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Anatomical and histochemical findings of Andean tuber peels. Potential for their nutraceutical application

Hallazgos anatómicos e histoquímicos de cáscaras de tubérculos Andinos. Potencial para su aplicación nutracéutica

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Abstract

The present study aimed to characterize the anatomical features and histochemical distribution of bioactive natural products in the peels of eight Andean tubers and to evaluate the potential of their extracts to promote the development of the probiotic *Lactobacillus acidophilus* biofilm, providing evidence of their potential applications. A comparative anatomical and histochemical analysis was conducted on the peels of *Ullucus tuberosus* Caldas (Basellaceae), two varieties of *Oxalis tuberosa* Mol. (Oxalidaceae), and five varieties of *Solanum tuberosum* L. subsp. *andigena* (Solanaceae) collected from traditional markets in the Argentine Puna. Additionally, aqueous, ethanolic and ethyl acetate extracts from the peels were tested for their ability to promote growth and biofilm formation of *Lactobacillus acidophilus* La-14 (SD5212). The tuber peels exhibited a thick cuticle, single-layered epidermis and cortical amyliferous parenchyma.

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In *O. tuberosa* and *U. tuberosus*, the vascular system consisted of open, collateral bundles, while *S. tuberosum* subsp. *andigena* showed a modified amphiphloic stele with abundant storage parenchyma. Idioblasts containing pigmented compounds such as anthocyanins and carotenoids were associated with tuber coloration. Histochemical assays revealed the presence of phenolic compounds, flavonoids, triterpenes, and proteins stored in epidermal and subepidermal tissues. All extracts promoted dose-dependent biofilm formation of the probiotic bacterium. The results indicate that Andean tuber peels are a natural source of bioactive metabolites with known antioxidant action and potential for the optimal development of the probiotic bacterium *L. acidophilus* La-14 (SD5212) and the future design of sustainable nutraceutical formulations.

Keywords: *Oxalis tuberosa; Solanum tuberosum* subsp. *andigena*; *Ullucus tuberosus*; nutraceutical potential; natural products.

Resumen

El presente estudio se centró en caracterizar la anatomía y la distribución histoquímica de metabolitos bioactivos en las cáscaras de ocho tubérculos andinos, así como en evaluar el potencial de sus extractos para favorecer el desarrollo de la bacteria probiótica *Lactobacillus acidophilus*, vislumbrando sus potenciales aplicaciones. Se realizó un análisis comparativo de las cáscaras de *Ullucus tuberosus* Caldas (Basellaceae), de dos variedades de *Oxalis tuberosa* Mol. (Oxalidaceae) y de cinco variedades de *Solanum tuberosum* L. subsp. *andigena* (Solanaceae), obtenidas en mercados tradicionales de la Puna argentina. Se evaluaron extractos acuosos, etanólicos y acetato de etilo por su capacidad para promover la formación de biofilm y el crecimiento de *L. acidophilus* La-14 (SD5212). Las cáscaras de los tubérculos analizados presentaron una cutícula gruesa, epidermis uniestratificada y parénquima cortical amilífero. En *O. tuberosa* y *U. tuberosus*, el sistema vascular consistió en haces colaterales abiertos; mientras que *S. tuberosum* subsp. *andigena* mostró una estela anfiflóica modificada con abundante parénquima de reserva. Los idioblastos con pigmentos, como antocianinas y carotenoides, se asociaron con la coloración de los tubérculos. Los análisis histoquímicos evidenciaron compuestos fenólicos, flavonoides, triterpenos y proteínas almacenadas en tejidos epidérmicos y subepidérmicos. Todos los extractos promovieron la formación del biofilm de la bacteria probiótica, de forma dosis-dependiente. Los resultados sugieren que las cáscaras de tubérculos andinos son una fuente natural de metabolitos de reconocida acción antioxidante y potencial para el desarrollo óptimo del probiótico *L. acidophilus* La-14 (SD5212) y el diseño futuro de formulaciones nutracéuticas sostenibles.

Palabras clave: *Oxalis tuberosa; Solanum tuberosum* subsp. *andigena*; *Ullucus tuberosus*; potencial nutracéutico; productos naturales.

INTRODUCTION

Native Andean roots and tubers (ART) often referred to as 'Andean treasures' play a pivotal role in the nutrition and economy of farmers of this region. Since ancient times, they have been consumed and used for nutritional and medicinal purposes (Campos *et al*., 2006; Clausen *et al*., 2010). The ART comprise around nine plant families: Asteraceae, Cannaceae, Basellaceae, Brassicaceae, Fabaceae, Nyctaginaceae, Oxalidaceae, Solanaceae, Tropaeolaceae, and Umbelliferae, including species with a remarkable genetic diversity and potential, such as achira (*Canna indica* L.), ahipa (*Pachyrhizus ahipa* (Wedd.) Parodi), arracacha (*Arracacia xanthorrhiza* Bancr.), maca (*Lepidium meyenii* Walp.), mashua (*Tropaeolum tuberosum* Ruiz & Pav.), mauka (*Mirabilis expansa* (Ruiz & Pav.) Standl.), oca (*Oxalis tuberosa* Molina), papas andinas (*Solanum tuberosum* L. subsp. *andigena*), ulluco (*Ullucus tuberosus* Caldas) and yacon (*Smallanthus sonchifolius* (Poepp.) H.Rob.) (Flores *et al*., 2003; Roca *et al*., 2007). The International Potato Centre (Lima, Peru) protects and maintains the remarkable genetic diversity of wild and domestic ART crops threatened by extinction or genetic weakening, their genebank contains more than 7,500 accessions collected from seven countries (Vollmer *et al*., 2022); while the INTA Germplasm Bank Network (Balcarce, Argentina) counts with more than 100 varieties of wild ART, including more than 40 varieties of Andean potatoes, collected from Argentina with the aim of preserving valuable materials for agriculture, food and Andean culture (Atencio, 2019; Lanari, 2022). These species are mainly distributed from the North of Argentina to Colombia and Venezuela, in the Andean region recognized as one of the most important centers of crop origin and diversity in the world, where most important food crops worldwide, such as potato tubers, were domesticated (García-Díaz *et al*., 2023).

Andean roots and tubers grow at high altitudes under extreme conditions of drought, freezing temperatures, and UV exposure (Flores *et al*., 2003), holding potential for exportation and further research in terms of adaptation and use in other regions of the world. They are drought-resistant and adaptable to various agro-climatic conditions. Many ART are categorized as neglected and underutilized species (NUS) despite offering high vitamin, micronutrient, starch content, dietary fibers, and antioxidants associated with health benefits and medicinal properties (Leidi *et al*., 2018). Moreover, peels of *S. tuberosum* exhibit healing properties for wounds (Rosas-Cruz *et al*., 2020) and burns (Camire *et al.*, 2009). However, all the knowledge regarding these species is at present dispersed and very restricted locally as part of a rich traditional heritage. The content of bioactive compounds in the ART and their effects on human health have not yet been fully proven, and there is a strong need to study the physiological and molecular mechanisms underlying their benefits (Leidi *et al*., 2018).

Nutraceuticals are substances that provide medical or health-promoting benefits, including the prevention and treatment of disease, such as food supplements, herbal products, probiotics, and prebiotics that are nowadays of great importance for both pharmaceutical and food industries (Dable-Tupa *et al*., 2020; Reque & Brandelli, 2021). Potatoes and other ART have high antioxidant and antimicrobial potential for effective nutraceutical formulations due to the presence of antioxidant polyphenols, such as chlorogenic acid, caffeoylquinic acid and its derivatives, flavonoids, flavonones, anthocyanins and coumarins, among others. Oca potatoes, mashua, ulluco and other ART constitute natural sources of antioxidants, establishing their value as functional foods. Many of their constituents neutralize free radicals, being important in the fight to prevent cancer, cardio and neurovascular diseases, also exhibiting antibacterial, antiviral, anti-inflammatory, antimutagenic, anticarcinogenic, antihyperglycemic, antiallergic, antithrombotic and vasodilatory properties (Campos *et al*., 2006; Andre *et al*., 2007; Leidi *et al*., 2018). Although there is an extensive bibliography referring to the morphology and anatomy of Andean tubers, few works address the location of interesting bioactive compounds. In potato (*Solanum* sp.) periderm and adjacent tissues (peels), have been reported to concentrate over 50% of total bioactive compounds (Schieber & Aranda Saldaña, 2009; Souza *et al*., 2009).

Lactic acid bacteria have a long history of application in fermented foods due to their beneficial effects on nutritional, organoleptic and product shelf-life. Lactic acid fermentation is one of the most investigated processes due to the potential applications of these bacteria in food, chemical, and pharmaceutical industries. Recently, growing awareness of the role of intestinal microbiota in human health has increased interest in lactic acid bacteria, and the nutraceutical industry is actively promoting the use of *Lactobacillus* species in food as probiotics (Erginkaya & Konuray-Altun, 2022; Abedin *et al*., 2024).

This study aimed to characterize the anatomical features and histochemical distribution of bioactive metabolites in the peels of *U. tuberosus* (Basellaceae), *O. tuberosa* (Oxalidaceae), and *S. tuberosum* subsp. *andigena* (Solanaceae) from the highlands of the Argentinian Puna, and to evaluate the potential of their extracts to stimulate the development of *Lactobacillus* probiotic strain La-14 (SD5212), in order to propose future studies of nutraceutical formulations based on Andean tubers.

MATERIALS AND METHODS

Plant material

Tubers were collected in the high mountain plateau of the Puna desert (2500-4000 m asl) and purchased in the Feria Nacional de la Papa Andina (Alfarcito, Salta, Argentina). Species were identified and classified by Dr María Inés Mercado (Foundation Miguel Lillo) in collaboration with Dr Andrea Clausen and Dr Ariana Digilio of the Banco Activo de Germoplasma EEA-INTA-Balcarce, Argentina.

The selected species were identified by their morphological characteristics as *Oxalis tuberosa* Mol. var. *oca rosa* and var. *oca blanca*, *Ullucus tuberosus* Caldas and five varieties of *Solanum tuberosum* L. subsp. *andigena* var. *miskila negra* o *azul*, var. *miskila colorada*, var. *chila*, var. *cuarentona* and var. *castilla blanca* (Fig. 1).

Anatomical and histochemical analysis

Five fresh tubers of each species and variety were used for optical microscopy studies. At the middle portion of the fresh tubers in a region without nodes (eyes), 2 cm segments of the organ were mounted on dental wax supports and subsequently transversely sectioned with a Microm HM 315 rotating microtome to obtain sections between 15 and 20 μm thick (Mercado & Ponessa, 2020).

For anatomical characterization, the sections were decolorized with 50% sodium hypochlorite, washed with distilled water, and subsequently stained with Astra blue-Safranin and mounted in water-glycerin (50:50) (Zarlavsky, 2014).

Sections of fresh material were subjected to the following histochemical tests: Sudan IV to detect lipids and ruthenium red for pectic substances (Zarlavsky, 2014), NADI reagent (1-naphthol and *N*,*N*-dimethyl-*p*-phenylene diamine, Sigma) to visualize terpenoids and/or essential oil (David & Carde, 1964), toluidine blue O for mucilage (Heslop-Harrison, 1981), iodine potassium for starch (IKI) and observed under polarized light for starch and oxalates (Johansen, 1940), Dragendorff reagent for alkaloids (Zarlavsky, 2014), Liebermann-Burchard for triterpenes and steroids (Harborne, 1999) and picric acid for proteins (Zarlavsky, 2014).

