UNIVERSITÀ DEGLI STUDI
DELL'INSUBRIA

"Production and Chemical/Biological Characterization of Active Compounds of Microbial Origin"

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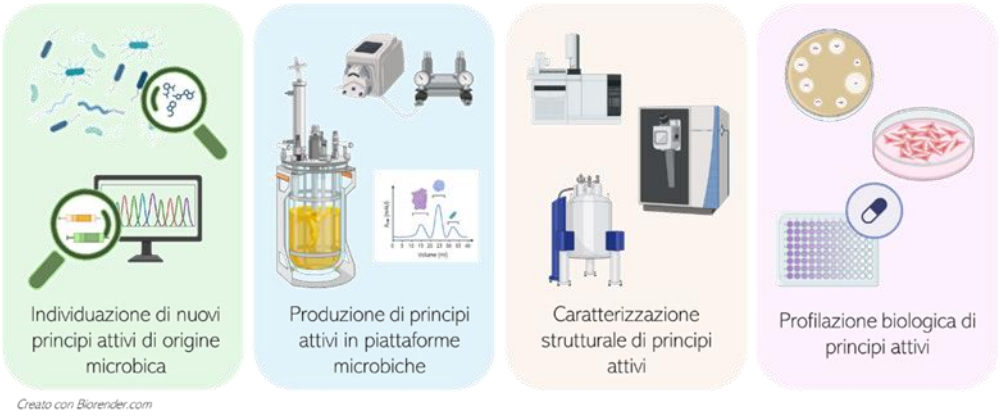
Keywords: natural products, fermentation, genomics, structural characterization, antimicrobial activity, antineoplastic activity.



Objectives: identification and selection of natural active compounds of microbial origin with activities and properties of interest for biotechnological applications, such as nutraceuticals and pharmaceuticals, using various discovery approaches ranging from phenotypic screening assays to genomic analyses; development of production processes (upstream and downstream) for active compounds in microbial expression platforms; structural and functional characterization of molecules (e.g., determination of antimicrobial or antioxidant activity profiles, assessment of toxicity profiles, evaluation of antineoplastic activity).

Location: c/o Department of Biotechnology and Life Sciences, Via Dunant 3, Varese

Organization: The facility is part of a network that brings together research groups with multidisciplinary expertise (fermentation chemistry, molecular biology, analytical chemistry, pharmacology/applied biology) to provide solutions related to the development of active ingredients of microbial origin for various biotechnological applications.

The facility is organized into four substructures:

a. Identification of new active ingredients of microbial origin;

b. Production of active ingredients in microbial expression platforms;

c. Structural characterization of active ingredients of microbial origin;

d. Biological profiling of active ingredients of microbial origin.

Linkage with CRIETT's Technology Platforms and the University's Scientific Platforms:

This facility operates in collaboration with:

- Sustainability Platform: Valorization of biomass and production of natural substances; Sustainability and protection of natural resources.
- Energy, Health, and Environment Technologies Platform: Materials and nanomaterials; Technologies, methods, and approaches for biomedical research.
- Frailty Platform: Frailty in the elderly: new therapies.
- Materials Analysis and Characterization Platform.
- Microscopy and Imaging Platform.

Subunit "Identification of New Microbial Bioactive Compounds"

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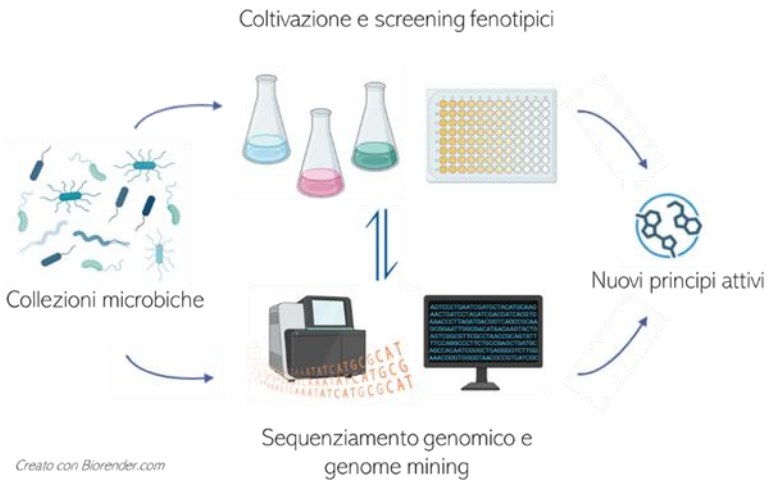
Keywords: microbial collections, functional screening, genomic sequencing, genome mining



