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Polysaccharides and rhamnolipids of *Pseudomonas aeruginosa* ONU-301 as antiphytoviral agents and promising biotechnological materials

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ABSTRACT

Rhamnolipids (RhLs) and polysaccharides (PS) produced by *Pseudomonas aeruginosa* are promising biosurfactants and bioactive compounds for agricultural and biotechnological applications. This study aimed to optimize RhL production by *P. aeruginosa* strain ONU 301 and evaluate the antiviral activity of its extracellular metabolites for plant protection. To improve the purification of RhLs from fermentation broth, a heterophase extraction method was developed, and its efficacy was confirmed by thin-layer chromatography. Among the tested metabolites, the PS fraction exhibited the highest antiviral activity, reducing tobacco mosaic virus (TMV) infectivity on *Datrura stramonium* leaves by 24–92% at concentrations ranging from 1 to 1000 µg/mL. The RhL fraction showed moderate activity, decreasing TMV infectivity by 19–59% at higher concentrations (10–1000 µg/mL). No antiviral activity was observed in the low-molecular-weight fraction after removal of RhL and PS biopolymers. Both fractions were non-phytotoxic, supporting their agricultural potential. Based on the obtained results, PS and RhLs from *P. aeruginosa* ONU-301 are proposed as promising bio-based nanomaterials for the development of complex liposomal formulations with broad-spectrum applications, particularly in eco-friendly plant protection strategies. Future research will focus on the biological and physicochemical characterization of these metabolites and the investigation of their potential applications in biotechnology, supramolecular chemistry, and agriculture.

Keywords: biosurfactant, liposomes, polysaccharides, rhamnolipids, plant viruses.

INTRODUCTION

Bacteria of the genus *Pseudomonas*, particularly *Pseudomonas aeruginosa*, produce a wide range of secondary metabolites with diverse biotechnological applications (Anayo et al., 2019; Franklin et al., 2011). Among them, rhamnolipids (RhLs) have been extensively studied due to their surface-active properties and broad applications in various fields, including cosmetology, pharmaceuticals, medicine and agriculture (Soberón-Chávez et al., 2020; Santos et al., 2024). Currently microbial RhLs are increasingly regarded as promising alternatives to synthetic surfactants (Randhawa and Rahman, 2014). Rhamnolipids produced by *Pseudomonas* spp. are promising components for the development of liposomal formulations, applied for drug delivery, due to their amphiphilic nature and biological activity (Sanches et al., 2021; Sharmila et al., 2025). RhLs have also been employed in the creation of complex structures – liposomal bionanocomposites (BNCs) that can promote the penetration of active biopolymers into plant cells, interfere with plant viral infections, and act as inducers of natural plant resistance (Kovalenko et al., 2019; 2023; Kyrychenko and Kovalenko 2025).

In addition to RhLs, *Pseudomonas* spp. produce at least three polysaccharides (alginate, Psl and Pel) (Franklin et al., 2011), which exhibit a broad spectrum of biological activity and have attracted commercial interest for different applications, including novel biological uses, particularly in plant protection (Kovalenko et

al., 1987; Podgorsky et al., 2010; Dedhia et al., 2022). Despite the great practical potential, the economic feasibility of liposomal BNCs is a critical concern, especially given the high cost of initial bionanomaterials such as rhamnolipids. Therefore, in addition to selecting effective producer strains, another key issue is the increasing RhLs yields. Achieving this requires improving culture productivity, optimizing purification processes, enrichment of target product fractions, and ensuring the safety of the resulting compounds. These factors are significantly influenced by the cultivation conditions of the producer strain and the methods used for metabolite extraction and purification.

In this context, the aim of this study was to optimize the production and purification of RhLs from *Pseudomonas aeruginosa* ONU 301, previously described as an active rhamnolipid producer (Abedalabas et al., 2014). The study also sought to develop an effective heterophase extraction method for isolation of high-purity RhLs and PS, assess the antiviral activity of the polysaccharide and rhamnolipid fractions obtained through this method, and evaluate their phytotoxic and/or phytostimulatory effects.

MATERIAL AND METHODS

Bacterial strain and cultivation conditions

The *Pseudomonas aeruginosa* ONU 301 strain was obtained from the culture collection of the Department of Microbiology, Virology and Biotechnology of I.I. Mechnikov Odessa National University.

To prepare the initial bacterial culture, *P. aeuroginosa* ONU 301 was grown for 7 days on a standard meat peptone agar (MPA) (Lab Time Ua, Ukraine). For large-scale metabolite production, a seed culture was prepared by transferring agar plugs from the initial culture into 50 ml of Hiss mineral medium supplemented with 2% glucose (Lyaskun et al., 2016). The cultures were incubated at 37 °C on a rotary shaker at 180 rpm for 3 days. Subsequently, the seed culture was aseptically transferred into 250–300 mL of the same medium in Erlenmeyer flasks and incubated under identical conditions for an additional 2 days. The resulting cultures were either maintained at room temperature for immediate use or stored at +4 °C for long-term preservation.

Isolation of metabolites

To separate biomass, the culture fluid (CF) of *P. aeruginosa* ONU 301 was centrifuged at 8000 ppm for 20 minutes. The resulting supernatant was concentrated fivefold using a rotary evaporator under vacuum at a temperature not exceeding 50 °C. Crude RhL precipitate (PP) was obtained from the concentrated CF by acidification with 2N HCl to pH 3.0 (Lyaskun et al., 2016). The PP was treated with hexane (1:10, v/v) to remove free fatty acids.