To visualize phenolic compounds and tannins, sections were treated with ferric chloride (Zarlavsky, 2014) and vanillin-hydrochloric acid (Tapia-Torres *et al*., 2014), respectively.

Fig. 1. General appearance of the tubers. **A-B)** *Oxalis tuberosa*. A. var. *rosa.* B. var. *blanca*. **C)** *Ullucus tuberosus*. **D-H)** *Solanum tuberosum* subsp. *andigena.* D. var. *miskila negra*. E. var. *miskila colorada*. F. var. *chila*. G. var. *cuarentona*. H. var. *castilla blanca*. Scale bars: 2 cm.

Fig. 1. Aspecto general de los tubérculos. **A-B)** *Oxalis tuberosa*. A. var. *rosa*. B. var. *blanca*. **C)** *Ullucus tuberosus*. **D-H)** *Solanum tuberosum* subsp. *andigena*. D. var. *miskila negra*. E. var. *miskila colorada*. F. var. *chila*. G. var. *cuarentona*. H. var. *castilla blanca*. Escalas: 2 cm.

To identify flavonoids and other hydroxycinnamic acids, sections were incubated with Neu's reagent (2-aminoethyl-diphenylborinate, Sigma) NP 1% in absolute ethanol (Neu, 1957), Benedict's reagent (Merck, 1980) and 10% KOH (Liakopoulos *et al.*, 2001). Flavonoids and hydroxycinnamic acids were detected by their differential fluorescence with NP reagent (Merck, 1980; Wagner & Bladt, 1996, Mondolot-Cosson *et al*., 1997). Unstained sections were used as controls.

For light microscopy, slides were visualized with a Zeiss Axiolab optic microscope equipped with a polarized light filter fitted with a Zeiss Axiocam ERc 5 s digital camera. For fluorescence microscopy, a Nikon Optiphot with UV light filters UV-1A: 365 nm excitation filters, 400 nm barrier filter was employed. The quantitative parameters were calculated using the software AxioVision 4.3.

Phytochemical extracts

Different dried extracts were obtained from peels of *O. tuberosa* (var. *oca rosa* and *oca blanca*), *U. tuberosus* and *S. tuberosum* subsp. *andigena* (var. *miskila azul* or *negra*, *miskila colorada*, *cuarentona*, *chila*, and *castilla blanca*). Full ethanol extract (EE), aqueous extract (AE) obtained by infusion (at 50 ºC during 30 min) and their sub-extracts obtained by partition of AE with ethyl acetate (EAS), and ethanol (ES) were prepared from each sample, and the key extraction conditions and their yields are listed in Table 1.

Bacterium, medium and bioassays

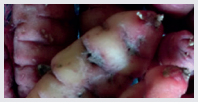
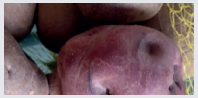
Probiotic bacterium: *Lactobacillus acidophilus* La-14 (SD5212) of human origin and commercially available in food and dietary supplements (Stah & Barrangou, 2013; Nada *et al.*, 2020; USP-NF, 2022) was used for the bioassay. The culture medium employed was PTYg (15 g/ L peptone, 10 g/ L triptone, 10 g/ L yeast extract, and 5 g/ L glucose, pH= 6.0).

Bacterial growth

Overnight culture was diluted to reach an appropriate bacterial density (10^5 CFU/mL). The diluted culture was placed in one of the 96 wells of a microtiter polystyrene plate. Solutions (DMSO-H_2O 50:50) containing the samples (EE, AE, EAS, ES of each plant species) were assayed by sixfold (n = 6) at 25, 50 and 100 μg/mL. Control wells contained the diluted culture (180 μL) and 20 μL of the solution DMSO-H_2O. The maximum level of DMSO to which the cells were exposed was 0.25%.

Table 1. Extraction conditions and yields of the Andean tuber peel extracts.

Tabla 1. Condiciones de extracción y rendimientos de los extractos de las cáscaras de los tubérculos Andinos.

Plant tubers	Varieties		Plant material: Wet peel weights (g)	Plant material: Dry peel weights (g)	Moisture loss (%)	Processed dry peel weights (g)	Extraction solvents	Codes	Extract weights (mg)	Extract yielding (%)
Oxalis tuberosa	*rosa*		87.91	11.93	86.43	8.00	Water 50 °C (3 x 100 mL), 30 min	AE	95.40	1.19
							Ethyl acetate (3 x 100 mL)	EAS	39.60	0.50
							Ethanol (3 x 100 mL)	ES	184.80	2.31
						3.93	Ethanol (3 x 75 mL)	EE	247.00	6.29
	blanca		96.11	11.81	87.71	8.00	Water 50 °C (3 x 100 mL), 30 min	AE	250.30	3.13
							Ethyl acetate (3 x 50 mL)	EAS	28.50	0.36
							Ethanol (3 x 100 mL)	ES	175.60	2.20
						3.81	Ethanol (3 x 75 mL), 3 days	EE	214.90	5.64
Ullucus tuberosus			163.70	28.40	82.65	19.00	Water 50 °C (3 x 100 mL), 30 min	AE	1834.10	9.65
							Ethyl acetate (3 x 100 mL)	EAS	21.60	0.11
							Ethanol (3 x 100 mL)	ES	2157.50	11.36
						9.40	Ethanol (3 x 70 mL), 3 days	EE	169.50	1.80
Solanum tuberosum subsp. *andigena*	*miskila negra*		126.45	18.19	85.61	12.00	Water 50 °C (3 x 100 mL), 30 min	AE	329.00	2.74
							Ethyl acetate (3 x 100 mL)	EAS	22.00	0.18
							Ethanol (3 x 100 mL)	ES	241.60	2.01
						6.19	Ethanol (3 x 75 mL), 3 days	EE	170.40	2.81
	miskila colorada		103.63	18.28	82.36	12.00	Water 50 °C (3 x 100 mL), 30 min	AE	240.30	2.00
							Ethyl acetate (3 x 100 mL)	EAS	18.80	0.16
							Ethanol (3 x 100 mL)	ES	205.60	1.71
						6.28	Ethanol (3 x 75 mL), 3 days	EE	154.90	2.47
	chila		112.16	22.47	79.97	15.00	Water 50 °C (3 x 100 mL), 30 min	AE	402.90	2.69
							Ethyl acetate (3 x 100 mL)	EAS	32.40	0.22
							Ethanol (3 x 100 mL)	ES	189.40	1.26
						7.47	Ethanol (3 x 75 mL), 3 days	EE	193.80	2.59
	cuarentona		88.89	15.24	82.86	10.00	Water 50 °C (3 x 100 mL), 30 min	AE	163.30	1.63
							Ethyl acetate (3 x 100 mL)	EAS	26.30	0.26
							Ethanol (3 x 100 mL)	ES	104.80	1.05
						5.24	Ethanol (3 x 75 mL), 3 days	EE	165.00	3.15
	castilla blanca		111.27	20.80	81.31	14.00	Water 50 °C (3 x 100 mL), 30 min	AE	307.00	2.19
							Ethyl acetate (3 x 100 mL)	EAS	32.10	0.23
							Ethanol (3 x 100 mL)	ES	2161.00	15.44
						6.80	Ethanol (3 x 75 mL), 3 days	EE	198.00	2.92

Bacteria were cultured in liquid medium at 37 ºC, and the bacterial growth was detected at an absorbance of 600 nm, using a microtiter plate reader (Power Wave XS2, Biotek, VT, USA).

Measurement of biofilm biomass

Biofilms formed after 24 h incubation were quantified using a micro method based on a protocol previously reported (O'Toole & Kolter, 1998) with some adaptations. Crystal violet (0.1%) bound to biofilm was removed from each well employing 200 μL ethanol for 15 min at 37 ºC with shaking. Absorbance (560 nm) of ethanol solutions of crystal violet was determined using a microtiter plate reader (Power Wave XS2. Biotek, Vermont, USA). Results of this assessment were given as the percentage of biofilm formation applying the following formula: Biofilm formation percentage = (As / A0) x 100, where A0 is the absorbance of the control, and As is the absorbance of each sample.

Oil spreading assay

The oil-spreading assay is a rapid method for surface-active substance detection, and it is a good choice to explore the air-liquid surface activities of *L. acidophilus* supernatants (from control and treated cultures). After 24 h incubation, bacterial cells were removed by centrifugation for 5 min and the supernatants were filtered through a 0.22 μm pore-size filter to obtain cell-free supernatants.

For the bioassay, 20 μL of mineral oil were placed on a crystallizer (250 mm in diameter) with demineralized water (100 mL). Then, 10 μL of each supernatant were gently put on the center of the oil film (Cartagena *et al.*, 2021). If biosurfactant is present in the supernatant, the oil is displaced, and a clearing zone is formed (Walter *et al.*, 2010). The diameter of this clearing zone on the oil surface correlates to surfactant activity. The diameters of clear halos (mm) visualized under visible light were measured in quintuplicate with respect to the control supernatant. Tween 80 (polysorbate 80, Merck, Darmstadt, Germany) was employed as a reference standard (positive control). The negative controls were the PTYg medium (without bacteria), and the solution of the samples assayed.

Statistical analysis

Data are presented as mean ± SD from at least five or six independent experiments. Tukey's test evaluated the statistical significance of differences between mean values. A value of $p < 0.05$ was considered significant.

RESULTS AND DISCUSSION

Anatomy

The analyzed tubers showed thick cuticle, pavement uniseriate epidermis with 1-2 layers of sub-epidermal angular to lamellar collenchyma (Fig. 2A, B; 3A-B, E-F, I-J), which was early replaced by a periderm in *S. tuberosum* (Fig. 2C; 4A-B, E-F, I-J, M-N, Q-R). Immediately below an amylaceous cortical parenchyma, compose by thin-walled isodiametric cells was found (Fig. 2A-C; 3A-B, E-F, I-J; 4A-B, E-F, I-J, M-N, Q-R).