This sub-unit is dedicated to the identification of new natural active compounds of microbial origin through the integration of advanced experimental and computational approaches, covering the entire process of molecule identification, from the selection of biological sources to the prediction of biosynthetic potential. Specifically, it specializes in the phenotypic screening of microorganisms to identify metabolites with biological activities of biotechnological and pharmaceutical interest, such as antibiotic and antioxidant activities. Experimental activities are based on a strain library comprising hundreds of microbial strains, including isolates from unique environmental matrices, with a particular abundance of actinomycetes and filamentous fungi known for the production of specialized metabolites. Experimental activities can be supplemented by next-generation genomic sequencing technologies and in silico analyses for the identification and characterization of biosynthetic clusters, facilitating the discovery of new active compounds and the exploitation of microbial diversity. Thanks to this integrated approach, the substructure serves as a reference infrastructure for basic research, preclinical studies, and applications in the biotechnology and pharmaceutical sectors.

Sub-unit "Production of Active Ingredients in Microbial Expression Platforms"

Coordinators: Prof.ssa Flavia Marinelli, Dott.ssa Francesca Berini, Prof. Enrico Caruso

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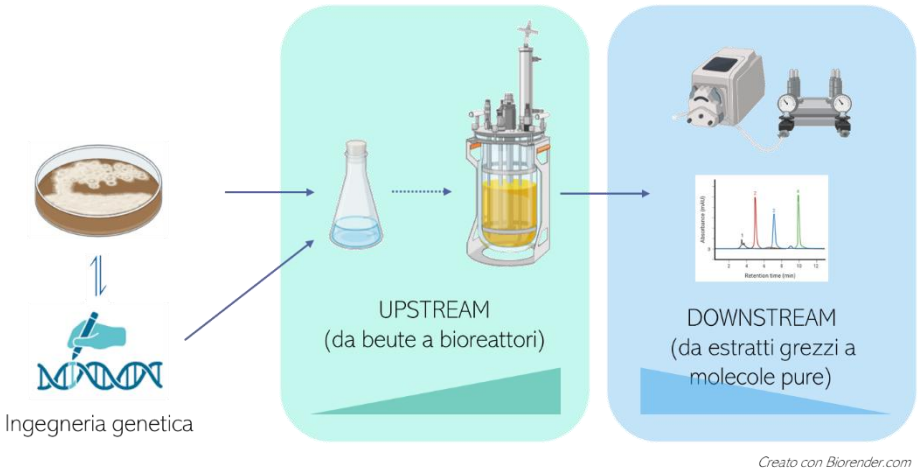
Keywords: fermentation, genetic engineering, upstream, downstream



This unit is dedicated to the production of active ingredients in microbial platforms and the development of scalable biotechnological processes for research and pre-industrial applications. The integration of expertise in microbiology, molecular biology, and process engineering allows for the systematic optimization of all production phases, ensuring process robustness and transferability. The facility is equipped for the development of fermentation systems that can be scaled up from flask to bioreactor scale, with a particular focus on optimizing growth and production parameters to maximize yields. It features thermostatic incubators, 3-liter fermenters, microscopes, and supporting instrumentation for upstream activities. Concurrently, advanced approaches to genetic manipulation of producer strains are applied to improve productivity and modulate the biosynthesis of active ingredients. Activities also include the development of downstream strategies for product recovery and purification. Our established expertise in the production of antibiotics and molecules with antioxidant activity provides a solid foundation for expanding into other classes of active ingredients of biotechnological and pharmaceutical interest.