Purification of rhamnolipids from the HCl precipitate was carried out in two stages. In the first stage, the precipitate was prepared for heterophase extraction. For this purpose, the preparation was dried in a vacuum desiccator over KOH to constant weight. To remove impurities, particularly free fatty acids, the dried material was treated with hexane (1:10, v/v), and the extract was discarded. The remaining defatted residue was then extracted twice with a methanol:methylene chloride mixture (2:1, v/v). The extracts obtained in this way were combined and evaporated to dryness to yield a crude RhL concentrate.

To further separate the amphiphilic and hydrophilic substances present in the residue, a heterophase extraction method was applied (Zapolsky and Baran, 1987). For this purpose, the dried RhL concentrate obtained in the previous stage was dissolved in the same methanol:methylene chloride mixture (2:1, v/v), divided into two equal portions and evaporated to dryness. One portion was dissolved in methylene chloride, and the other in ethyl acetate. Then, aliquots of water acidified with HCl (pH 2) were added to both extracts. The mixtures were vigorously shaken for 20 min. The organic and aqueous phases were separated by centrifugation (3000 rpm). The aqueous phases were re-extracted with the respective organic solvents – methylene chloride or ethyl acetate, respectively. All organic extracts were combined and evaporated to dryness. The aqueous fractions were analyzed spectrophotometrically in the UV range (220–310 nm).

Polysaccharides were recovered from the RhL-extraction residue by adding two volumes of ethanol and the subsequent protein removal with isopropanol : chloroform (1:5) mixture. The precipitates were collected by centrifugation (3000 rpm, 10 min.). The obtained RhL and PS fractions, along with the residual fraction after their extraction, were freeze-dried at –50 °C for

24 hours, weighed, and subjected to biological (antiviral and growth-stimulating) activity assays.

Qualitative analysis of RhLs was performed using thin-layer chromatography (TLC) on Silufol plates (Kavalier, Czech Republic) with a mobile phase of chloroform:acetic acid:water (85:15:1, v/v/v). Chromatograms were visualized with a 5% ethanolic solution of phosphomolybdic acid (Ando and Saito, 1987).

Antiviral activity assay

The antiviral activity of the isolated metabolites was evaluated against tobacco mosaic virus (TMV) using the half-leaf method on *Datura stramonium* L. plants. For this purpose, metabolite solutions at final concentrations of 1, 10, 10 and 1000 μg/ml were mixed (1:1, v/v.) with TMV suspension (10 μg/ml), purified according to Gooding and Hebert (1967), and incubated for 30 minutes at room temperature. Control samples were prepared by mixing the TMV suspension with 0.01 M potassium-sodium phosphate buffer (PPB, pH 7.0) solution in equal volumes. Experimental and control samples were applied to opposite halves of the same *D. stramonium* leaf using a glass spatula. Leaves were previously powdered with fine carborundum to enhance inoculation efficiency. Inoculated plants were maintained under controlled greenhouse conditions for 7 days, after which the number of local lesions was recorded

The degree of inhibition (I, %) was calculated using the formula:

$$I = [(C–E)/C] \times 100\ (\%) \qquad (1)$$

where: E is the average number of lesions on treated leaves, and C is the average number of lesions in the control.

Each treatment involved at least 10 leaves and was performed in triplicate.

Phytostimulatory activity assay

Aqueous solutions of the isolated RhL and PS fractions (10–100 μg/ml) were tested for phytostimulatory or phytotoxic effects on watercress plants (*Lepidium sdativum* L.). Seeds were placed in Petri dishes on filter paper moistened with test solutions and incubated in a climate-controlled chamber (25 °C, 10.000 lux, 16 h photoperiod). Seed germination and biomass accumulation were assessed on day 5 of a 7-day period. Control seeds were treated with distilled water under the same conditions. The experiment was repeated 3 times.

Statistical analysis

Statistical analysis of the experimental data was conducted using parametric tests for pairwise comparisons (Dospekhov, 1985) in Microsoft Excel 2010. Results were expressed as mean ± standard deviation (SD) based on three independent experiments. The unpaired, two-tailed Student's t-test was applied to assess differences between test samples and controls. Statistical significance was determined at a 95% confidence level ($p < 0.05$).

RESULTS AND DISCUSSION

Culturing of P. aurugenosa 301 ONU

The *P. aeruginosa* ONU 301 demonstrated robust growth on nutrient medium, forming a dense, whitish surface film typical of this species (Figure 1a). As observed for other *Pseudomonas* strains, the optimal growth temperature on solid media was 27–28 °C. In liquid culture, bacteria were grown in Hiss mineral medium supplemented with glucose, under aerobic conditions at 37 °C. After 24 h of incubation, intensive foaming was observed in the inoculated flask (Figure 1b, right), unlike the uninoculated control (Figure 1b, left). This foaming indicates the active production of surface-active compounds, including biosurfactants.

Metabolite isolation and characterization

As previously noted, this study, in addition to the widely investigated glycolipids (biosurfactants) produced by *P. aeruginosa*, also focused on extracellular polysaccharides, which have demonstrated potential antiviral properties. This broader approach enabled the assessment of a more diverse metabolite profile of *P. aeruginosa* ONU 301. The experimental workflow for metabolite extraction and analytical characterization is summarized in Figure 2.

Fractionation of the culture fluid revealed that free fatty acids extracted with hexane represented a minor component, approximately 0.6% of the dry weight of CF (Figure 3). In contrast, the yields of RhL and PS fractions were significantly higher, reaching 3.56% and 4.47%, respectively.