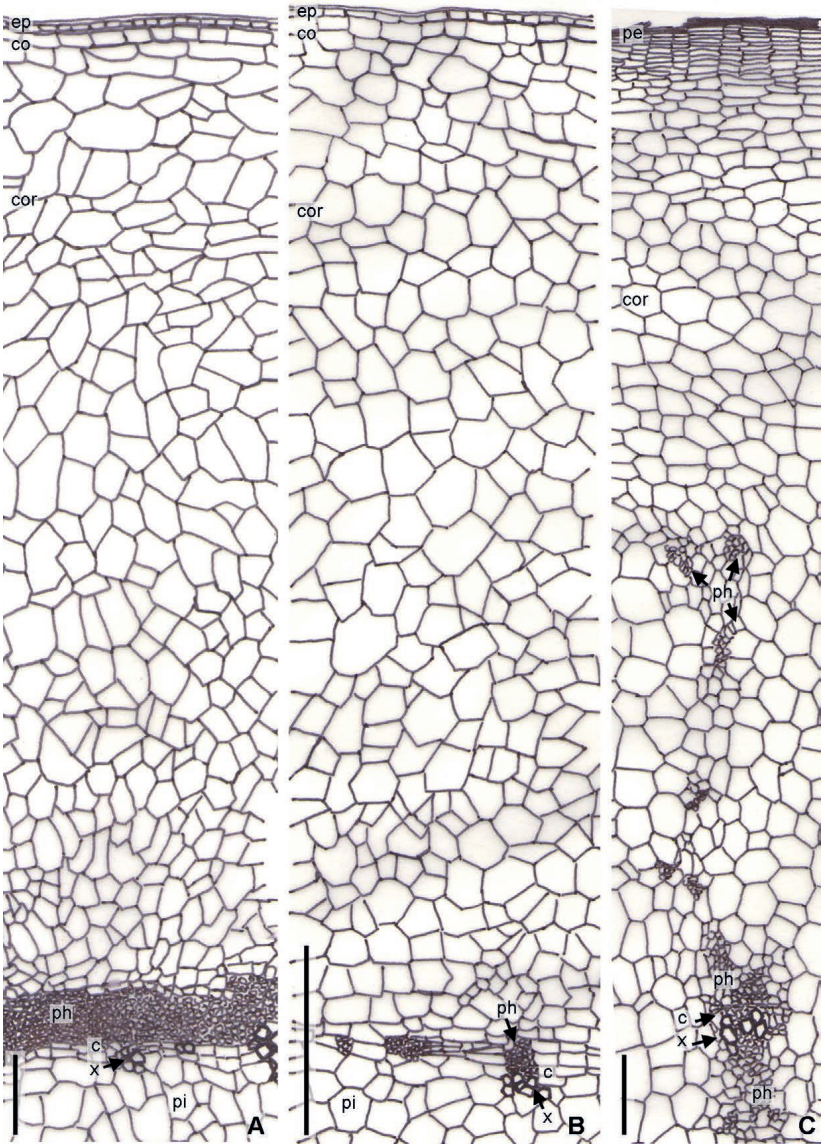


Fig. 2. Transversal section of the tubers. **A)** *Oxalis tuberosa*. **B)** *Ullucus tuberosus*. **C)** *Solanum tuberosum* subsp. *andigena*. Scale bars: 500 μm. References: c, cambium; co, collenchyma; cor, cortex; ep, epidermis; pe, peridermis; ph, phloem; pi, pith; x, xylem.

Fig. 2. Sección transversal de los tubérculos. **A)** *Oxalis tuberosa*. **B)** *Ullucus tuberosus*. **C)** *Solanum tuberosum* subsp. *andigena*. Escalas: 500 μm. Referencias: c, cámbium; co, colénquima; cor, corteza; ep, epidermis; pe, peridermis; ph, floema; pi, medula; x, xilema.

In *O. tuberosa* and *U. tuberosus*, the vascular system was constituted by open, isolated collateral vascular bundles. In the ocas early secondary growth occurs exhibiting phloem as a continuous ring (Fig. 2A); whereas in *Ullucus* bundles are arranged in a cycle and separated by large areas of reserve parenchyma, each bundle showed well-developed phloem and a few xylem vessels (Fig. 2B). *S. tuberosum* subsp. *andigena*, exhibited an unusual secondary growth, the vascular cylinder formed a modified amphiphloic with stellate parenchyma pith and scattered phloem and xylem strands separated by abundant reserve parenchyma rich in starch (Fig. 2C). In *U. tuberosus* and *S. tuberosum*, the vascular system and the pith occupies more than 70% to 80% of the surface of the tuber section; meanwhile in *Oxalis* it represents approximately 50% of the diameter of the cross section. Similar characteristics were previously described (Sabba & Lulai, 2002).

The epidermis/peridermis and subjacent tissues (subepidermal collenchyma and amylaceous cortical parenchyma) compose tuber peels. In control un-stained samples, the colored varieties showed pink-violet or orange-yellow idioblasts or cells containing colored droplets (Fig.3A-B, E-F, I-J; 4A-B, E-F, I-J, M-N, Q-R). These idioblasts play a crucial role in determining the macroscopically observed color, indicating the presence of anthocyanins, carotenes, and/or isoflavones (Schieber & Aranda Saldaña, 2009; Souza *et al.*, 2009).

As stated by Valcárcel-Yamani *et al.* (2013), Zhu & Cui (2020), Hernández-Uribe *et al.* (2020), Cruz *et al.* (2023) starch grains displayed significant variability in size and shape. *Oxalis* starch grains were characterized by ellipsoidal, ovoid, and polyhedral shapes, ranging from 8 to 75 μm in length, with visible deposition bands and an eccentric hilum (Fig. 3C-D, G-H). *Ullucus* showed ellipsoidal, polyhedral, pyriform and irregular shaped starch grains, ranging from 2 to 58 μm in length. These grains featured notable lamelae and an eccentric hilum, sometimes fissured (Fig. 3K-L). Finally, Andean potatoes grains were predominantly ellipsoidal to ovoid, occasionally spherical or polyhedral, and varied in length from 2 to 57 μm, they exhibited evident lamellae and an eccentric hilum (Fig. 4C-D, G-H, K-L, O-P, S-T).

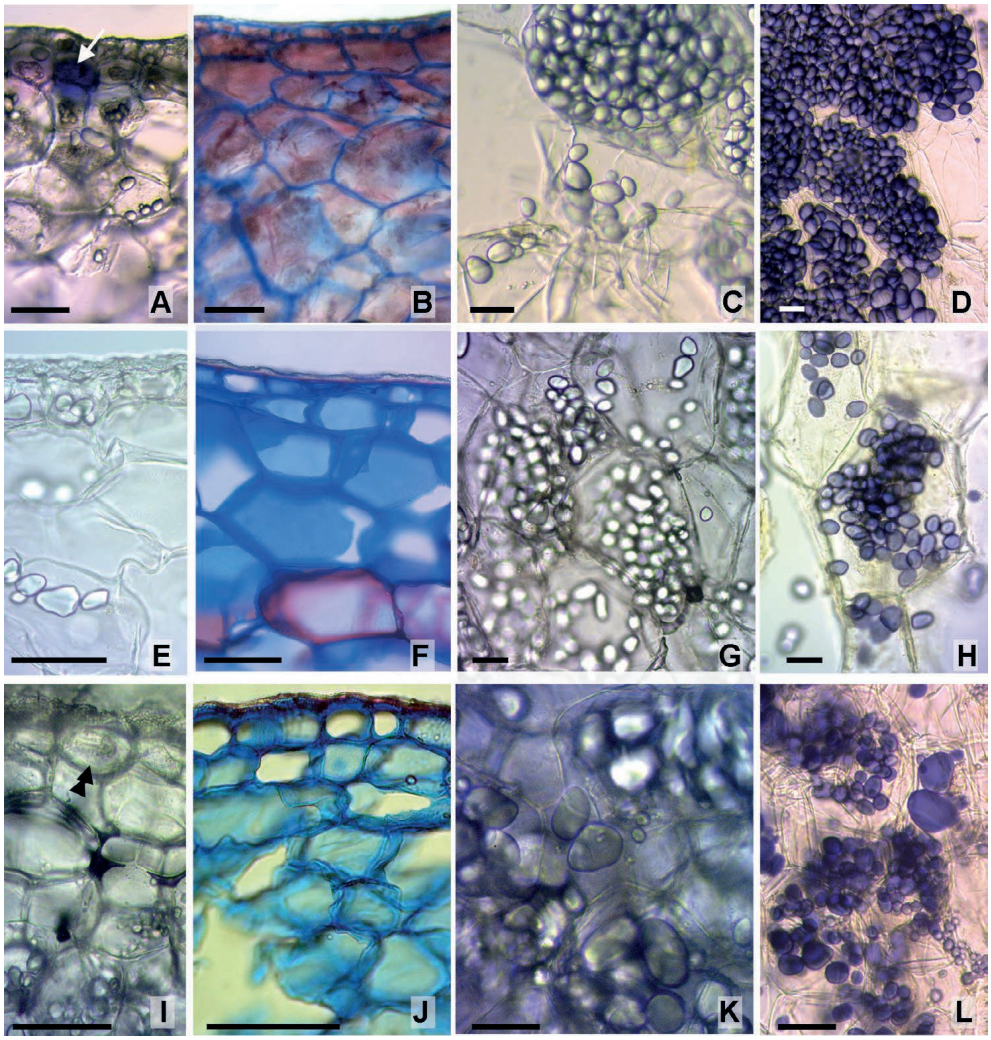


Fig. 3. Transversal section of the tuber peels. **A-H)** *Oxalis tuberosa*. A-D. var. *rosa*. E-H. var. *blanca*. **I-L)** *Ullucus tuberosus*. A, E, I. Unstained control. B, F, J. Astra blue - safranin. C, G, K. Starch grains under bright light. D, H, L. Starch grains stained with Lugol's solution. Scale bars A-B, E-F, I-J, 100 μm; C-D, G-H, K-L, 50 μm. References: arrow, pink-violet idioblast; double arrowhead, orange-yellow idioblast.

Fig. 3. Sección transversal de las cáscaras de los tubérculos. **A-H)** *Oxalis tuberosa*. A-D. var. *rosa*. E-H. var. *blanca*. **I-L)** *Ullucus tuberosus*. A, E, I. Control sin teñir. B, F, J. Azul de Astra - safranina. C, G, K. Granos de almidón a luz brillante. D, H, L. Granos de almidón teñidos con Lugol. Escalas: A-B, E-F, I-J, 100 μm; C-D, G-H, K-L, 50 μm. Referencias: flecha, idioblasto rosa-violeta; doble punta de flecha, idioblasto naranja-amarillo.

