materials (e.g., biopolymers, as well as small molecular weight bioactive compounds) outstands as a viable strategy to possibly fulfill a circular economy model, thus improving the sustainability of the whole agri-food production chain (Dahiya et al., 2018). In this realm, an intriguing and emerging application is given by the fabrication of bio-based plant plugs for the horticultural sector to replace conventionally employed plastic-based containers (Fuentes et al., 2021). The latter typically suffer from hampered recyclability owing to contamination by organic matter and chemicals after usage in plant nurseries (Schettini et al., 2013). To tackle this drawback, many scientists have successfully developed biodegradable plugs from food losses, alone or in combination with other naturally derived polymers. The effectiveness of these biodegradable plugs in intensifying both growth and yield of tested crops as compared to the fossil-based counterparts was recently summarized in our perspective paper (Mapelli et al., 2022).
Pectin is a polysaccharide extracted from both food losses and residues of the food industry, such as fruit peel, which has recently emerged as a valuable alternative to non-degradable materials, especially in the packaging sector (Qiang et al., 2024). Its chemical structure consists of chains formed by α (1-4) linked galacturonic acid

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monomers with different degrees of esterification (Viscusi et al., 2021). Apart from being a GRAS (generally recognized as safe) product, pectin holds several interesting features, such as compostability, biodegradability of originating sources, film-forming properties, and large availability on the market (Butler et al., 2023). Notwithstanding this, the design and further fabrication of pectin-based plugs intended for horticultural purposes is still an unexplored research topic. To the best of our knowledge, Viscusi and colleagues have been the only ones to present an initial framework for describing the primary steps in the bio-plug manufacturing process (Viscusi et al., 2021). These steps include the preparation of the pectin solution, molding, and the final drying stage. Unfortunately, they limited their testing to a single pectin-based formulation. Consequently, the relationship between the composition of the pectin solution and the key functional properties exhibited by the resulting solid product remains unexplored.
Therefore, this explorative study aimed to produce and characterize pectin-based films as a function of their composition (e.g., concentration of plasticizer, presence/absence of fillers) in terms of mechanical (e.g, puncture resistance), water solubility, and oxygen-barrier properties, to possibly move a step forward in generating biodegradable plugs. Additional tests were also executed to assess the responses (height and nitrate concentration in leaves) of lettuce, selected as a model plant, to the administration of potential plant-growth-promoting (PGP) bacterial strains. The outcomes of this work pave the way for future activities in the frame of the "BBPlug" project, where PGP bacteria will be incorporated in pectin-based compostable plugs, together with bioactive compounds extracted from fresh-cut vegetable waste, for "in-field" monitoring of the plant performance.

2. Materials and Methods

2.1. Raw materials and chemicals

GENU® pectin free-flowing powder (degree of esterification = typically 35%) extracted from citrus peel was supplied from CP Kelco (Atlanta, Georgia, USA) and used as the main polymeric phase to yield biofilms. Glycerol was purchased from Merck KGaA (Darmstadt, Germany) and used as a plasticizing agent, whereas NaOH in the form of beads was obtained from VWR Chemicals (Milan, Italy). Microfibrillated cellulose (MFC), acting as a reinforcing agent throughout biofilms preparation, was produced at the Paper and Fibre Research Institute (PFI, Trondheim, Norway) using two different types of cellulose, namely i) elemental chlorine-free (ECF) fully-bleached sulfate pulp mainly based on juvenile *Picea abies*, and ii) ECF fully-bleached sulfate cellulose mainly based on mature *Picea abies* with up to 5% by weight (w/w) *Pinus sylvestris* (Cozzolino et al., 2014). Salicylic acid (99%), KNO_3, and H_2SO_4 (95 – 97% v/v) were purchased from Fisher Scientific (Rodano, Italy) and Carlo Erba Reagents (Cornaredo, Italy), and were used for determining the nitrate concentration in tested plants.

