Activity and Regulation of Symbiotic Peptides: A Frontier in Chemical Ecology

Eva Kondorosi

2018 Balzan Prize in Chemical Ecology

ADDENDUM 2025: Supporting Young Scientists within the Framework of the Balzan Research Project

Eva Kondorosi's project was originally planned to end in 2022, but there were funds were remaining at the time, so they have been used in the following ways: to support PhD students, to train students as scientific collaborators, to mentor students and provide assistance in attending various educational programs, to support young scientists through scholarships from the funds from Eva Kondorosi's Balzan Research Project, to assist young researchers in attending conferences and delivering invited lectures, and to carry out field work. A list of publications can be found at the end of this document.

I. Supervision of PhD students

PhD degrees obtained:

2021 Senlei Zhang. Developing Tools for the Functional Analysis of NCR Peptides in *Medicago Truncatula***.**

2022 Szilárd Kovács. Identification and characterization of New *Medicago Truncatula* Symbiotic Genes and Functional Analysis of Two Point-Mutant Alleles of the NSP2 Gene.

2022 Ting Wang. Incompatible Symbiotic Interactions Between *Sinorhizobium Meliloti* Strain RM41 and the Ecotypes of the Host *Medicago Truncatula.*

2023 Rui Dániel Lima. Evolution and Functional Analysis of Nodule-Specific Cysteine-Rich NCR Peptides.

PhDs in preparation:

M. Anas Al Bouni (ongoing)

Alexandra Pál (ongoing)

II. Training of students in Scientific Collaboration

Krisztina Borbély and **Boglárka Kovács.** Development of label-free detection of NCR peptides and protein interactions in collaboration with the Nanobiosensorics Research Group led by Róbert Horváth in Budapest. Borbély also attended the **12th Environmental Chemistry Symposium** in Siófok, Hungary (10-11 October 2024), where she did the following presentation: Label-Free Biophysical Methods for the Study of Molecular Interactions and Cell Adhesion Processes. She will also participate in the joint conference of the Hungarian Physiological Society, the Hungarian Biophysical Society, the Hungarian Microcirculatory and Vascular Biology Society and the Hungarian Bioinformatics Society, in Szeged, Hungary (2-5 September 2025) with the presentation "From protein–peptide interactions to cell adhesion - label-free biophysical insights into peptide activity".

Bettina Szerencsés, PhD student at Szeged University, co-authored the following articles:

Bettina Szerencsés, Csaba Papp, Alexandra Pál, Sándor Jenei, Nelli Németh, Csaba Vágvölgyi, Ferhan Ayaydin, Gabriella Endre, Éva Kondorosi, Ilona Pfeiffer. Plant-derived nodule-specific cysteine-rich peptides as potent antifungal agents against *Cryptococcus neoformans*: mechanisms of action, chimeric peptide enhancement, and immunomodulatory effects. Curr Res Microb Sci. 2025; 9: 100407. Published online 2025 May 23. doi: 10.1016/j.crmicr.2025.100407

Bettina Szerencsés, Attila Gácser, Gabriella Endre, Ildikó Domonkos, Hilda Tiricz, Csaba Vágvölgyi, János Szolomajer, Dian H. O. Howan, Gábor K. Tóth, Ilona Pfeiffer, Éva Kondorosi. Symbiotic NCR Peptide Fragments Affect the Viability, Morphology and Biofilm Formation of *Candida* Species. Int J Mol Sci. 2021 Apr; 22(7): 3666. Published online 2021 Apr 1. doi: 10.3390/ijms22073666

Dian Howan, PhD student at the Szeged University, co-authored the following articles:

Howan, Dian H. O., Sándor Jenei, János Szolomajer, Gabriella Endre, Éva Kondorosi, and Gábor K. Tóth. 2023. "Enhanced Antibacterial Activity of Substituted Derivatives of NCR169C Peptide." *INTERNATIONAL JOURNAL OF MOLECULAR SCIENCES* 24 (3): 2694. doi:10.3390/ijms24032694.

Lima, Rui M., Balaji Baburao Rathod, Hilda Tiricz, **Dian H. O. Howan**, Bouni Mohamad Anas Al, Sandor Jenei, Edit Timar, Gabriella Endre, Gabor K. Toth, and Eva Kondorosi. 2022. "Legume Plant Peptides as Sources of Novel Antimicrobial Molecules Against

III. Mentoring and participation in educational programs

Workshops and training and research programs for students organized in the framework of Kondorosi's Balzan research project (including lab work)

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The following students benefitted from mentoring: Eliza Morvai (2021), Diana Tóth (2022), Emma Tikász (2022), Ármin Szeles (2023), Mihály Nádasdy (2023-2024, as well as recipient of the Gold prize for his research project in a Hungarian competition in 2025), Luca Polgár (2024), Benedek Sallai (2024), Maté Ádám Gera (2024), Dóra Arany (2024-2025), Veronka Szűcs (2025), Kata Kőhida (2025-2026).