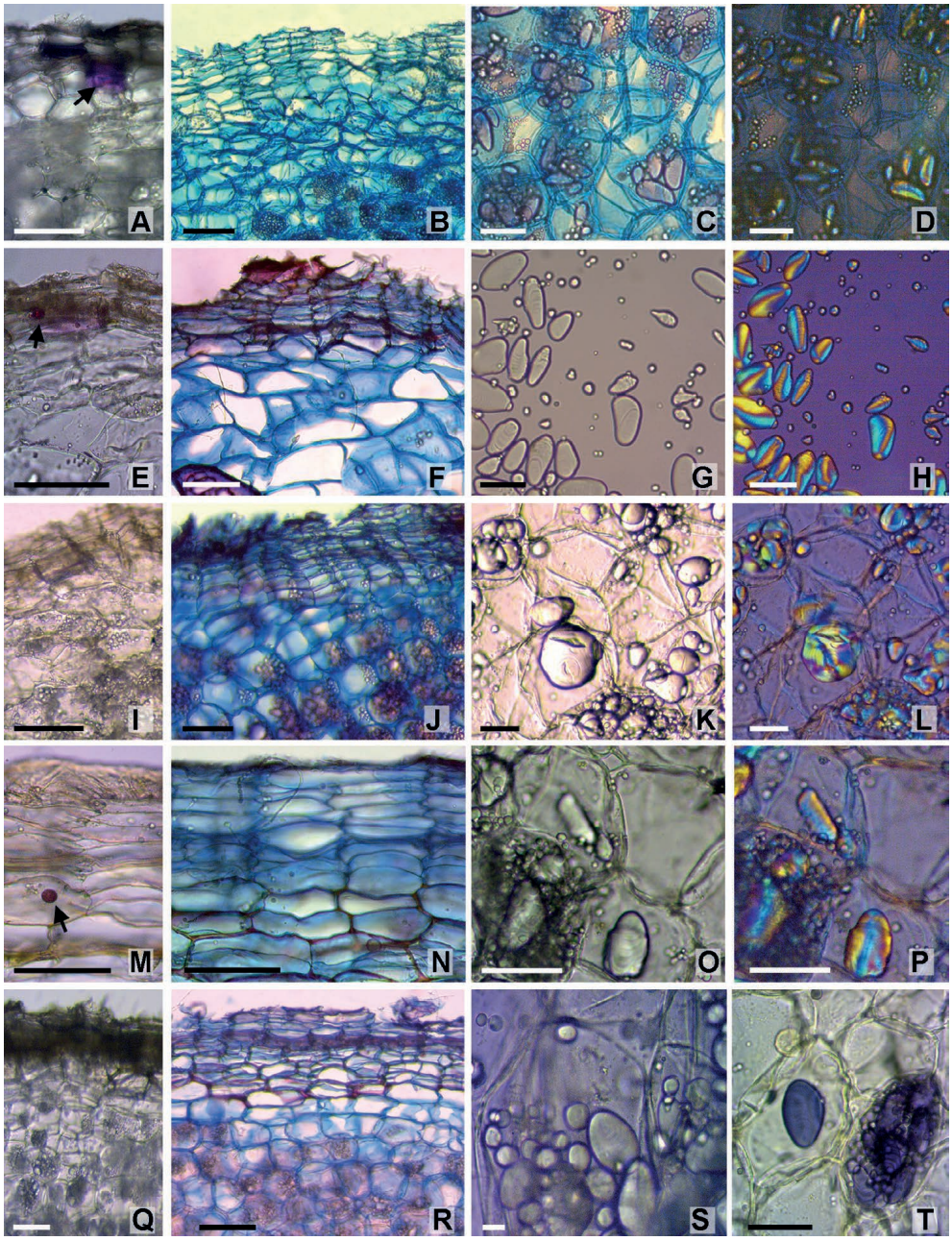


Fig. 4. Transversal section of tuber peels of *Solanum tuberosum* subsp. *andigena*. **A-D)** var. *miskila negra*. **E-H)** var. *miskila colorada*. **I-L)** var. *chila*. **M-P)** var. *cuarentona*. **Q-T)** var. *castilla blanca*. A, E, I, M, Q. Unstained control. B, F, J, N, R. Astra blue - safranin. C, G, K, O, S. Starch grains under bright light. D, H, L, P. Starch grains under polarized light. T. Starch grains stained with Lugol's solution. Scale bars, A-B, E-F, I-J, M-N, Q-R, 100 μm; C, D, G, H, K, L, 50 μm. References: arrow, pink-violet idioblast or content.

Fig. 4. Sección transversal de las cáscaras de los tubérculos de *Solanum tuberosum* subsp. *andigena*. **A-D)** var. *miskila negra*. **E-H)** var. *miskila colorada*. **I-L)** var. *chila*. **M-P)** var. *cuarentona*. **Q-T)** var. *castilla blanca*. A, E, I, M, Q. Control sin teñir. B, F, J, N, R. Azul de Astra - safranina. C, G, K, O, S. Granos de almidón a luz brillante. D, H, L, P. Granos de almidón a luz polarizada. T. Granos de almidón teñidos con Lugol. Escalas: A-B, E-F, I-J, M-N, Q-R, 100 μm; C, D, G, H, K, L, 50 μm. Referencias: flecha, idioblasto rosa-violeta o contenido.

Histochemical analysis

Table 2 provides a summary of the histochemical tests conducted on the examined tuber peels. Oca and ulluco samples exhibited positive results for lipids on the external walls of the epidermis due to the presence of a thick cuticle. In addition, all the Andean potato varieties showed similar results both in the external and internal periclinal walls of the periderm attributable to suberin depositions (Fig. 5A-D). The middle lamellae of cell walls in the periderm, epidermis, and cells of the cortical parenchyma pectins were detected (Fig. 5E-H).

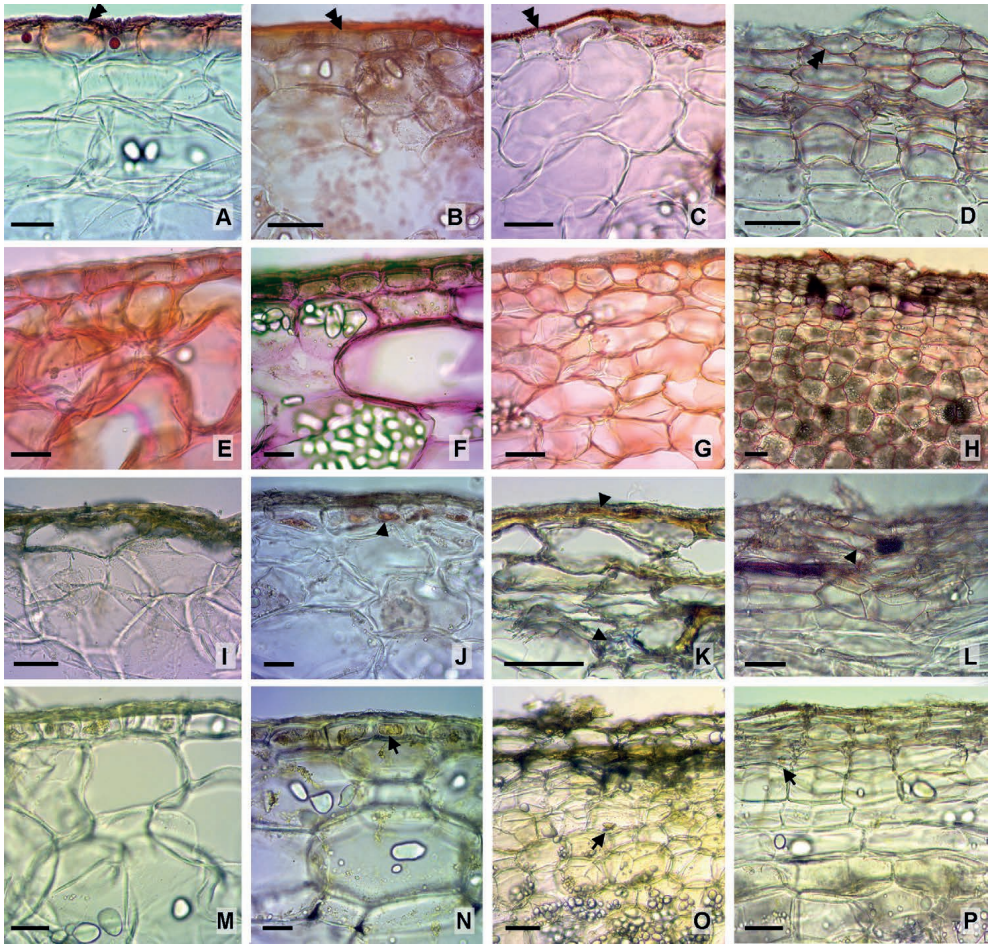


Fig. 5. Transversal section of the tuber peels. Histochemical tests. **A-D)** Sudan IV for detection of lipids (red, double arrowhead). **E-H)** Ruthenium red to identify pectins (bright red). **I-L)** Vanillin-HCl for tannins (red, arrowhead). **M-P)** Protein with picric acid (arrow). A, E, I, M. *Oxalis tuberosa* var. *rosa*. B, F, J, N. *O. tuberosa* var. *blanca*. C, G, K, O. *Ullucus tuberosus*. D, H, L, P. *Solanum tuberosum* subsp. *andigena* var. *miskila negra*. Scale bars: 50 μm.

Fig. 5. Sección transversal de las cáscaras de los tubérculos. Ensayos histoquímicos. **A-D)** Sudan IV para detección de lípidos (rojo, doble punta de flecha). **E-H)** Rojo de rutenio para identificación de pectinas (rojo intenso). **I-L)** Vainillina-HCl para taninos (rojo, punta de flecha). **M-P)** Proteínas con ácido pícrico (flecha). A, E, I, M. *Oxalis tuberosa* var. *rosa*. B, F, J, N. *O. tuberosa* var. *blanca*. C, G, K, O. *Ullucus tuberosus*. D, H, L, P. *Solanum tuberosum* subsp. *andigena* var. *miskila negra*. Escalas: 50 μm.

Table 2. Histochemical identification of compound classes in peels of Andean tubers.

Tabla 2. Identificación histoquímica de las clases de compuestos en las cáscaras de tubérculos andinos.

Staining reagents	Target compounds	Revealed color	PLANT TUBERS							
			Oxalis tuberosa		*Ullucus tuberosus*	*Solanum tuberosum* subsp. *andigena*				
			rosa	blanca		miskila negra	miskila colorada	chila	cuarentona	castilla blanca
Dragendorff	Alkaloids	Orange / orange red	-	-	-	-	-	-	-	-
Ferric chloride	Dihydroxyphenols	Dark-green or grey	++	++	++	++	++	++	++	++
NP/Benedict	Caffeic and/or chlorogenic Acid	Blue / blue-green fluorescence	+	+	+	+	+	+	+	+
Benedict	O-dihydroxyflavonoids	Orange / yellow fluorescence	++	++	+	-	-	-	-	+
NP	Flavonoids and flavones	Yellow / orange fluorescence	++	++	+	-	-	-	-	+
KOH	Ferulic and p-coumaric acid	Blue fluorescence	+	+	+	+	+	+	+	+
Sudan IV	Lipids	Red	++	++	++	++	++	++	++	++
Toluidine blue	Mucilages/Polysaccharides	Blue	-	-	-	-	-	-	-	-
Ruthenium red	Pectin	Red	++	++	++	++	++	++	++	++
Picric acid	Proteins	Bright yellow	+	+	+	+	+	+	+	+
Vainillin HCl	Tannins	Red	-	+	-	++	++	++	-	-
Liebermann Burchard	Triterpenes	Red	+	+	+	+	-	+	+	+
	Steroids	Blue	-	-	-	-	-	-	-	-
Lugol	Starch	Blue-black	++	++	++	++	++	++	++	++

References: NP: Natural products reagent. **++**: Very positive reaction, **+**: Positive reaction, **-**: Negative reaction.
Benedict's reagent for o-dihydroxy flavonoids and cumarins. Ferric chloride for dihydroxyphenols (catechol type phenols).

Referencias: NP: reactivo de productos naturales. **++**: reacción muy positiva, **+**: reacción positiva, **-**: reacción negativa.
Reactivo de Benedict para flavonoides o-dihidroxilados y cumarinas. Cloruro férrico para dihidroxifenoles (fenoles tipo catecol).

Regarding tannins, the content of the epidermal cells of *O. tuberosa* var. *blanca* displayed a reddish coloration with vanillin-HCl reflecting the presence of these compounds (Fig. 5J); while the var. *colorada* of *Oxalis* and the *Ullucus* tubers showed negative results (Fig. 5I-K). The *miskila negra*, *miskila colorada* and *chila* varieties of *S. tuberosum* subsp. *andigena* showed similar results for tannins at the periclinal walls of the periderm cells (Fig. 5L, only positive results for var. *miskila negra* are shown).

Crystalline structures in the epidermal cells of oca and in cortical cells of ulluco and Andean potatoes reacted positively with picric acid, revealing their protein nature (Fig. 5M-P). Finally, the Liebermann-Buchard test was positive for triterpenes displaying an ephemeral reddish color in the epidermis of oca and ulluco, and in isolated parenchyma cells from the cortex of Andean potatoes, except in the *miskila colorada* variety (instantaneous reaction, images not shown). Both mucilages and alkaloids tests yielded negative results across all the tested samples.

Positive phenol detection using ferric chloride was consistent across all cases. In oca, phenols were predominantly observed (Fig. 6A-B). Conversely, in ulluco, the grey-green coloration appeared in both the external and internal periclinal walls of the epidermis (Fig. 6C). Finally, phenolic depositions were evident in the periderm cells and intercellular spaces of the cortex in Andean potato varieties (Fig. 7A-E).