2.2. Preparation of film-forming solutions and film formation

1.5% (w/w) of pectin powder was slowly dispersed in distilled water at 90 °C under strong magnetic stirring (780 rpm) until complete dissolution. The so-obtained solutions, whose pH was about 3.5 (BASIC 20 +, Crison, Barcelona, Spain) were cooled down to 25 °C in a water bath and subsequently added with different amounts of glycerol (6.7 - 33.3 $g_{Glycerol}/g_{Pectin}$) under agitation for 10 min. For the sake of comparison, glycerol-free solutions (0 $g_{Glycerol}/g_{Pectin}$) were also prepared and used for film formation. In the first set of experiments investigating the effect of a different pH on the tested properties of the achieved films, the pectin-based solutions were added with a few drops of a 1M NaOH solution up to reaching pH = 7. The second set of experiments involved the addition of MFC at variable concentrations (2.7 - 8.1 mg_{MFC}/g_{Pectin}) only to the native (pH = 3.5) solution containing 6.7 $g_{Glycerol}/g_{Pectin}$. This formulation was chosen as it possessed the best mechanical properties among glycerol-containing samples (see Results and Discussion section).
To obtain pectin-based films, 150 g of each film-forming solution was poured into rectangular plastic templates (10 x 23 cm^2) and subsequently left to dry under controlled conditions at 25°C and 50% relative humidity (RH). All films were stored for 7 days in a polycarbonate vacuum (- 0.06 MPa) desiccator (Lab Companion line, Jeio Tech Co., Ltd., Daejeon, South Korea) under dry conditions before being analyzed.

2.3. Films characterization: puncture, water solubility, and oxygen permeability tests

Puncture tests were conducted according to ASTM F1306A using a Z005 dynamometer (Zwick Roell, Ulm, Germany) coupled to the software TestXpert V10.11 for data analysis. For each tested formulation, circular specimens (6 cm in diameter) were subjected to puncturing by means of a hemispherical probe with a 3.2 mm diameter. All trials were executed at a cross-head speed of 25 mm min^{-1} using a 100 N cell load. "Puncture force vs. distance" plots were recorded and the resistance of samples to punctural stresses was assessed in terms of elastic modulus (E, in MPa), maximum force (F_{MAX}, in N), and work of rupture (W, in mJ).
Biopolymer films were also tested for their solubility in water by visual inspection. Circular specimens of each film (1 cm in diameter) were dipped into distilled water at 25 °C with a solid-to-liquid ratio of 1:100 (g/mL) while

magnetically stirred at 780 rpm. Film solubility in water was measured as the time required for the complete dissolution of the sample. Each formulation was tested using three independent replicates.
Pectin films generated from formulations at pH 3.5 and at 6.7 $g_{Glycerol}/g_{Pectin}$ of glycerol load, with or without MFC addition (2.7 mg_{MFC}/g_{Pectin}), were analyzed in terms of oxygen barrier properties on a 50 cm^2 surface sample using a TotalPerm permeability analyzer (ExtraSolution®Srl, Capannori, Italy) based on the isostatic method and equipped with an electrochemical sensor for oxygen detection. The oxygen transmission rate (OTR, in cm^3 m^{-2} day^{-1}) was determined at 23°C and at two levels of relative humidity, that are, 0% RH and 50% RH, according to the standard methods ASTM D3985 and F1927, respectively. A carrier flow (N_2) of 10 mL min^{-1} and a one-atmosphere oxygen partial pressure difference between both sides of the specimen were applied (Carullo et al., 2023).