Participation in programs of the National Academy of Scientist Education

Over 2500 students (students/year: ~300 /2023, ~600/2024, ~2000/2025) were assisted in the following programs:

Summer Schools

- The Academy of Sciences of Moldova Chisinau, Moldova12-15 June 2023.

Eva Kondorosi: Biological Nitrogen Fixation in Rhizobium-legumes symbiosis; Nitrogen crisis and a path to sustainable agriculture.

- M. Anas Al Bouni (PhD student) was supported to participate in the **First V4SDB Developmental Biology Student Summer School** (6-12 June 2022), Tihany, Hungary.

Interdisciplinary program for mentors and mentees

52 participants on 28 May 2025
in Lovas, Hungary

IV. Scholarships with funds from Eva Kondorosi's Balzan Research Project

The following young scientists received scholarships with funds from Eva Kondorosi's Balzan Research Project: **Dr. Salome Kyralova** (postdoc from Charles University Prague); **Dr. Hilda Tiricz Lima** (Hungarian female scientist returning from maternity leave); **Dr. Balaji Rathod** (postdoc from India); **Dr. Senlei Zhang** (Chinese citizen, first a PhD student and then postdoc in the group); **Dr. Ting Wang** (Chinese citizen, first a PhD student and then postdoc in the group); **Dr. Jun Li** (postdoc from China, ongoing); **M. Anas Al Bouni** (PhD student from Syria, ongoing); **Alexandra Pál** (Hungarian PhD student, ongoing).

IV. Conferences and Invited Scientific Lectures

Festival of Science and Philosophy (13 April 2019), Foligno, Italy. Eva Kondorosi: *Fertilizers from the Air.*

21st International Conference on Nitrogen Fixation - ICNF 2019 (10-15 October 2019), Wuhan, China. Gabriella Endre, Peter Kaló, Eva Kondorosi. Kondorosi was Chair of the Plenary Session, *Legume nodule development* and Closing session and Plenary Speaker on *From cellular infection to symbiotic nitrogen fixation*

The Fourth Adam Kondorosi Symposium (26-27 November 2019), Gif-sur-Yvette, France.

19th Vienna Congress com·sult (30-31 January 2022), Vienna, Austria. Introductory notes by Eva Kondorosi for Harald zur Hausen, Nobel Laureate in Physiology or Medicine and for Katalin Kariko, Nobel Laureate in Physiology or Medicine 2023.

ESOF 2022 (16 July 2022 online) in SS3.14 *Current Research Trends Towards Solving Antimicrobial Resistance.* Eva Kondorosi: *Global Shortage of Innovative Novel Antibiotics. Do Antimicrobial Peptides Provide Solutions?*

14th European Nitrogen Fixation Conference (online participation), Aarhus, DK. Lima et al: Medicago truncatula *nodGRPs are crucial for nitrogen-fixing nodule development*. (poster)

FEMS Conference on Microbiology (30 June – 2 July 2022), Belgrade, Serbia. M. Anas Al Bouni (PhD student) was supported with funds from Eva Kondorosi's Balzan Research Project to present a poster. M.A. Al Bouni et al.: *Legume Antimicrobial Peptides Against Human Pathogens*.

Alexandra Pál (PhD student) received support to participate in the meeting organized with **Corteva** representatives. The discussion focused on how science and scientists can be better involved and supported in meeting the needs of agricultural production, with a particular focus on climate change and sustainable agriculture.

11th International Interdisciplinary Research Laboratory (InteR-La+B) on ENVIRONMENT (13 September 2022), Milan. The young scientists invited to participate included **Rui Daniel Lima** (PhD student), **Hilda Tiricz** (postdoc), Senlei Zhang (postdoc), and Ting Wang (PhD Student). Unfortunately, bureaucratic problems prevented Senlei Zhang and Ting Wang from participating. Presentations were done by Rui Daniel Lima, *Enhancing nitrogen-fixation and overcoming microbial infections*, and Eva Kondorosi, *Plants for the benefit of the environment and sustainable future.*

2023 Scott-Moncrieff Lecture by Eva Kondorosi, *Serendipity in symbiotic nitrogen fixation* (10 February 2023), John Innes Centre, Norwich, UK.