When examined under a fluorescence microscope, untreated material from both ocas and ulluco exhibited blue autofluorescence at the cuticles, which is an indicative of cutinization and suberization (Donaldson, 2020). The cell walls of the collenchyma and cortical parenchyma of *Ullucus* also displayed a similar pattern with green-bluish fluorescence (Fig. 6D-F). Similarly, Andean potatoes showcased an intense blue autofluorescence, characteristic of suberin on the anticlinal walls of the periderm cells. Notably, in some varieties, an additional blue fluorescence in the cell walls of some the cortical parenchyma was observed (Fig. 7F-J). The cell wall fluorescence aligns with the findings suggested by Donaldson (2020) and Siemińska-Kuczer *et al.* (2022), which attributed it to phenolic acid derivatives crosslinked as cell walls components.

Upon further examination with NP and Benedict's reagent to identify flavonoids, flavones or flavanones, diverse blue-green fluorescence patterns were revealed in the cuticles of *Oxalis* (Fig. 6 G-H, J-K), and the cortical cells wall of *Ullucus* (Fig. 6I, L), as well as, in the internal strata of the peridermis and the cortical parenchyma cell walls of the Andean potato varieties (Fig. 7K-T). These findings strongly suggest the presence of phenolic acids derivatives (Donaldson, 2020). While, an orange and yellow fluorescence was observed at the subepidermal collenchyma and cortical parenchyma of both *O. tuberosus* varieties; at the cuticle of *Ullucus* (Fig. 6G-I) and at the internal layers of the peridermis of *S. tuberosum* subsp. *andigena* var. *cuarentona* and var. *castilla blanca* (Fig. 7N-O).

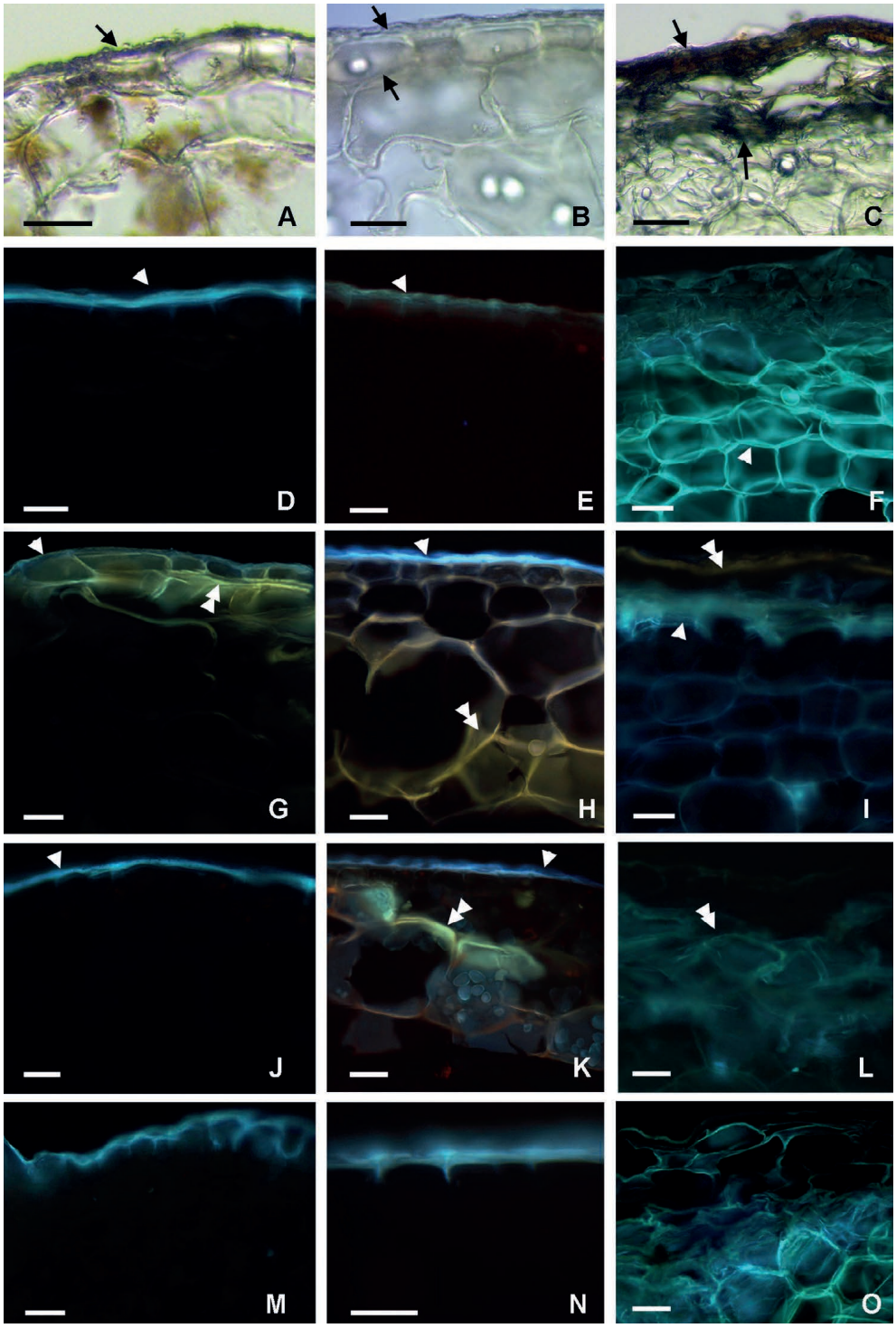


Fig. 6. Transversal section of the tuber peels. Histochemical test. **A-C)** Detection of phenols (arrow) with ferric chloride, observed under bright light field. **D-O)** Fluorescence microscopy. D-F. Untreated control samples. Autofluorescence at 365 nm. G-I. NP reactive to identify phenolic acids and derivatives (greenish blue, arrowhead), flavones, flavanones, and flavonoids (orange, reddish yellow, double arrowhead). J-L. Benedict's reagent for the identification of flavones and flavanones and flavonoids (orange, reddish, yellow, double arrowhead). M-O. Detection of ferulic and *p*-coumaric acids by increased blue fluorescence after incubation with KOH. A, D, G, J, M. *Oxalis tuberosa* var. *rosa*. B, E, H, K, N. *O. tuberosa* var. *blanca*. C, F, I, L, O. *Ullucus tuberosus*. Scale bars: 50 µm.

Fig. 6. Sección transversal de las cáscaras de los tubérculos. Ensayo histoquímico. **A-C)** Detección de fenoles (flecha) con cloruro férrico, observados a campo claro. **D-O)** Microscopía de fluorescencia. D-F. Muestras control sin tratar. Autofluorescencia a 365 nm. G-I. Reactivo NP para identificar ácidos fenólicos y derivados (verde-azulado, punta de flecha), flavonas, flavanonas y flavonoides (naranja, amarillo rojizo, doble punta de flecha). J-L. Reactivo de Benedict para la identificación de flavonas, flavanonas y flavonoides (naranja, amarillo rojizo, doble punta de flecha). M-O. Detección de ácidos ferúlico y *p*-cumárico por aumento de fluorescencia azul tras incubación con KOH. A, D, G, J, M. *Oxalis tuberosa* var. *rosa*. B, E, H, K, N. *O. tuberosa* var. *blanca*. C, F, I, L, O. *Ullucus tuberosus*. Escalas: 50 μm.

In accordance with established references, this fluorescence may be indicative of the presence of flavones/flavanones and flavonoids, respectively (Donaldson, 2020).

In all samples, an increase in the fluorescence of the epidermis and periderm cell walls is noted, upon incubation with potassium hydroxide. This observation suggests the potential presence of ferulic and *p*-coumaric acids, which is aligned with the findings reported by Liakopoulos *et al.* (2001) (Fig. 6M-O and 7U-Y).

The medicinal properties attributed to Andean tubers are believed to be linked, at least in part, to the presence of antioxidant polyphenols. Notable among these are chlorogenic acid, caffeoylquinic acid and its derivatives, flavonoids, flavonones, anthocyanins, and coumarins, as outlined by various studies (Lachman & Hamouz, 2005; Campos *et al.*, 2006). The research conducted by Campos *et al.* (2006) and Andre *et al.* (2007) underscored the antioxidant potential of Andean tubers, such as oca and ulluco, positioning them as valuable contributors to functional foods. These compounds possess the ability to neutralize free radicals, thereby playing a crucial role in the prevention of cancer, cardiovascular diseases, and neurovascular diseases. For potatoes (*Solanum* sp.), it has been demonstrated that these bioactive compounds are commonly concentrated in the periderm and adjacent tissues, accumulating at levels exceeding 50%. This phenomenon has been documented in previous studies (Schieber & Aranda Saldaña, 2009; Souza *et al.*, 2009).