2.4. Plant material, application of PGP bacteria, and physiological analyses

The experiments on lettuce plants were carried out in the experimental greenhouse of the University of Milan. Lettuce seeds (*Lactuca sativa* var. longifolia) were sown in pots filled with a peat-based commercial substrate (VigorPlant) and the ability of bacteria to promote plant growth was assessed by inoculating three different strains, namely *Rhizobium sp.*, *Bacillus sp.*, and *Kosakonia sp.* - coded as GR12, LR01, and VR04, respectively - in the substrate before seedlings germination. Strains were administered at the concentration of 10^8 cell/g of soil. When plants reached the proper size for transplant, disruptive and non-disruptive analyses were performed. Chlorophyll content, flavonol content, and nitrogen flavonol index (NFI) were determined *in vivo* on expanded lettuce leaves by using a portable multi-pigment meter MPM-100 (ADC BioSCientific Ltd.). Lettuce plants were harvested by cutting at ground level and plant height was measured from the cut to the uppermost point of the plant. Afterward, approximately 1 g of leaf tissue was sampled and homogenized in a mortar with 3 mL of water. The mixture was centrifuged at 4000 rpm for 15 min at 25°C (ALC centrifuge-model PK130R) and the achieved supernatant was used for the analyses. Nitrate concentration was determined in the leaf extracts by a colorimetric method (Cataldo et al., 1975). In a 15 mL tube, 80 µL of 5% (w/v) salicylic acid solution in concentrated H_2SO_4 was added to 20 µL of plant extract. After rapid mixing, 3 mL of 1.5 N NaOH was added. The absorbance of the final mixture was read at 410 nm via a spectrophotometer (Evolution 300, Thermo Electron Corporation). Nitrate content was calculated and expressed as mg kg^{-1} referring to a KNO_3 standard calibration curve.

2.5. Statistical analysis

All analyses were repeated five times unless otherwise specified. The mean value and standard deviation (SD) were calculated from the experimental data. Statistically significant differences among the averages were evaluated using a one-way analysis of variance (ANOVA) and Tukey's test ($p \leq 0.05$). Statistical analysis was carried out using IBM SPSS Statistics 20 software (IBM Corp., Armonk, New York, USA).

3. Results and Discussion

3.1. Effect of the composition of film-forming solutions on the functionality of pectin-based films

Figure 1 shows the averaged 'force vs deformation' curves obtained from the puncture tests of pectin-based films, as a function of both the glycerol load and the pH of the film-forming solutions.

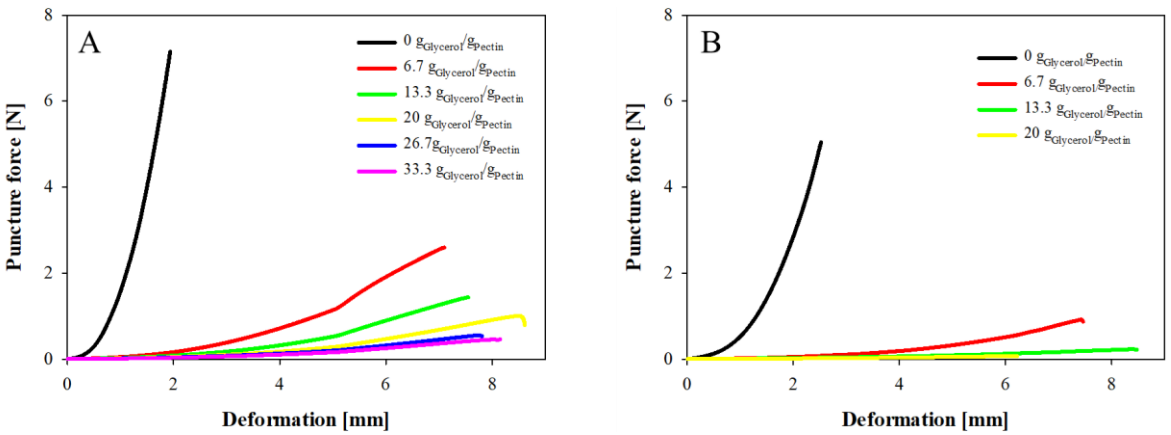

Figure 1: "Puncture force vs deformation" curves for pectin-based films, obtained from film-forming solutions with increasing load of glycerol at pH = 3.5 (A) or pH = 7 (B).