Sub-unit "Structural Characterization of Active Ingredients of Microbial Origin"

Coordinators: Prof. Enrico Caruso; Prof. Francesco Della Monica, Prof. ssa Lorella Izzo, Dott. Orlando Santoro

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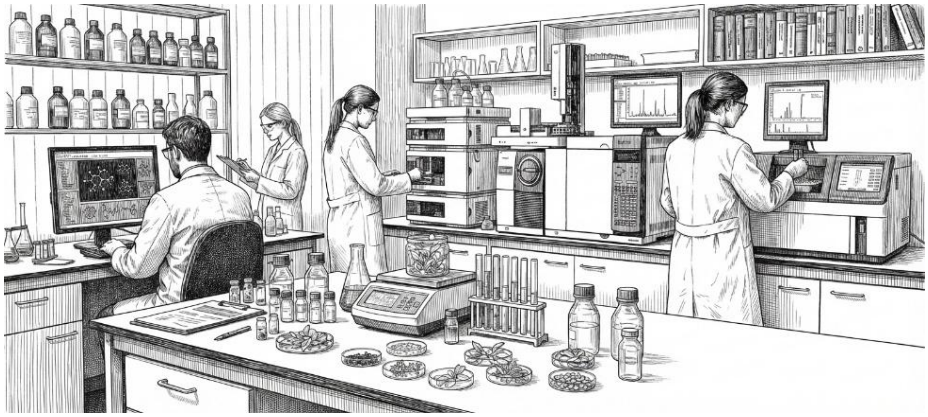
Keywords: chromatographic techniques, spectroscopic techniques, NMR, TGA



The substructure provides integrated support for the advanced stages of the discovery and development process, enabling the identification of the extract component(s) associated with the biological activity of interest, while also assessing their purity and chemical stability. Specifically, the purity of the extract can be assessed using chromatographic techniques such as HPLC coupled with analytical systems like UV-vis and mass spectrometry. For more volatile compounds, or those that can be made more volatile through derivatization, analyses can be performed using gas chromatography (GC) coupled with mass spectrometry (GC-MS). The substructure also possesses the expertise to apply analytical identification techniques such as spectroscopic methods (UV-vis, fluorescence, and IR) and one- and two-dimensional nuclear magnetic resonance (NMR), which, together with data from mass spectrometry, can aid in determining the exact structure of the active ingredients. Finally, the unit's activities include the evaluation of thermal stability—using thermogravimetric analysis (TGA)—and light stability (photobleaching phenomenon) of the purified active ingredients.

Sub-unit: "Biological Profiling of Microbial-Origin Active Ingredients"

Coordinators: Prof. ssa Marzia Gariboldi, Prof. Enrico Caruso, Dott. ssa Francesca Berini, Prof.ssa Flavia Marinelli

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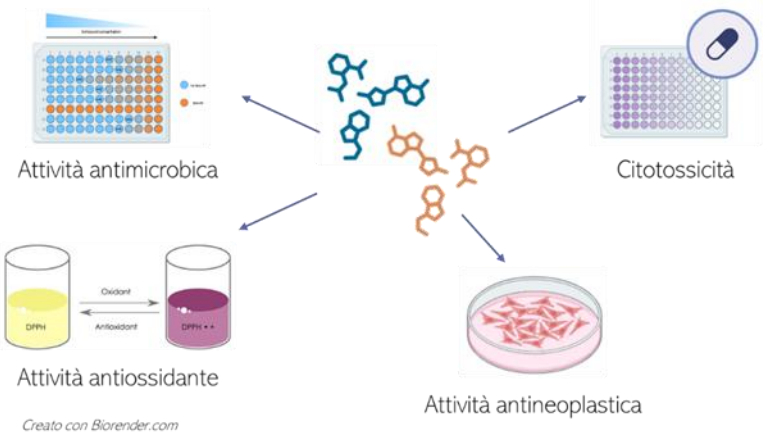
Keywords: antineoplastic activity, antimicrobial activity, antioxidant activity, cytotoxicity