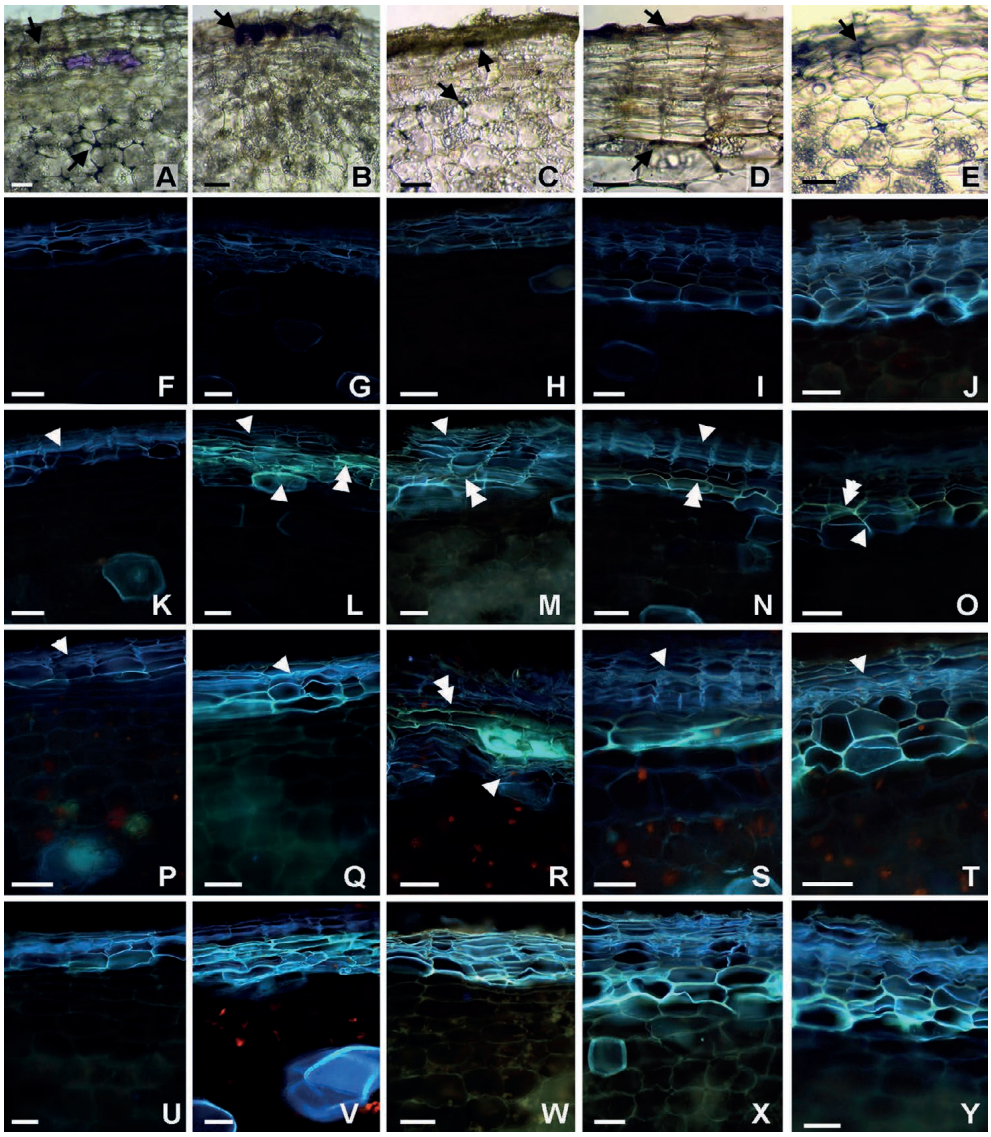


Fig. 7. Transversal section of the tuber peels of *Solanum tuberosum* subsp. *andigena*. Histochemical test. **A-E)** Detection of phenols (arrow) with ferric chloride, under bright light field. **F-Y)** Fluorescence microscopy F-J. Untreated control samples. Autofluorescence at 365 nm. K-O. NP reactive to identify phenolic acids and derivatives (greenish blue, arrow head), flavones, flavanones and, flavonoids (orange, reddish yellow, double arrowhead). P-T. Benedict's reagent for the identification of flavones, flavanones, and flavonoids (orange, reddish yellow, double arrowhead). U-Y. Detection of ferulic and *p*-coumaric acids by increased blue fluorescence after incubation with KOH. A, F, K, P, U. var. *miskila negra*. B, G, L, O, V. var. *miskila colorada.* C, H, M, R, W. var. *chila*. D, I, N, S, X. var. *cuarentona*. E, J, O, T, Y. var. *castilla blanca*. Scale bars: 50 μm.

Fig. 7. Sección transversal de las cáscaras de *Solanum tuberosum* subsp. *andigena*. Ensayo histoquímico. **A-E)** Detección de fenoles (flecha) con cloruro férrico, a campo claro. **F-Y)** Microscopía de fluorescencia. F-J. Muestras control sin tratar. Autofluorescencia a 365 nm. K-O. Reactivo NP para identificar ácidos fenólicos y derivados (verde-azulado, punta de flecha), flavonas, flavanonas y flavonoides (naranja, amarillo rojizo, doble punta de flecha). P-T. Reactivo de Benedict para la identificación de flavonas, flavanonas y flavonoides (naranja, amarillo rojizo, doble punta de flecha). U-Y. Detección de ácidos ferúlico y *p*-cumárico por aumento de fluorescencia azul tras incubación con KOH. A, F, K, P, U. var. *miskila negra*. B, G, L, O, V. var. *miskila colorada*. C, H, M, R, W. var. *chila*. D, I, N, S, X. var. *cuarentona*. E, J, O, T, Y. var. *castilla blanca*. Escalas: 50 μm.

Human probiotic *Lactobacillus acidophilus* strain

All peels' extracts resulted in non-inhibitory effects for the probiotic *L. acidophilus* La-14 (Fig. 8 and 9, Supplementary material, Fig. A-F). Some extracts significantly increased the biofilm biomass ($p < 0.05$). The highest stimulations were exerted by EAS and EE of *S. tuberosum* subsp. *andigena* var. *chila* (64.84% and 55.44% at 50 μg/mL, respectively), as shown in Fig. 8. In the case of *oca rosa* (Fig. 9), EAS increased the *L. acidophilus* biofilm by 43.14%; while the *Ullucus tuberosus* EAS increased by 41% at 100 μg/mL (in both cases), and EE at 25 μg/mL similarly promoted biofilm formation (41.36%), as shown in Fig. SA-F.

Regarding the planktonic bacterial development, the *O. tuberosa* var. *oca blanca* AE and the *S. tuberosum* subsp. *andigena* var. *blanca* EE at 25 μg/mL increased planktonic growth by 35.93% and 27.98% (respectively), in relation to the control culture (without the natural product addition). Therefore, it turned out to be a growth promoter on the *L. acidophilus* strain (Supplementary material, Fig. A-F).

According to Table 2, *S. tuberosum* subsp. *andigena* var. *chila* and *O. tuberosa* var. *oca rosa* represent valuable natural sources of dihydroxyphenols, ferulic and *p*-coumaric acid (Lachman & Hamouz, 2005; Campos *et al.*, 2006). In the case of potato, the presence of *o*-dihydroxyflavonoids, flavonoids and flavones are well known (Lachman & Hamouz, 2005; Campos *et al.*, 2006). To the best of our knowledge, this study provides original insights into the potential role of major metabolites of Andean tuber peels in the modulation of biofilm formation by probiotic bacterium *L. acidophilus* La-14 (SD5212).

The bioactive extracts analyzed in this study had been previously characterized by UV spectroscopy, showing absorption maxima consistent with flavonoid-type compounds (band I: 300–390 nm; band II: 250–280 nm). These findings are in agreement with the histochemical evidence obtained for tuber peel tissues. Preliminary qualitative tests for phenolic compounds and flavonoids ($FeCl_3$ and $AlCl_3$ reagents) further supported the presence of these chemical classes. Among the evaluated extracts, EAS showed the highest activity in previous assays involving the lactic acid bacterium *Lacticaseibacillus paracasei* CO1LVP105. Orphèe *et al.* (2025) reported that this extract exhibited a total phenolic content of 226.39 ± 18.25 mg/g dry peel and a total flavonoid content of 398.04 ± 18.02 mg/g dry peel. Future studies integrating quantitative phytochemical analyses and their correlation with microbial endpoints will be necessary to fully elucidate the functional relationships suggested in this work.

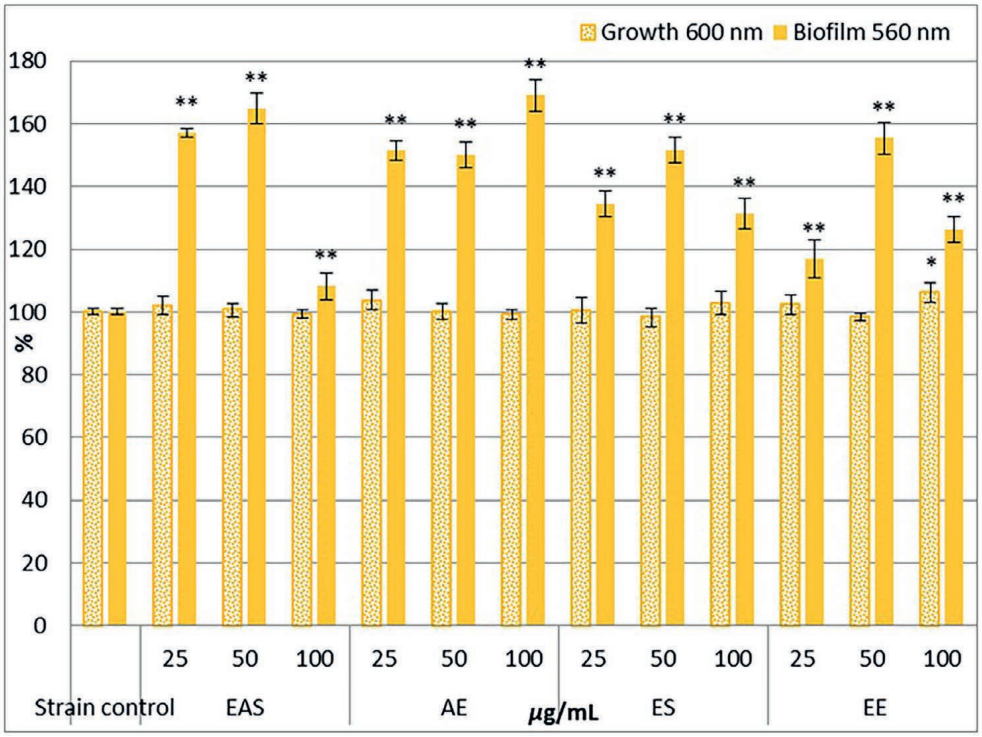


Fig. 8. Effects of *S. tuberosum* subsp. *andigena* var. *chila* on *L. acidophilus* La-14 (SD5212). **EAS:** Ethyl acetate sub-extract, **AE:** Aqueous extract, **ES:** Ethanol sub-extract, **EE:** Ethanol extract. Bars with asterisks (* for growth and ** for biofilm formation) showed significant differences with respect to each control ($p < 0.05$, n=6). The results are expressed as percentages (%). Growth (%) = (As/A0) × 100, where A0 is the absorbance of the control and As is the absorbance of each sample (measured at 600 nm). Biofilm formation (%) = (As/A0) × 100, where A0 is the absorbance of the control and As is the absorbance of each sample (measured at 560 nm).

Fig. 8. Efectos de *S. tuberosum* subsp. *andigena* var. *chila* sobre *L. acidophilus* La-14 (SD5212). **EAS:** subextracto en acetato de etilo; **AE:** extracto acuoso; **ES:** subextracto etanólico; **EE:** extracto etanólico. Las barras con asteriscos (* para crecimiento y ** para formación de biofilm) indican diferencias significativas respecto a cada control ($p < 0,05$, n=6). Los resultados se expresan como porcentajes (%). Crecimiento (%) = (As/A0) x 100, donde A0 es la absorbancia del control y As es la absorbancia de cada muestra (medidas a 600 nm). Formación de biofilm (%) = (As/A0) x 100, donde A0 es la absorbancia del control y As es la absorbancia de cada muestra (medidas a 560 nm).

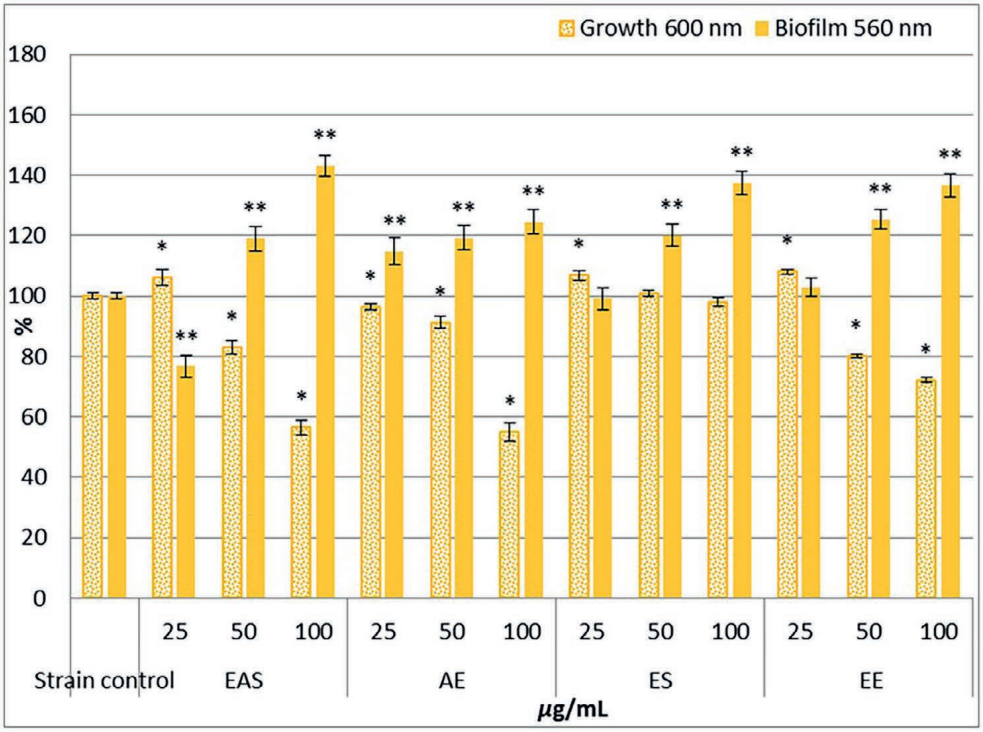


Fig. 9. Effects of *Oxalis tuberosa* var. *oca rosa* on *L. acidophilus* La-14 (SD5212). **EAS:** Ethyl acetate sub-extract, **AE:** Aqueous extract, **ES:** Ethanol sub-extract, **EE:** Ethanol extract. Bars with asterisks (* for growth and ** for biofilm formation) showed significant differences with respect to each control ($p < 0.05$, n=6). The results are expressed as percentages (%). Growth (%) = (As/A0) × 100, where A0 is the absorbance of the control and As is the absorbance of each sample (measured at 600 nm). Biofilm formation (%) = (As/A0) × 100, where A0 is the absorbance of the control and As is the absorbance of each sample (measured at 560 nm).