A plasticizer load-dependent effect was disclosed, inasmuch as the maximum force needed to pierce the films gradually increased until reaching a peak value in samples devoid of glycerol. However, films produced at pH = 7 showed a lower toughness as compared to those produced at more acidic conditions. In the former case, films obtained from formulations with a glycerol load within 26.7 – 33.3 $g_{Glycerol}/g_{Pectin}$ were not tested as they collapsed upon detaching from the plastic templates (data not shown). These results can be ascribed to the change in the dissociation degree of carboxyl groups along the pectin chains as a function of the pH (Chen et al., 2021). In particular, a reduction in the electrostatic repulsion among adjacent pectin chains occurs at lower values of pH (i.e., 3.5), thus facilitating chain association via hydrogen bonding and, hence, increasing film strength.
The analysis of the curves of Figure 1 enabled the values of E, F_{MAX}, and W to be extrapolated for the tested pectin-based films (Table 1). All the puncture parameters significantly ($p < 0.05$) decreased when the glycerol load in the film-forming solution was raised within the tested range. Nevertheless, statistical similarities between samples at different pH were recorded only for the elastic modulus and the maximum force in the absence of glycerol. Such a detected loss in biofilms' mechanical properties upon glycerol addition can be explained by invoking the free volume theory (Farris et al., 2010). Being a small molecule, glycerol is capable to accumulate inside the intermolecular polymeric voids and reduce the number of available sites for polymeric chain interaction (e.g., hydrogen bonds). This ultimately leads to an improvement in pectin chain mobility, that is, greater flexibility. Similar results were highlighted in a recent work investigating the influence of glycerol load on the functional properties of arrowroot starch-based films (Tarique et al., 2021). Remarkably, the authors observed an incipient reduction in the tensile parameters - elastic modulus, elongation at break, and tensile strength - of control films when increasing the glycerol load in the film-forming solution up to 45% (w/w on starch basis).

*Table 1: Values of the main parameters retrieved from puncture tests of the pectin-based films investigated in this work. The results are expressed as mean ± SD. For each investigated parameter, different lowercase letters within the same column express significant differences ($p < 0.05$) among mean values, whereas the symbol * indicates a significant difference ($p < 0.05$) between pH = 3.5 and pH = 7 within the same film.*

Glycerol load	E [MPa]		F_{MAX} [N]		W [mJ]	
[$g_{Glycerol}/g_{Pectin}$]	pH = 3.5	pH = 7	pH = 3.5	pH = 7	pH = 3.5	pH = 7
0	5.6 ± 2.6^{b}	3.0 ± 0.2^{c}	7.3 ± 2.9^{c}	5.0 ± 0.5^{c}	$5.9 \pm 2.8^{b,*}$	$5.2 \pm 0.4^{c,*}$
6.6	0.5 ± 0.1^{a}	0.6 ± 0.1^{b}	$2.6 \pm 0.1^{b,*}$	$0.9 \pm 0.2^{b,*}$	$6.3 \pm 0.3^{b,*}$	$2.2 \pm 0.5^{b,*}$
13.3	0.3 ± 0.1^{a}	0.2 ± 0.1^{a}	$1.4 \pm 0.2^{a,*}$	$0.2 \pm 0.1^{a,*}$	$3.8 \pm 0.5^{ab,*}$	$0.7 \pm 0.2^{a,*}$
20	0.2 ± 0.1^{a}	0.2 ± 0.1^{a}	$1.0 \pm 0.1^{a,*}$	$0.2 \pm 0.1^{a,*}$	$2.3 \pm 0.1^{a,*}$	$0.3 \pm 0.1^{a,*}$
26.7	0.2 ± 0.1^{a}	-	0.6 ± 0.1^{a}	-	1.6 ± 0.1^{a}	-
33.3	0.2 ± 0.1^{a}	-	0.5 ± 0.1^{a}	-	1.3 ± 0.1^{a}	-