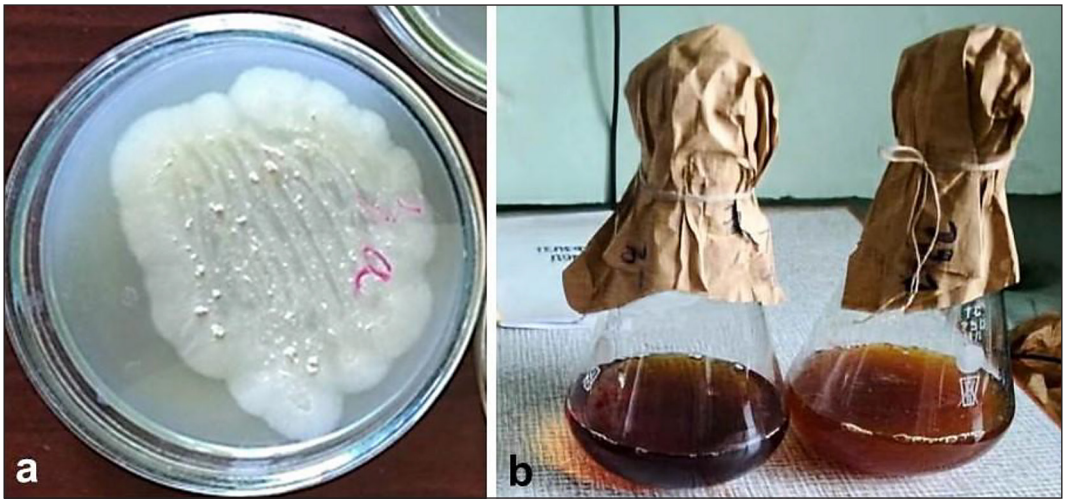

Figure 1. Growth of *P. aeruginosa* ONU 301 on agar plate (a) and in liquid culture (b): inoculated culture medium (right); uninoculated control (left); incubation time – 72 h

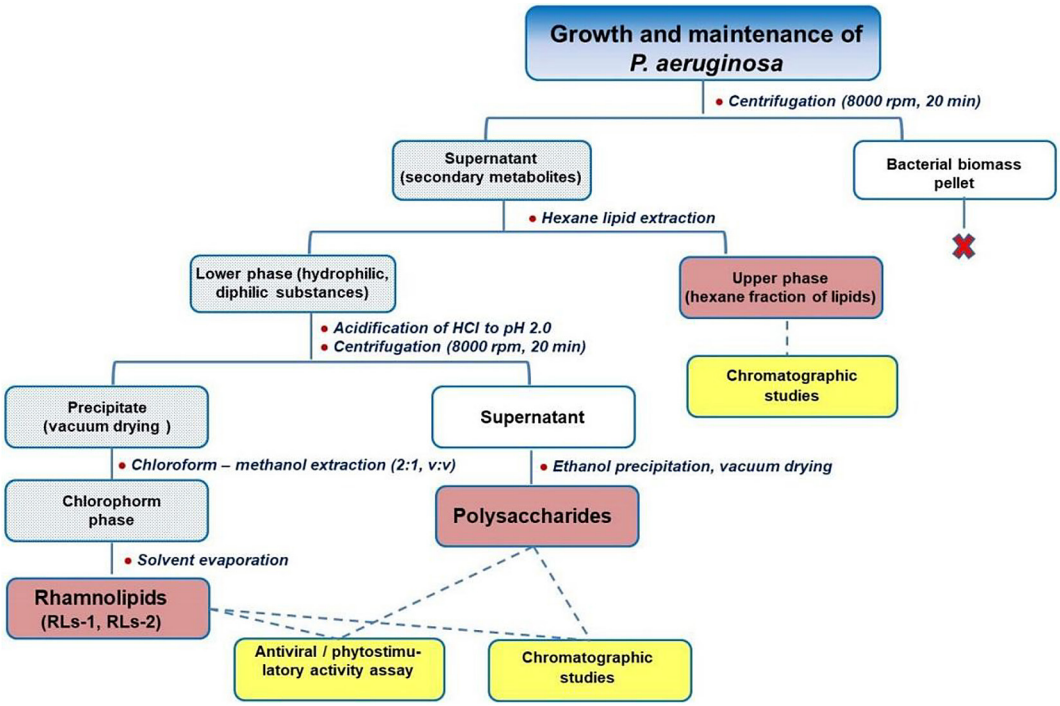

Figure 2. Schematic representation of the workflow for the isolation and analysis of secondary metabolites produced by *P. aeruginosa* ONU 301

The total dry residue in the culture fluid of *P. aeruginosa* ONU 301 reached approximately 60 g/L. This value reflects the biosynthetic capacity of the strain, which may be further improved through optimization of culture conditions. Notably, non-target (concomitant) lipids were present in trace amounts and accounted for 0.32% of the total lipid content. This suggests that the strain efficiently directs metabolic flux toward rhamnolipid production, with minimal synthesis of non-specific lipid byproducts. These characteristics make *P. aeruginosa* ONU 301 a potentially valuable microbial producer of biosurfactants and other biologically active compounds for biotechnological applications.

RhLs isolated from the CF of *P. aeruginosa* ONU 301 by acid precipitation at pH 3.0 may contain the co-precipitated impurities (Invally et al., 2019). During acidification, organic and amphiphilic substances can precipitate along with the target lipids or surface-active compounds. The presence of such contaminants is undesirable for further applications, particularly in the development of