Fig. 9. Efectos de *Oxalis tuberosa* var. *oca rosa* sobre *L. acidophilus* La-14 (SD5212). **EAS:** subextracto en acetato de etilo; **AE:** extracto acuoso; **ES:** subextracto etanólico; **EE:** extracto etanólico. Las barras con asteriscos (* para crecimiento y ** para formación de biofilm) indican diferencias significativas respecto a cada control ($p < 0,05$, n=6). Los resultados se expresan como porcentajes (%). Crecimiento (%) = (As/A0) x 100, donde A0 es la absorbancia del control y As es la absorbancia de cada muestra (medidas a 600 nm). Formación de biofilm (%) = (As/A0) x 100, donde A0 es la absorbancia del control y As es la absorbancia de cada muestra (medidas a 560 nm).

ART or their bioactive and antioxidant constituents, such as phenolic compounds (Campos *et al.*, 2012; Verediano, *et al.*, 2021) may act as prebiotics that is "a substrate that is selectively utilized by host microorganisms conferring a health benefit" (Gibson *et al.*, 2017). Thus, the lactic acid bacteria and ART mixtures or ART isolated compounds may also serve as "synbiotic" that is a mixture of probiotics and prebiotics (dietary supplements), which selectively stimulating the development and/or activating the metabolism of one or a limited number of health-promoting bacteria in the gastrointestinal tract, and thus improving host welfare (Palai *et al.*, 2020).

On the other hand, in many cases the biofilm's stimulating effects did not obey to a significant increase in growth, but rather to a probably adaptive behavior to chemical stress, mediated by a cell-cell communication mechanism (Quorum sensing), as it was published by Verni *et al.* (2022).

Capable of colonizing almost every environment, the microorganisms have evolved a wide range of biological responses to environmental stressors, and biofilms constitute a protective physical barrier that confers tolerance to antimicrobial agents (disinfectants and antibiotics) by reducing the diffusion of those toxic compounds. Moreover, they effectively reduce the grazing by protozoa. Biofilms are multicellular complex microbial communities held together by a self-produced extracellular matrix. They form highly diverse and complex structures that can attach to interfaces, grow and aggregate in layers. Particularly, biofilm-growing probiotic bacteria have the ability to improve thermo-tolerance and freeze-drying resistance, and one of their important features is their capacity to replace pathogenic biofilm by annulling competitors (Berlanga & Guerrero, 2016).

The stimulating effects exerted by Andean tuber extracts on the *L. acidophilus* La-14 biofilm would be keys to the development of probiotic formulations, given the antiproliferation, anti-angiogenesis and antitumor properties of this bacterium, as previously published (Nada *et al.*, 2020).

Surface activity

The probiotic strain *L. acidophilus* La-14 (SD5212) showed a higher surfactant activity than tween 80, increased in the presence of phenol and phenolic compounds present in the EAS, as shown in Table 3.

It is important to note that PhOH and its solvent system, previously added to the culture media, did not exert any surface activity by themselves. Therefore, the substantial increase in the supernatant surface activity, which has valuable adaptive and biological implications, would be due to an increase in surface-active substances by an induction to its biosynthesis, which is in line with our previous studies (Cartagena *et al.*, 2021; Mesurado *et al.*, 2021; Verni *et al.*, 2020, 2022).

Table 3. *Lactobacillus* surfactant activity.

Tabla 3. Actividad tensioactiva/surfactante de *Lactobacillus*.

Samples	Oil spreading halos (mm)	Surfactant activity*
L. acidophilus La-14 (SD5212)	110 ± 1 a	100%
L. acidophilus La-14 (SD5212) + PhOH	159 ± 3 b	145%
L. acidophilus La-14 (SD5212) + EAS	161 ± 1 b	146%
Tween 80	50 ± 3 c	62.5%

References: * Calculated by oil spreading halos. **PhOH:** Phenol solution at 100 μg/mL as a stressor agent. **EAS:** Ethyl acetate subextract of oca rosa (50 μg/mL). Different letters indicate statistically significant differences ($p < 0.05$, n=5).

Referencias: * Calculado mediante halos de dispersión de aceite. **PhOH:** Solución de fenol a 100 μg/mL como un agente estresor. **EAS:** Subextracto de acetato de etilo de oca rosa (50 μg/mL). Diferentes letras indican diferencias estadísticamente significativas ($p < 0,05$, n=5).

The 46% increase in the bacterial surfactant formation mediated by SEC would improve the solubility processes of fat-soluble ingredients and the bioavailability of nutraceutical formulations, which will be addressed in future studies *in vitro* and *in vivo*. Further researches incorporating structurally relevant Andean tuber-derived phenolics or phenolic mixtures would be necessary to better support the suggested interpretations.

CONCLUSIONS

The study offers thorough and significant insights on the Andean tubers` peels, the histochemical localization of bioactive natural products, mainly phenolic compounds, and their modulation on the biofilm formation and surface-associated phenotypes of the probiotic bacterium *L. acidophilus* La-14 (SD-521214) under the tested conditions.

Recovering tuber peels aligns with the principles of the circular economy, a paradigm that promotes the reuse of products and natural resources, minimizing waste and byproducts.

Given the known and novel Andean tuber properties, is essential to promote global strategies for their conservation, cultivation, and sustainable exploitation.

SUPPLEMENTARY MATERIAL

Fig. SA-F) Effects of Andean tuber peel extracts on *Lactobacillus acidophilus* La-14 (SD5212) growth and biofilm. A-F: EAS: Ethyl acetate sub-extract, AE: Aqueous extract, ES: Ethanol sub-extract, EE: Ethanol extract. Bars with asterisks (* for dot bars and ** for full bars) showed significant differences with respect to each control ($p < 0.05$, n=6). The results are expressed as percentages (%). Growth (%) = (As/A0) × 100, where A0 is the absorbance of the control wells and As is the absorbance of each sample (measured at 600 nm). Biofilm formation (%) = (As/A0) × 100, where A0 is the absorbance of the control wells and As is the absorbance of each sample (measured at 560 nm).

Fig. SA-F) Efectos de los extractos de cáscaras de tubérculos andinos en el crecimiento y la formación de biofilm de *Lactobacillus acidophilus* La-14 (SD5212). A-F: EAS: subextracto de acetato de etilo, AE: extracto acuoso, ES: subextracto etanólico, EE: extracto etanólico. Las barras con asteriscos (* para barras puntadas y ** para barras llenas) muestran diferencias significativas respecto a cada control ($p < 0,05$, n=6). Los resultados se expresan como porcentajes (%). Crecimiento (%) = (As/A0) x 100, donde A0 es la absorbancia del control y As es la absorbancia de cada muestra (medidas a 600 nm). Formación de biofilm (%) = (As/A0) x 100, donde A0 es la absorbancia del control y As es la absorbancia de cada muestra (medidas a 560 nm).