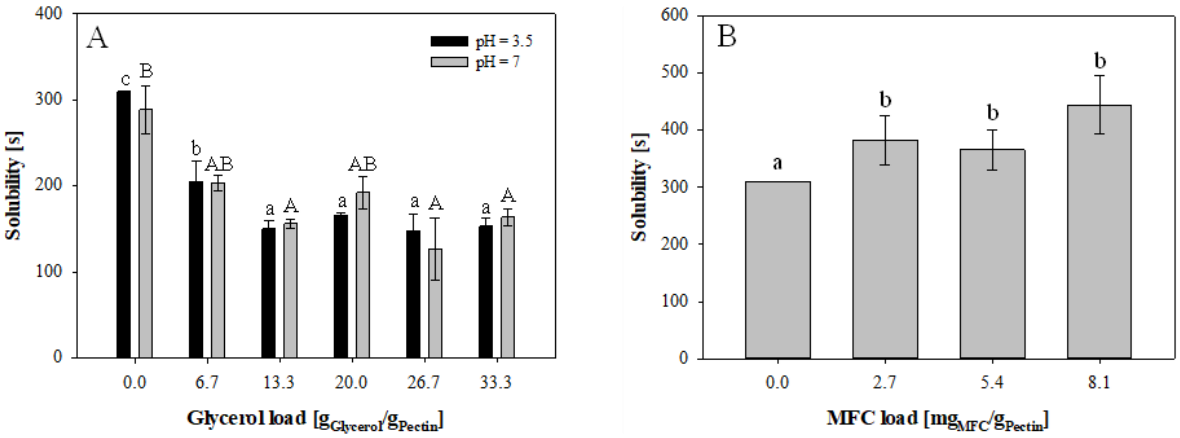

Figure 2: Water solubility of pectin-based films, obtained from film-forming solutions with (A) increasing load of glycerol and at different pH, or (B) increasing load of MFC and at pH = 3.5 (B). In the latter case, the glycerol load was kept constant at 6.7 $g_{Glycerol}/g_{Pectin}$. The results are expressed as mean ± SD. In the left-sided graph, different lowercase and uppercase letters above the bars indicate significant differences among the mean values ($p \leq 0.05$) at pH = 3.5 and pH = 7, respectively.

The plasticizer load dramatically affected the water solubility properties of the investigated films (Figure 2). The time needed to completely dissolve glycerol-free films was about 310 s and 290 s for formulations obtained at pH = 3.5 and pH = 7, respectively (Figure 2A). A significantly ($p < 0.05$) faster dissolution of films was yielded

when employing the lowest glycerol concentration within film-forming solutions, above which no statistical differences could be detected among samples, regardless of the tested pH. A reversed trend was unveiled when dealing with film-forming solutions enriched with MFC (Figure 2B). In this case, a worsening effect towards water solubilization times was induced (+28% on average as compared to control samples), owing to the intrinsic inability of cellulose and its derived forms such as MFC to dissolve in water (Seiler et al., 2020).

*Table 2: Values of oxygen transmission rate (OTR) of the pectin-based films (6.7 $g_{Glycerol}/g_{Pectin}$, pH = 3.5), with or without MFC addition, as a function of the relative humidity. The results are expressed as mean ± SD. Different lowercase letters within the same column express significant ($p < 0.05$) differences between mean values. When reported, the symbol * indicates a significant difference ($p < 0.05$) within the same row (effect of RH).*

MFC load [mg_{MFC}/g_{Pectin}]	OTR [$cm^3\ m^{-2}\ day^{-1}$] 23°C – 0% RH	23°C – 50% RH
0	$27.9 \pm 5.4^{b,*}$	$271.5 \pm 15.4^{b,*}$
2.7	$8.9 \pm 2.1^{a,*}$	$234.1 \pm 12.9^{a,*}$

The incorporation of MFC within the film-forming solution enhanced the O_2 barrier performance of the bare pectin-based film, with a more marked reduction ($p < 0.05$) in OTR values under dry conditions (-68% at 0% RH) rather than in humid environments (-14% at 50% RH), due to the water-sensitive nature of pectin. Overall, our results are consistent with previous literature findings which demonstrated the capability of micro- and nano-fillers to reduce the O_2 permeability of different biopolymers, such as starch, gelatin, pectin, and chitosan (Hoffmann et al., 2019; Hoyos-Merlano et al., 2022; Mellinas et al., 2020).