15th European Nitrogen Fixation Conference 2023 (31 August – 3 September), Naples, Italy. The following papers were delivered: **M. Anas Al Bouni,** *Unravel Nodule GRPS in Plant Symbiosis*; **Alexandra Pál,** *Exploring the Function of the MtnodGRP3C Gene in the Development of the Symbiotic Nodule*; **Rui Daniel Lima,** *Production of Mutants for Nodule-Specific Peptides and Their Screen for Symbiotic Phenotype*.

14th International Agriculture Symposium "AGROSYM 2023" (5-8 October 2023), Jahorina Mountain, Bosnia and Herzegovina. **Alexandra Pál,** *Methylobacterium symbioticum* in Bluen Has a Positive Effect on Nutrition Stimulation in Cultivated Plants.

Straub Symposium at the Biological Research Centre (30-31 May 2024), Szeged, Hungary**. Alexandra Pál et al.,** *Investigation of the role of the MtnodGRP3C gene in nitrogen-fixing symbiosis*; **Mohamad Anas Al Bouni et al.,** *Uncovering the fate of some nodule specific glycine rich proteins in* Medicago truncatula; **Rui M. Lima et al.**, *Silencing of nodule-specific genes affects the development of nitrogen fixing symbiosis.*

Workshop: Single-Cell RNA-Seq Data Analysis: A Practical Introduction, (25-27 September 2024), budapest, Hungary. Participants: Rui M. Lima.

Flow Cytometry Days by Bio-Science (5 September 2025) Budapest, Hungary. Participants: Rui M. Lima.

Straub Symposium at the Biological research Centre (20-31 May 2025), Szeged, Hungary**. Sándor Jenei et al.**, *Beneficial microbes to help faba bean growth in climate change.*

Plant Biology Europe 2025 Conference (25-28 June 2025), Budapest, Hungary. Oral presentation by **M. Rui Lima,** *Symbiotic NCR-Like Peptides and Bacteroid Differentiation in the* Amorpha fruticosa Mesorhizobium amorphae *Symbiosis*

Upcoming Conferences:

16th European Nitrogen Fixation Conference 2025 (26-29 August 2025), Oxford, England. **Alexandra Pál et al.,** *The Glycine-Rich Peptide nodGRP3C Regulates Bacteroid Differentiation and Nodule Function in* Medicago truncatula; **Hilda Tiricz,** *Mapping the protein interactome of NCR peptides in* Medicago truncatula *symbiosis;* **Mohamad Anas Al Bouni et al.,** *MtnodGRP1L Is Essential for Efficient Symbiotic Nitrogen Fixation in* Medicago truncatula; **Rui M. Lima et al.,** *Exploring a Hidden Symbiosis: What* Amorpha fruticosa *Reveals*; **Sándor Jenei et al.,** *Enhancing Raba Bean cultivation in Drought conditions: Toward a sustainable Inoculum Prototype*; **Eva Kondorosi,** *Co-evolved Peptides Driving Nitrogen-Fixing Symbiosis: Insights into NCR and NODGRP Functions in* Medicago Truncatula.

V. Field Work

Spring – Autumn 2023. Location: fields near Hódmezővásárhely, Hungary.

In the course of the team's work, a *Methylobacterium symbioticum* strain was applied on various cultivated plants (barley, maize, sunflower) in plot experiments with the corresponding control plots. The photosynthetic activity of the plants during their development was monitored and the yield quantity and quality was analyzed, followed by a statistical evaluation in comparison with the controls. The results of these comparative plot experiments confirmed the positive effect of this bacterium strain on plant development and yield. Participating student**: Alexandra Pál**

April – June 2024. Locations: *Vicia* fields at several locations in Hungary

The following samples were collected: *Vicia faba* – organic farming: Mórahalom, Somogyvámos and Zsámbok; field cultivation: Orosháza; teaching farm: University of Debrecen, Nyíregyháza Research Institute. Specimens of a protected plant, *V. narbonensis*, were collected in the Kőrös-Maros National Park from two saline sites: Zsadány - Gát and Zsadány - Kis-Sárrét, and from three *V. sativa* cultivation sites in Szeged. In each case, bacteria were isolated separately from the soil region around the roots (rhizosphere), from the root surface and root tissue (endophytic bacteria), and from the nodule (symbiotic rhizobium-type bacteria and other nodule endophytic bacteria). Out of a total of more than 500 isolates, nearly 400 grew after inoculations, half of which were successfully grown individually and identified at least at the genus level using the rDNA-ITS amplification and sequencing method. The project aims to develop a prototype soil inoculant containing drought-resistant rhizobacterial strains to enhance the resilience and reliability of faba bean cultivation.