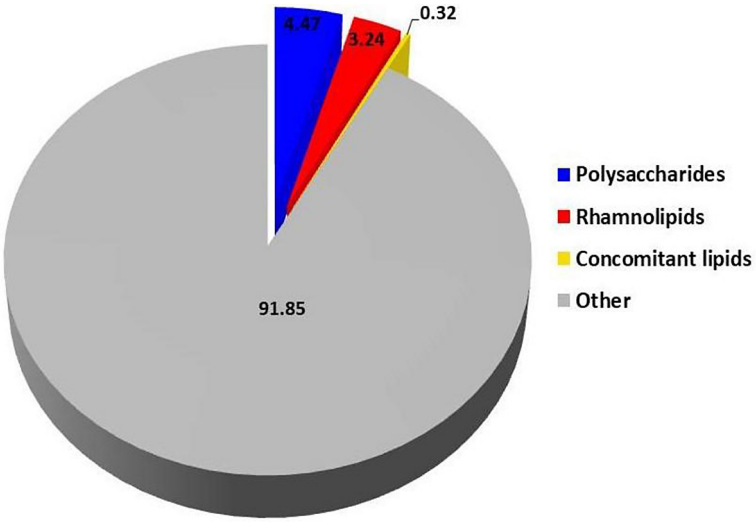

Figure 3. Relative content (%) of the main metabolite fractions in the CF of *P. aeruginosa* ONU 301

bio-based nanocarriers – an area actively explored in this study to obtain functionally effective and compositionally pure formulations. Therefore the study prioritized further purification of the acid precipitates to obtain fractions enriched in target metabolites. For this purpose, a heterophase extraction method was employed as described by Zapolsky and Baran (1987), with purification conducted in two stages. The workflow for RhLs purification is detailed in Figures 4 and 5. Stage

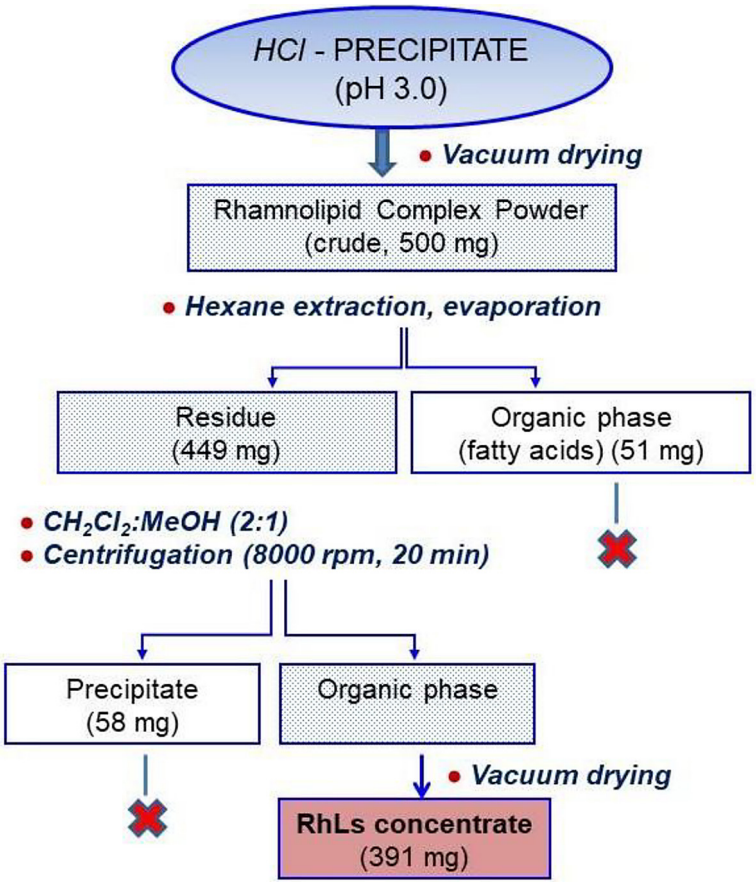

Figure 4. Obtaining the *P. aeruginosa* ONU 301 rhamnolipid concentrate for heterophase extraction.
× – discarded fractions

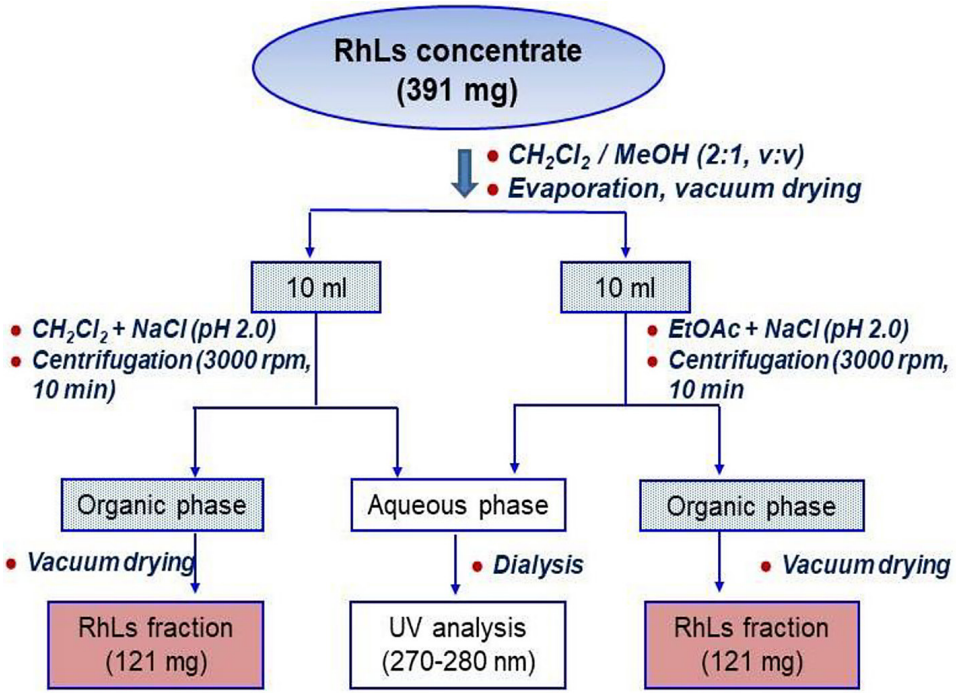

Figure 5. Heterophase extraction of RhLs from the partially purified concentrate o*f P. aeruginosa* ONU 301

1 focused on initial purification and preparation of the rhamnolipid concentrate (Figure 4). In this stage, the acid (HCl) precipitate was vacuum-dried over crystalline KOH to a constant weight. The dried material was then treated with hexane to remove free fatty acids. Subsequently, RhLs were extracted from the defatted residue using a methylene chloride:methanol mixture (2:1, v/v). The resulting precipitate was removed by centrifugation (8000 rpm, 20 min) and the organic phase was evaporated. The yield of obtained RhLs concentrate was 391 mg, representing 78.2% of the initial precipitate.