3.2. Effect of PGP bacteria inoculation on growth parameters of lettuce plant

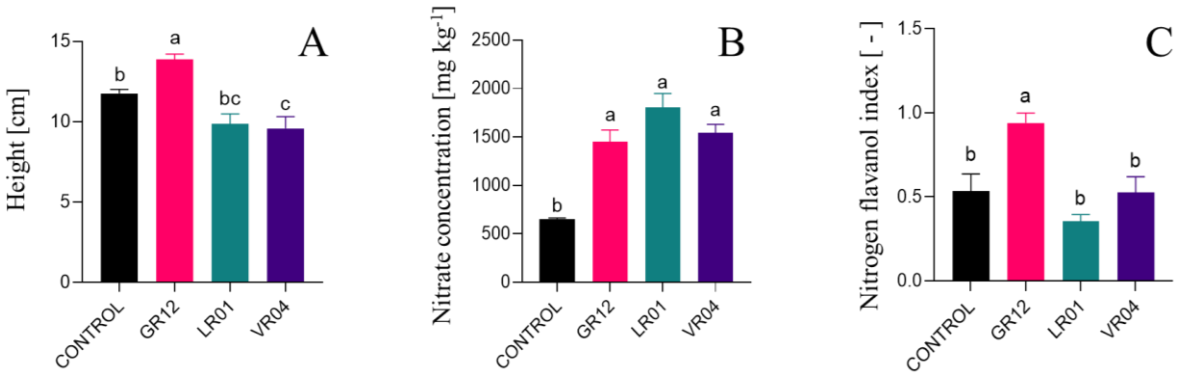

Figure 3: Values of (A) plant height, (B) nitrate concentration, and (C) nitrogen flavonol index in lettuce leaves from both control and inoculated plants. The results are expressed as mean ± SD. For each tested parameter, different letters above the bars indicate significant differences among the mean values ($p \leq 0.05$).

Preliminary trials of *in vivo* colonization of tested plants demonstrated a strong ability of the GR12 strain to colonize lettuce seedling roots (data not shown). Looking at the histograms of Figure 3A, the GR12 strain induced a significant increase (+17.7%, $p < 0.05$) in plant height, whereas an opposite behavior resulted in response to the application of VR04, owing to a slight but significant reduction (-18.4%, $p < 0.05$) in this parameter as compared to the control plants. Interestingly, a significant ($p < 0.05$) increase (+150% on average) in nitrate concentration within leaves was observed upon microbial inoculation, irrespective of the employed strain (Figure 3B). This result seems to suggest either a greater availability of nitrogen in the substrate or an improved uptake of the element by tested plants. Lastly, the *in vivo* analysis of the leaf nitrogen status, expressed as NFI and calculated as the ratio of chlorophyll to flavonols, showed a significantly higher ($p < 0.05$) value only in response to GR12 strain application (+80% over control samples, Figure 3C).

4. Conclusions

This research study shed light on the possibility of using naturally derived materials (e.g., pectin) as alternative substrates to conventional plastics in the fabrication of plant nursery plugs. The basic characterization of the pectin-based film prototypes allowed unraveling the effect of pH, glycerol, and microfibrillar cellulose load on the puncture, water solubility, and oxygen barrier properties thereof. As far as the PGP inoculation tests are concerned, an overall beneficial effect of GR12 strain on plant development parameters during the early stages

of growth was disclosed. Nevertheless, additional studies must be put in place to i) fully characterize the self-standing films (e.g., tensile behavior, water permeability, and moldability), ii) assess their biodegradability in soils of different compositions, and iii) understand their effect on the growth performance of multiple crops when loaded with PGP bacteria, in order to possibly shift towards commercial applications.

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