A unit dedicated to evaluating the biological properties of natural active compounds, aimed at functionally validating their potential as antibiotics, anticancer drugs, and nutraceutical molecules. Activities include profiling antimicrobial activity through susceptibility testing on bacteria and fungi, evaluating antioxidant activity via radical assays, and analyzing antineoplastic potential. The latter involves assessing the antiproliferative effect of the active compounds on human tumor cell lines of various origins, cultured in two-dimensional and three-dimensional (3D) models, as well as studying the molecular mechanisms involved, including cell death, cell cycle, invasiveness, and other processes associated with aggressive tumor phenotypes. The unit's activities also include the in vitro study of the potential toxicity of active compounds. The unit is equipped with the main instruments for microbial growth and cell culture, including CO_2 incubators, microscopes, a flow cytometer, a plate reader, and a fluorescence microscope, as well as chambers for cell culture under hypoxic conditions, which allow for the reproduction of the microenvironment of solid tumors and the study of their response to treatments.

Publications:

1. Andreo-Vidal, A., Yushchuk, O., Marinelli, F., & Binda, E. (2023). Cross-talking of pathway-specific regulators in glycopeptide antibiotics (teicoplanin and A40926) production. *Antibiotics* (Basel, Switzerland), 12(4), 641. https://doi.org/10.3390/antibiotics12040641
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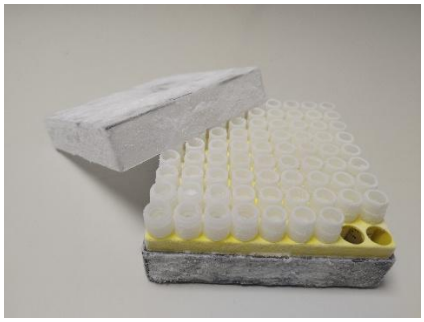
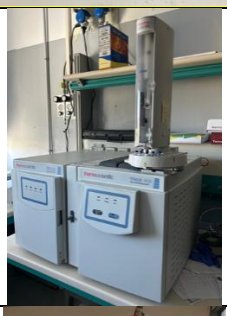
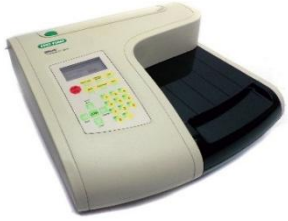
9. Stepanyshyn, A., Rückert-Reed, C., Busche, T., Yaruta, B., Andreo-Vidal, A., Marinelli, F., Kalinowski, J., & Yushchuk, O. (2024). Complete genome assembly of *Amycolatopsis bartoniae* DSM 45807t allows the characterization of a novel glycopeptide biosynthetic gene cluster. *Genes*, 15(12), 1651. https://doi.org/10.3390/genes15121651
10. Tallarita, R., Randisi, F., Jacobsen, L. M., Marras, E., Riva, M., Modoni, G., Fimmen, J., Bandaru, S. S. M., Schulzke, C., & Gariboldi, M. B. (2025). Anticancer effect of nature-inspired indolizine-based pentathiepines in 2D and 3D cellular model. *Cancers*, 17(14), 2393. https://doi.org/10.3390/cancers17142393
11. Yushchuk, O., Andreo-Vidal, A., Marcone, G. L., Bibb, M., Marinelli, F., & Binda, E. (2020). New molecular tools for regulation and improvement of a40926 glycopeptide antibiotic production in *Nonomuraea gerenzanensis* ATCC 39727. *Frontiers in microbiology*, 11, 8. https://doi.org/10.3389/fmicb.2020.00008
12. Yushchuk, O., Vior, N. M., Andreo-Vidal, A., Berini, F., Rückert, C., Busche, T., Binda, E., Kalinowski, J., Truman, A. W., & Marinelli, F. (2021). Genomic-led discovery of a novel glycopeptide antibiotic by *Nonomuraea coxensis* DSM 45129. *ACS chemical biology*, 16(5), 915–928. https://doi.org/10.1021/acschembio.1c00170
13. Yushchuk, O., Berini, F., Zhong, L., Rückert-Reed, C., Bernasconi, E., Bartolone, L., Busche, T., Kalinowski, J., Süssmuth, R. D., & Marinelli, F. (2025). A rare peptide scaffold in kineomicins, the glycopeptide antibiotics produced by *Actinokineospora auranticolor* DSM 44650. *Communications chemistry*, 8(1), 134. https://doi.org/10.1038/s42004-025-01534-x
14. Yushchuk, O., Rückert-Reed, C., Busche, T., Marinelli, F., & Kalinowski, J. (2026). Updated sequence of ramoplanin biosynthetic gene cluster from *Actinoplanes ramoplaninifer* ATCC 33,076. *Journal of applied genetics*, 10.1007/s13353-025-01035-6. Advance online publication. https://doi.org/10.1007/s13353-025-01035-6
15. Zhukrovska, K., Binda, E., Fedorenko, V., Marinelli, F., & Yushchuk, O. (2024). The impact of heterologous regulatory genes from lipodepsipeptide biosynthetic gene clusters on the production of teicoplanin and A40926. *Antibiotics* (Basel, Switzerland), 13(2), 115. https://doi.org/10.3390/antibiotics13020115