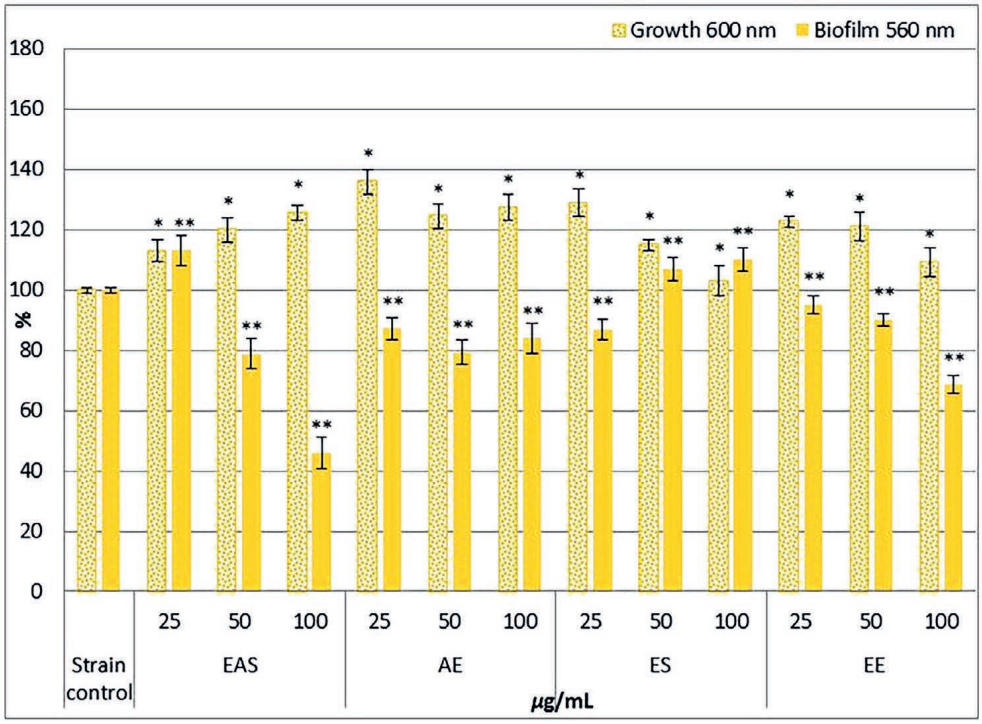


Fig. SA. *Oxalis tuberosa* var. *oca blanca.*

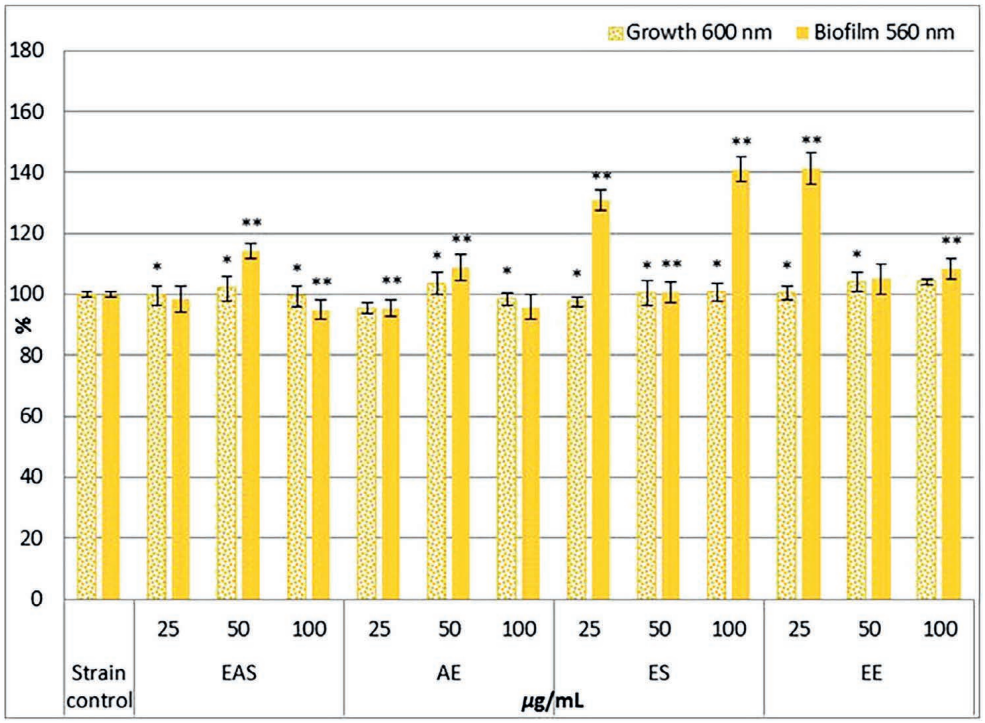


Fig. SB. *Ullucus tuberosus.*

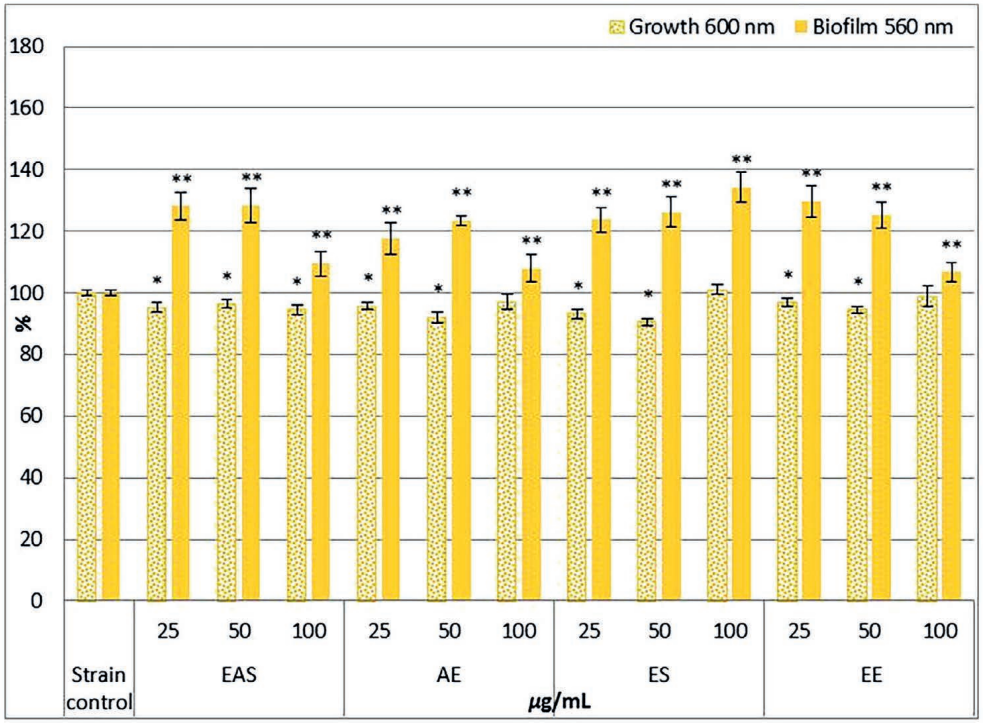


Fig. SC. *Solanum tuberosum* subsp. *andigena* var. *miskila negra.*

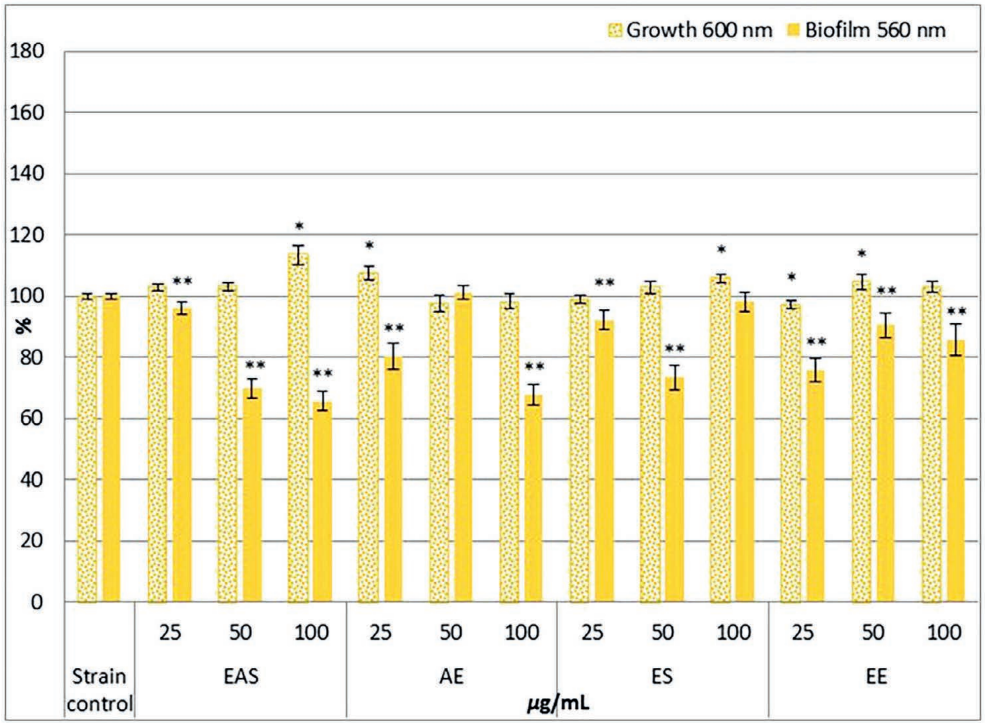


Fig. SD. *Solanum tuberosum* subsp. *andigena* var. *miskila colorada.*

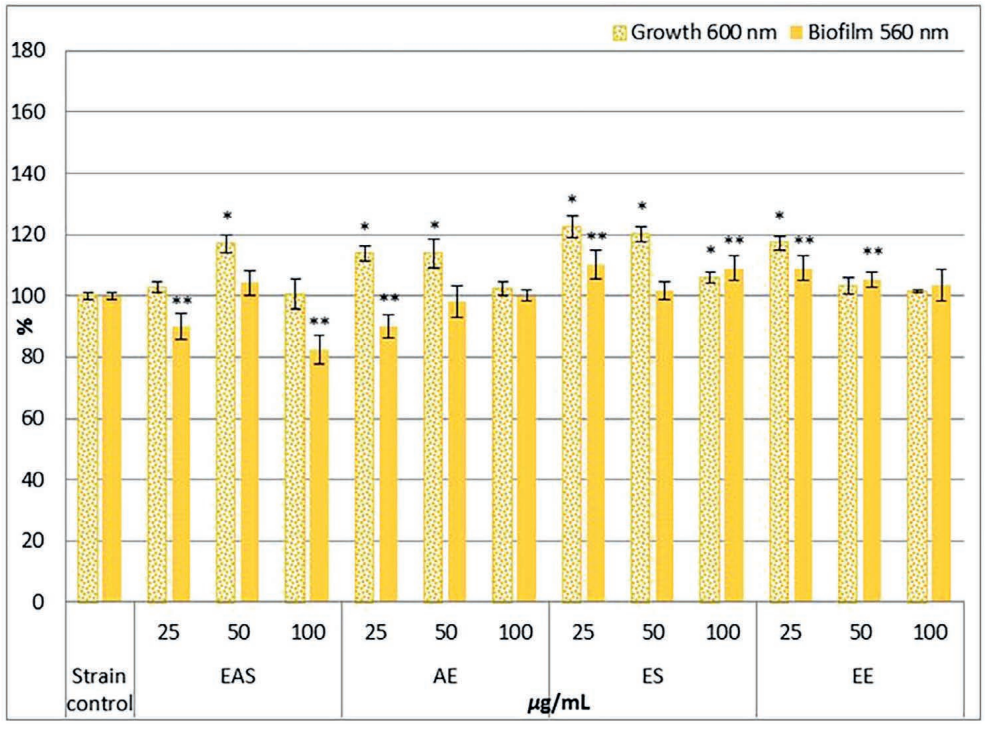


Fig. SE. *Solanum tuberosum* subsp. *andigena* var. *cuarentona.*

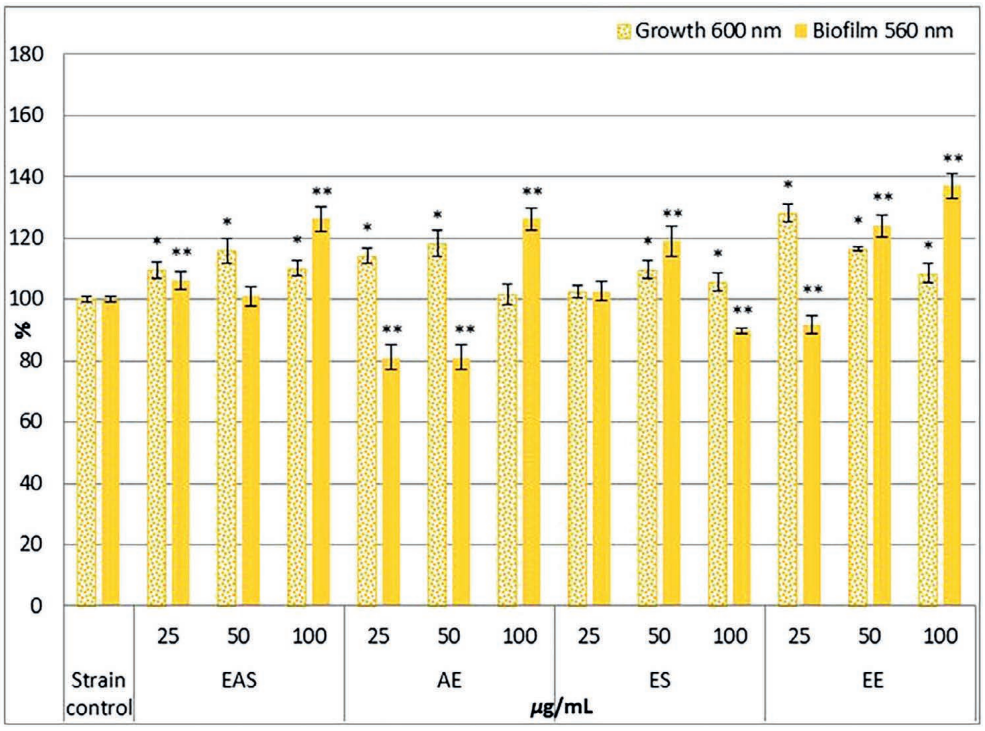


Fig. SF. *Solanum tuberosum* subsp. *andigena* var. *blanca*.

AUTHOR CONTRIBUTIONS

CHO: Investigation, Data analysis, Writing-Original draft preparation. MIM: Conceptualization; Methods, Data analysis, Resources, Writing-Reviewing and Editing. EC: Conceptualization, Methods, Data analysis, Resources, Supervision, Writing-Reviewing and Editing.

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CONFLICT OF INTEREST

The authors declare no conflicts of interest.

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