In stage 2, the partially purified RhLs were subjected to heterophase extraction to separate and remove hydrophilic impurities (Figure 5). The RhLs concentrate was re-extracted twice with methylene chloride:methanol (2:1, v/v), and the combined extracts were aliquoted (10 mL each), evaporated, and vacuum-dried.

Subsequent extraction was performed using two solvent systems: (1) methylene chloride with 1 M NaCl (pH 2.0, adjusted with 2 N HCl), and (2) ethyl acetate with the same acidified NaCl solution. Phase separation was achieved by centrifugation (3000 rpm, 10 min). Organic phases were collected and air-dried under a fume hood, while aqueous phases were desalted by dialysis against distilled water and analyzed by UV spectroscopy. Absorbance in the 270–280 nm range with a maximum at 270–280 nm suggested the presence of aromatic amino acid-containing (peptides and/or proteins) impurities (data not shown).

The total RhL yield from methylene chloride and ethyl acetate extractions was 242 mg (121 mg per extract), corresponding to 61.9% of the partially purified concentrate and 48.4% of the original crude precipitate. Purification efficiency was confirmed by TLC, comparing the RhLs fractions from all extraction stages (Figure 6).

In the chromatogram, RhL-1 and RhL-2 were present in the crude precipitate (track 4). After treatment with hexane (track 1), the RhL-1 band became more prominent. The intensity and resolution of the RhL-1 and RhL-2 bands increased markedly after heterogeneous extraction with CH_2Cl_2 (track 2) and EtOAc (track 3). A similar, though less pronounced, pattern was observed for RhL-2. These results demonstrate the high efficiency of heterophase extraction method applied for enrichment of RhL fractions and removal of interfering substances. The purified RhLs obtained in this study hold practical interest for the development of supramolecular liposomal structures. The method described here offers a promising approach for the application-level production of rhamnolipid components suitable for use in the formulation of liposomal-based BNCs and other nanotechnology-based applications.

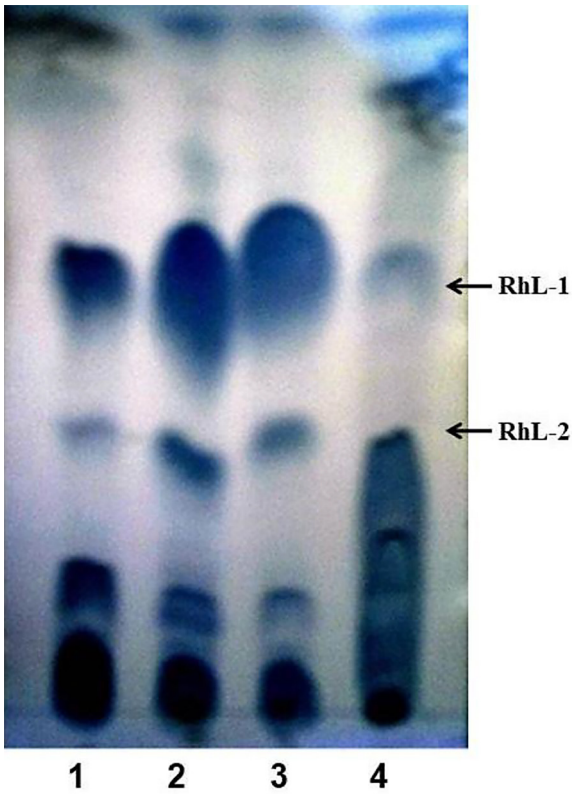

Figure 6. TLC analysis of rhamnolipids of *P. aeruginosa* ONU 301. 1 – partially purified sample after hexane treatment; 2 – extract with dichloromethane; 3 – extract with EtOAc; 4 – crude precipitate obtained from CF after acidification (pH 3.0)

Antiviral activity of metabolites

Among the tested metabolites, PS and RhL fractions demonstrated antiviral activity against TMV on *D. stramonium* leaves (Table 1). Notably, the PS fraction exhibited stronger antiviral effects than RhLs. At concentrations ranging from 1 to 1000 µg/ml, PS inhibited the formation of virus-induced local lesions by 24–92%, showing a clear dose-dependent response. In contrast, RhLs showed moderate antiviral activity, with statistically significant suppression of TMV infectivity observed only at concentrations of 100–1000 µg/ml, where inhibition reached 36–59%. Fractions containing low-molecular-weight compounds remaining in the CF after removal of RhLs and PS showed no significant antiviral effects (data not shown).

Phytotoxicity testing of metabolite fractions

The PS and RhL fractions isolated from *P. aeruginosa* ONU-301 culture fluid were tested for phytotoxicity using watercress (*Lepidium sativum* L.) as a model plant. The effects on seed germination and biomass accumulation were assessed over a 7-day period. The results showed that both metabolite fractions, at concentrations of 10 and 100 µg/ml (effective against TMV, see Table 1), did not inhibit seed germination or seedling growth (Figure 7). On the contrary, both PS and RhL stimulated biomass accumulation in seedlings at these concentrations. Combined with their antiviral activity, these results suggest that the studied biopolymers are promising components for the development of bioactive nanocomposites with a broad spectrum of action.