• **A) Identification of new active compounds of microbial origin**	
	**A strain library** comprising several hundred microbial strains, primarily actinomycetes and filamentous fungi, taxa known for their ability to produce bioactive compounds of biotechnological interest. The collection is constantly enriched with isolates from various environmental matrices with varying degrees of human impact and which remain relatively unexplored for the discovery of new natural products, including approximately 200 isolates from Antarctic waters, approximately 150 isolates from Arctic waters, approximately 40 isolates from urban environments with high human impact, etc. The strain library therefore represents a resource to be drawn upon for the search for new bioactive compounds with a variety of biotechnological applications.
	**Three Infors HT Multitron and Sartorius Stedim Certomat thermostatic shakers**, for the simultaneous temperature- and agitation-controlled incubation of more than 100 flasks (reference size: 300 mL). The incubators ensure a reproducible environment for cell growth thanks to their high internal temperature uniformity (ranging from a minimum of 5°C to a maximum of 60°C) and overall robustness, which allow for 24/7 operation. The incubators serve as a valuable resource both for the initial screening phase in the search for new active compounds and for the production of active compounds of interest.
	**An MPM Instruments M 80 – TB thermostatic incubator**, featuring a double-insulated door and thermal insulation made of natural mineral fiber, to ensure excellent sealing and constant maintenance of the desired incubation temperature within the range of 5–80°C. The incubator's 80 L capacity allows for the simultaneous incubation of approximately 500 plates (reference diameter: 90 mm).
CRIETT https://www.uninsubria.it/ricerca/strutture-la-ricerca/centri-speciali/centro-di-ricerca-e-trasferimento-tecnologico-criett	**The unit may utilize the genomic sequencer managed** by CRIETT to sequence the genomes of microbial species of interest, a prerequisite for applying genome mining approaches to identify biosynthetic clusters encoding the products of interest.

• **B) Production of active ingredients using microbial expression platforms**	
	**3-liter bioreactors (Applikon P100)** with a maximum operating volume of 2 liters per reactor are ideal for the cultivation of microbial cells, including filamentous microorganisms, and the small-scale production of active ingredients. Equipped with electrodes for continuous monitoring of critical parameters such as pH, temperature, foam level, and dissolved oxygen concentration (dO2), these fermenters offer precise, real-time control of culture conditions, enabling the optimization of cell growth and metabolite production in controlled environments.
	The **FPLC ÄKTA Start** is a compact, automated entry-level system for the laboratory-scale purification of peptides and nucleic acids. Given its high adaptability, it can be used to separate natural peptide products (such as glycopeptide antibiotics) using various purification strategies, including affinity chromatography and ion-exchange chromatography.
	**Millipore ultrafiltration systems**, featuring selectively permeable membranes, are suitable for filtering culture broths and for the concentration and partial purification of the biomolecules present therein. Thanks to their effectiveness, simplicity, and low operating costs, they serve as a starting point for downstream processes aimed at recovering the active ingredients of interest.
	The facility has access to the **centrifugation systems** at the DBSV, including three supercentrifuges capable of simultaneously processing approximately 1.5 L of sample per instrument (which can also be adapted to accommodate rotors with larger capacities), useful for the separation of biomass and spent broth, as well as for supporting subsequent fractionation/purification steps, and a Savant SpeedVac system for the vacuum concentration of samples in various solvents.