V. PUBLICATIONS

1. Szerencsés, Bettina ; Papp, Csaba ; Pál, Alexandra ; Jenei, Sándor ; Németh, Nelli ; Vágvölgyi, Csaba ; Ayaydin, Ferhan ; Endre, Gabriella ; Kondorosi, Éva ; Pfeiffer, Ilona. 2025. "Plant-derived nodule-specific cysteine-rich peptides as potent antifungal agents against Cryptococcus neoformans: mechanisms of action, chimeric peptide enhancement, and immunomodulatory effects" CURRENT RESEARCH IN MICROBIAL SCIENCES 9 Paper: 100407 , 11 p. (2025)
2. Zhang, Senlei, Ting Wang, Rui M. Lima, Aladár Pettkó-Szandtner, Attila Kereszt, J. Allan Downie, and Eva Kondorosi. 2023. "Widely Conserved AHL Transcription Factors Are Essential for NCR Gene Expression and Nodule Development in Medicago." *NATURE PLANTS* 9: 280–288. doi:10.1038/s41477-022-01326-4.
3. Howan, Dian H. O., Sándor Jenei, János Szolomajer, Gabriella Endre, Éva Kondorosi, and Gábor K. Tóth. 2023. "Enhanced Antibacterial Activity of Substituted Derivatives of NCR169C Peptide." *INTERNATIONAL JOURNAL OF MOLECULAR SCIENCES* 24 (3): 2694. doi:10.3390/ijms24032694.
4. Lima, Rui M., Balaji Baburao Rathod, Hilda Tiricz, Dian H. O. Howan, Bouni Mohamad Anas Al, Sandor Jenei, Edit Timar, Gabriella Endre, Gabor K. Toth, and Eva Kondorosi. 2022. "Legume Plant Peptides as Sources of Novel Antimicrobial Molecules Against Human Pathogens." *FRONTIERS IN MOLECULAR BIOSCIENCES* 9. doi:10.3389/fmolb.2022.870460.
5. Downie, J. Allan, and Eva Kondorosi. 2021. "Why Should Nodule Cysteine-Rich (NCR) Peptides Be Absent from Nodules of Some Groups of Legumes but Essential for Symbiotic N-Fixation in Others?" *FRONTIERS IN AGRONOMY* 3. doi:10.3389/fagro.2021.654576.
6. Nicoud, Quentin, Quentin Barrière, Nicolas Busset, Sara Dendene, Dmitrii Travin, Mickaël Bourge, Romain Le Bars, et al. 2021. "Sinorhizobium Meliloti Functions Required for Resistance to Antimicrobial NCR Peptides and Bacteroid Differentiation." *MBIO* 12 (4). doi:10.1128/mBio.00895-21.
7. Szerencsés, Bettina, Attila Gácser, Gabriella Endre, Ildikó Domonkos, Hilda Tiricz, Csaba Vágvölgyi, János Szolomajer, et al. 2021. "Symbiotic NCR Peptide Fragments Affect the Viability, Morphology and Biofilm Formation of Candida Species." *INTERNATIONAL JOURNAL OF MOLECULAR SCIENCES* 22 (7). doi:10.3390/ijms22073666.
8. Jenei, Sandor, Hilda Tiricz, Janos Szolomajer, Edit Timar, Eva Klement, Bouni Mohamad Anas Al, Rui M. Lima, et al. 2020. "Potent Chimeric Antimicrobial Derivatives of the Medicago Truncatula NCR247 Symbiotic Peptide." *FRONTIERS IN MICROBIOLOGY* 11. doi:10.3389/fmicb.2020.00270.
9. Lima, Rui M, Salome Kylarová, Peter Mergaert, and Éva Kondorosi. 2020. "Unexplored Arsenals of Legume Peptides with Potential for Their Applications in Medicine and Agriculture." *FRONTIERS IN MICROBIOLOGY* 11: 1307. doi:10.3389/fmicb.2020.01307.
10. Mergaert, Peter, Attila Kereszt, and Eva Kondorosi. 2020. "Gene Expression in Nitrogen-Fixing Symbiotic Nodule Cells in Medicago Truncatula and Other Nodulating Plants." *PLANT CELL* 32 (1): 42–68. doi:10.1105/tpc.19.00494.
11. Zhang, Senlei, Éva Kondorosi, and Attila Kereszt. 2019. "An Anthocyanin Marker for Direct Visualization of Plant Transformation and Its Use to Study Nitrogen-Fixing Nodule Development." *JOURNAL OF PLANT RESEARCH* 132 (5): 695–703. doi:10.1007/s10265-019-01126-6.

In progress: two manuscripts are under review and three are in preparation.