The results of this study demonstrate that *P. aeruginosa* ONU 301 produces and secretes into the CF a range of metabolites with significant biotechnological potential, particularly as components of liposome-based antiviral formulations. Although high-molecular-weight fractions containing neutral polysaccharides and alginates have demonstrated stronger antiviral activity than rhamnolipids (Franklin et al., 2011), the latter

Table 1. Inhibition of TMV infectivity by PS and RhL fractions

*Fractions	Concentration (mg/ml)	Lesions per leaf		**Inhibition (%)
		Left (treated)	Right (control)	
PS	1	32	42	24 ± 8
	10	10	30	67 ± 9
	100	4	21	81 ± 10
	1000	3	38	92 ± 13
RhLs	1	22	23	4 ± 11
	10	13	16	19 ± 12
	100	18	28	36 ± 9
	1000	10	24	59 ± 7

Note: *fractions were isolated from 24-hour culture of *P. aeruginosa* ONU-301, **values are presented as means ± standard deviations (SD) from triplicate experiments. Differences were considered statistically significant at $P < 0.05$.

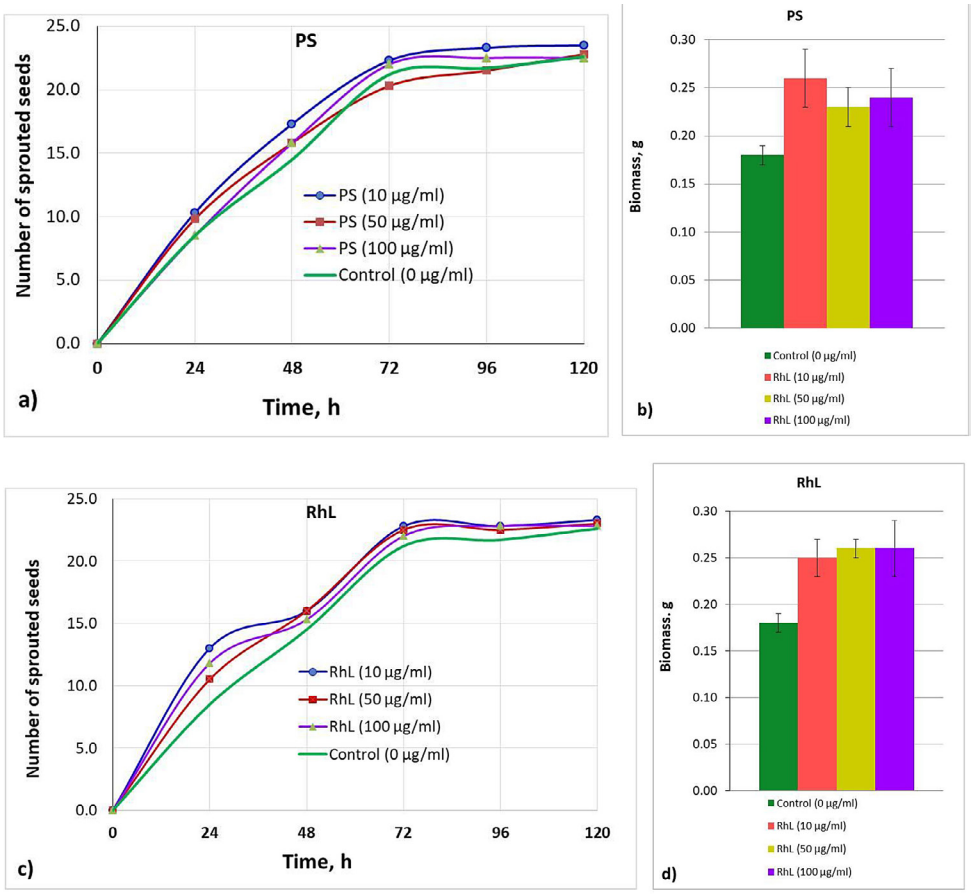

Figure 7. Effect of PS (a, b) and RhL (c, d) fractions from *P. aeruginosa* ONU-301 on seed germination and biomass accumulation in watercress seedlings

remain valuable for their self-assembling properties, which enable the formation of supramolecular structures with antiviral activity.

In agriculture, RhLs have been investigated as eco-friendly bioremediation agents capable to remove of petroleum hydrocarbons, heavy metals, and extracellular polymeric substances from soils and wastewater without harming autochthonous microbial communities (Lavanya, 2024; Kabeil et al., 2025). In addition to bioremediation, RhLs can also function as biocontrol agents, showing algicidal, mycoplasmicidal, and zoosporicidal activity (Kabeil et al., 2025). Moreover, rhamnolipid biosurfactants have been studied for their antimicrobial and antiphytoviral properties (Pierre et al., 2025). While there is no evidence that RhLs directly inhibit bacteria and viruses responsible for plant diseases, they have been reported to stimulate plant defense mechanism (Haferburg et al., 1987; Kovalenko et al., 2023; Pierre et al., 2025). RhLs can act as elicitors of systemic acquired resistance (SAR), activating defense gene and stimulating the production of MAP kinase, reactive oxygen species (ROS), defense-related compounds (salicylic acid, jasmonic acid and ethylene), and callose depositions – all of which help restrict viral spread (Vatsa et al. 2010; Pierre et al., 2023). Furthermore, RhLs have shown protective effects against soil-borne virus infection in plants (Fomitcheva et al., 2024). These properties illustrate the versatility of RhLs in sustainable agriculture, and, together with results obtained in this study, emphasize their importance in developing liposome-based formulations with antiviral and growth stimulation activity. Polysaccharides, in contrast to RhLs, have been more extensively studied as antiviral agents. It has been shown that PS from microbial, algal, and plant sources exhibit significant antiviral activity and, at the same time, low toxicity (Sano, 1995; Claus-Desbonnet et al., 2022). They act as inhibitors of infection through direct inactivation of virions, by aggregation of viral particles or by blocking virus-cell interactions, such as adsorption and internalization (Sano, 1995; Damonte et al., 2004).