• C) Structural characterization of active ingredients of microbial origin	
	**Two HPLC systems equipped with a diode array detector and a column-coupled detector** for the analysis of active ingredients at all stages of the production and purification process. In addition to these two instruments, the facility also has access to similar equipment owned by CRIETT.
	**Vacuum oven** for drying or treating heat- or oxidation-sensitive samples at low temperatures, thanks to the reduced pressure that facilitates the evaporation of solvents (including flammable ones).
	**Fourier-transform infrared (FT-IR) spectrometer**. As part of the DBSV's instrumentation, it is useful for identifying the functional groups of active ingredients. The instrument can analyze samples in solution and, thanks to the ATR (Attenuated Total Reflection) module, also solid or liquid substances.
	**Spectrofluorometer:** a type of fluorometer capable of recording emission spectra by scanning across a range of wavelengths. The emission is typically collected at a 90° angle to the incident light. It is used to determine wavelengths and the quantum yield of fluorescence.
	The **gas chromatograph coupled with a mass spectrometer (GC-MS),** operated by the DBSV, is used for the separation, identification, and quantification of volatile substances with molecular masses ranging from 1.2 to 1,100 u. Chromatographic analysis allows for the separation of components in a mixture (even a complex one), while spectroscopic analysis enables the identification of each substance by studying fragmentation patterns.
	**Rotary evaporator** for removing solvents from a solution. Thanks to the reduced pressure, evaporation occurs at low temperatures, thereby preventing thermal degradation of the substances.

	**Liquid chromatography coupled with mass spectrometry (LC-MS)** operated by the CRIETT platform for the separation, identification, and quantification of non-volatile and/or thermolabile substances. Similar to GC-MS, chromatographic analysis allows for the separation of the components of a mixture, while mass spectrometry ensures the identification of the molecules.
	**Nuclear Magnetic Resonance Spectrometer** operated by CRIETT for the structural analysis of active ingredients. This technique allows for the determination not only of atomic connectivity but also of the presence of any impurities. The instrument operates in solution and enables the acquisition of one- and two-dimensional spectra by analyzing signals from 1H, 13C, and heteronuclei such as 19F and 31P.
	**Thermogravimetric Analysis (TGA)** for assessing the thermal stability of active ingredients. The instrument, part of CRIETT's equipment, records changes in the sample's weight as the temperature rises, allowing for the assessment of any degradation, loss of solvent molecules, and/or the presence of inorganic residues. Measurements can be performed in either an inert atmosphere or in air.

• **D) Biological profiling of active ingredients of microbial origin**	
	The **FACSCalibur** flow cytometer, an essential instrument at the DBSV for conducting assays to characterize the mechanisms of action underlying anticancer activity (cell death, ROS production, uptake, etc.).
	The **iMark plate reader**, manufactured by BIO-RAD, is a DBSV instrument that can be used to perform viability assays to evaluate the antiproliferative effect of identified natural active compounds, as well as ELISA tests and protein quantification for Western blot analysis.
CRIETT https://www.uninsubria.it/ricerca/strutture-la-ricerca/centri-speciali/centro-di-ricerca-e-trasferimento-tecnologico-criett	The sub-unit may use the confocal microscope operated by CRIETT to assess the subcellular localization of fluorescent natural products.
	The **Billups-Rothenberg hypoxic chamber** can be used to simulate controlled hypoxic conditions by supplying a gas mixture with an oxygen concentration of 0.1%.
	**Nikon Eclipse TS2R fluorescence microscope**. This is a DBSV instrument that allows users to capture images to be combined with data obtained from the flow cytometer.
	**Jasco V630 UV-Vis Spectrophotometer**, a versatile instrument for conducting assays aimed at identifying antimicrobial and antioxidant activities, among others. In addition to this spectrophotometer, the unit can utilize spectrophotometers and spectrofluorometers from the DBSV, which are available at both the Varese and Busto Arsizio locations.
	The substructure is also compatible with the DBSV **Tecan Infinite 200 PRO plate reader for absorbance**, fluorescence, and luminescence measurements. Thanks to its compatibility with multi-well plates, it enables the simultaneous analysis of numerous samples, making it an essential tool for facilitating and accelerating the biological profiling of the molecules under study.