Additionally, PS may act as biological signaling molecules in plants and induce plant defense mechanisms against pathogen infection, showing elicitor activity (Kovalenko et al., 2008; Abdul Malik et al., 2022; Aitouguinane et al., 2022). While sulfated polysaccharides from algae and plants are well characterized, the antiviral potential of microbial PS, including those from *P. aeruginosa*, remains underexplored. This study provides the first report on the antiviral activity of polysaccharides from *P. aeruginosa* ONU 301, which may contribute to the existing knowledge and represent a promising direction for future biotechnological applications. Taken together, the results of this study indicate the broad potential of *P. aeruginosa* ONU 301 metabolites for use in antiviral and plant-compatible biotechnology applications. The lack of toxicity, and combination of antiviral and growth-stimulating properties, make them ideal candidates for sustainable agriculture, offering environmentally friendly alternatives to synthetic agrochemicals.

CONCLUSION

This study demonstrates the significant potential of *P. aeruginosa* ONU-301 as a microbial producer of RhLs and PS, and presents the first report on the antiviral activity observed in the polysaccharide derived from its culture fluid. The potential application of both metabolites produced by the same strain appears particularly promising for the development of broad-spectrum BNCs. These metabolites demonstrated notable antiviral efficacy against TMV across a wide range of concentration and were found to be non-toxic to plants. The heterophase extraction method developed in the study provided an effective approach for RhLs purification and suggests its suitability for large-scale production. Future investigations will be aimed at the optimization of bacterial culture conditions to improve the upstream production of extracellular and structural metabolites, the biosynthetic potential of which remains largely underexploited. Continued efforts in this direction are essential to fully realize the biotechnological value of this strain.

Acknowledgments

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REFERENCES

1. Abdul Malik, N.A., Kumar, I.S., Nadarajah, K. (2020). Elicitor and receptor molecules: orchestrators of plant defense and immunity. *International Journal of Molecular Sciences, 21*(3), 963. https://doi.org/10.3390/ijms21030963
2. Abedalabas, M., Galkin, M.B, Filipova T.O. (2014). Rhamnolipids biosynthesis in *Pseudomonas aeruginosa* ONU 301 in the presence of exogenous signaling quinolone. *Microbiology & Biotechnology*, *1*, 15–23.
3. Aitouguinane, M., Alaoui-Talibi, Z.E., Rchid, H., Fendri, I., Abdelkafi, S., El-Hadj, M. D.O., Boual, Z., Dubessay, P., Michaud, P., Traïkia, M., Pierre, G., El Modafar, C., Delattre, C. (2022). Polysaccharides from Moroccan green and brown seaweed and their derivatives stimulate natural defenses in olive tree leaves. *Applied Sciences*, *12*(17), 8842. https://doi.org/10.3390/app12178842
4. Anayo, O.F., Scholastica, E.C., Peter, O.C., Nneji, U.G., Obinna, A., Mistura, L.O. (2019). The beneficial roles of Pseudomonasin medicine, industries, and environment: A review. *IntechOpen eBooks*. https://doi.org/10.5772/intechopen.85996
5. Ando, S., Saito, M. (1987). Chromatography lipid, biomedical research and chemical diagnostic. Elsevier.: Amsterdames.
6. Claus-Desbonnet, H., Nikly, E., Nalbantova, V., Karcheva-Bahchevanska, D., Ivanova, S., Pierre, G., Benbassat, N., Katsarov, P., Michaud, P., Lukova, P., Delattre, C. (2022). Polysaccharides and their derivatives as potential antiviral molecules. *Viruses, 14*(2), 426. https://doi.org/10.3390/v14020426
7. Damonte, E., Matulewicz, M., Cerezo, A. (2004). Sulfated seaweed polysaccharides as antiviral agents. *Current Medicinal Chemistry*, *11*(18), 2399–2419. https://doi.org/10.2174/0929867043364504
8. Dedhia, N., Marathe, S.J., Singhal, R.S. (2022). Singhal, food polysaccharides: A review on emerging microbial sources, bioactivities, nanoformulations and safety considerations, *Carbohydrate Polymers, 287*, 119355. https://doi.org/10.1016/j.carbpol.2022.119355.
9. Dospechov B.A. (1985). Field experience methodology (with the basics of statistical processing of

research results). M: Agropromizdat. (in Russian).

10. Fomitcheva, V., Strauch, C.J., Bonse, S., Bauer, P., Kühne T., Niehl A. (2024). Bio-control of soil-borne virus infection by seed application of *Glycyrrhiza glabra* extract and the rhamnolipid Rhapynal. *Planta 260*, 94. https://doi.org/10.1007/s00425-024-04529-5
11. Franklin, M.J., Nivens, D.E., Weadge, J.T., Howell, P.L. (2011). Biosynthesis of the Pseudomonas aeruginosa Extracellular Polysaccharides, Alginate, Pel, and Psl. *Frontiers in Microbiology*, *2*, 167. https://doi.org/10.3389/fmicb.2011.00167
12. Gooding, Jr.G.V. & Hebert, T.T. (1967). A simple technique for purification of tobacco mosaic virus in large quantities. – *Phytopathology*, *57*, 1285
13. Invally, K., Sancheti, A., Ju, L. (2019). A new approach for downstream purification of rhamnolipid biosurfactants. *Food and Bioproducts Processing, 114*, 122–131. https://doi.org/10.1016/j.fbp.2018.12.003
14. Kovalenko A.G. (1987). Antiviral properties of microbial polysaccharides – an overview. *Zbl. Mikrobiol. 143*(3), 301–310. (in German).
15. Kovalenko, O., Kyrychenko, A., Lubenets, V., Pokynbroda, T., Banya, A., Chervetsova, V., Karpenko, O. (2023). Thiosulphonate-rhamnolipid-glycanic complexes as inducers of virus resistance in hypersensitive plants. *Biologia Plantarum*, *67*, 159–165. https://doi.org/10.32615/bp.2023.014
16. Kovalenko, O.G., Kyrychenko, A., Kovalenko, O. (2019). Callus cultures of beans infected with virus as a model for testing antiviral compaunds. *Journal of Botanical Research*, *1*(2), 19–24. https://doi.org/10.30564/jrb.v1i2.1252
17. Kovalenko, O.G., Vasilev, V.M., Adamchuk-Chala N.I. Tytova, L.V., Karpenko, E.V. (2022). Antiviral agents and biological preparations for agriculture based on artificial glycan-glycolipid complexes. *Journal of Ethology & Animal Science*, *4*(1), 1–6. https://doi.org/10.23880/jeasc-16000123
18. Lyaskun, D.O., Shulga, O.M., Midyana, G.H., Zayarnuyk, N.L., Vildanova, R.I. (2016). Method of isolation of the surfactant complex of the Pseudomonas aeruginosa JRV-L strain. *Visnyk of the National University "Lviv Polytechnic", 841*, 181–186. (in Ukrainian)
19. Pierre, E., Marcelo, P., Croutte, A., Dauvé, M., Bouton, S., Rippa, S., Pageau, K. (2023). Impact of rhamnolipids (RLs), natural defense elicitors, on shoot and root proteomes of brassica napus by a tandem mass tags (TMTs) labeling approach. *International Journal of Molecular Sciences, 24, 2390*. https://doi.org/10.3390/ijms24032390
20. Pierre, E., Shaw, E., Corr, B., Pageau, K., Rippa, S. (2025). Applications of rhamnolipid surfactants in agriculture. *Plant Stress*, 100749. https://doi.org/10.1016/j.stress.2025.100749
21. Podgorsky, V.S., Kovalenko, O.G., Vasyliev, V.N., Isakova, V.O. (2010). Fodder and baker yeasts using for production of biologically active glycans. *Biotechnologia Acta, 3*(6), 49–58.
22. Randhawa, K.K.S., Rahman, P.K.S.M. (2014). Rhamnolipid biosurfactantsâ: past, present, and future scenario of global market. *Frontiers in Microbiology*, *5*, 454. https://doi.org/10.3389/fmicb.2014.00454
23. Sanches, B.C.P., Rocha, C.A., Bedoya, J.G. M., Da Silva, V.L., Da Silva Silva, P.B., Fusco-Almeida, A.M., Chorilli, M., Contiero, J., Crusca, E., Marchetto, R. (2021). Rhamnolipid-based liposomes as promising nano-carriers for enhancing the antibacterial activity of peptides derived from bacterial toxin-antitoxin systems. *International Journal of Nanomedicine*, *16*, 925–939. https://doi.org/10.2147/ijn.s283400
24. Sano, Y. (1995). Antiviral activity of polysaccharides against infection of tobacco mosaic virus. Macromolecular *Symposia, 99*(1), 239–242. https://doi.org/10.1002/masy.19950990125
25. Santos, S.C.D., Torquato, C.A., De Alexandria Santos, D., Orsato, A., Leite, K., Serpeloni, J.M., Losi-Guembarovski, R., Pereira, E.R., Dyna, A.L., Barboza, M.G.L., Arakawa, M.H.F., Bitencourt, J.a.P., Da Cruz Silva, S., Da Silva Sá, G.C., Rodrigues, P. D., Quintella, C.M., Faccin-Galhardi, L.C. (2024). Production and characterization of rhamnolipids by Pseudomonas aeruginosa isolated in the Amazon region, and potential antiviral, antitumor, and antimicrobial activity. *Scientific Reports*, *14*(1). https://doi.org/10.1038/s41598-024-54828-w
26. Sharmila, R., Sathya Priya, R., Yuvaraj, M., Palanisamy Murali Arthanari, Suganthi, N., Saranya, M., Jaiby Cyriac. (2025). *Liposomes in Plant Nanotechnology*. In: Al-Khayri, J.M., Anju, T.R., Jain, S.M. (eds) Plant Nanotechnology Fundamentals and Methodologies. Nanotechnology in Plant Sciences, 1. Springer, Cham. https://doi.org/10.1007/978-3-031-81896-7_12
27. Soberón-Chávez, G., González-Valdez, A., Soto-Aceves, M.P., Cocotl-Yañez, M. (2020). Rhamnolipids produced by Pseudomonas: from molecular genetics to the market. *Microbial Biotechnology*, *14*(1), 136–146. https://doi.org/10.1111/1751-7915.13700
28. Vatsa, P., Sanchez, L., Clement, C., Baillieul, F., Dorey, S. (2010). Rhamnolipid biosurfactants as new players in animal and plant defense against microbes. *International Journal of Molecular Sciences, 11*(12), 5095–5108. https://doi.org/10.3390/ijms11125095
29. Zapolsky, A.K. Baran A.A. (1987). *Coagulants and flocculants in processes water purification.* Properties. Receiving Application. Leningrad: Chemistry.