FESR 2014-2020 POR BasilicataEuropa

REGIONE BASILICATA

Unione Europea
Fondo Europeo di Sviluppo Regionale

I Giornata di informazione e comunicazione progetti di COOPERAZIONE INTERREGIONALE e TRANSNAZIONALE

New molecules with antimicrobial, anticancer and antiviral activity: alternative solutions for three of the main social-health emergencies

AAA:SAFE SOS

UNIBAS Matera 03 febbraio 2023

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PARTNER

Lead Partner

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IRCCS CROB

Scientific Hospitalization and Treatment Institute - Oncological Reference Center of Basilicata - Referent dr. Rocco Galasso

University of Catania - Referent prof. Daria Nicolosi

University of Giessen – Germany - Referent prof. Andreas Vilcinskas

University of Ioannina - Greece - Referent prof. Maria Eleni Lekka

University of Novi Sad – Serbia - Referent prof. Zeljko Popovic

REGIONE BASILICATA Basilicata Region - Department of Health and Personal Policies

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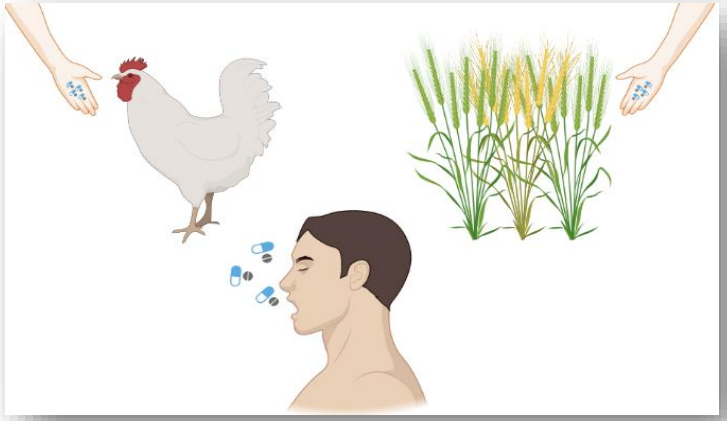
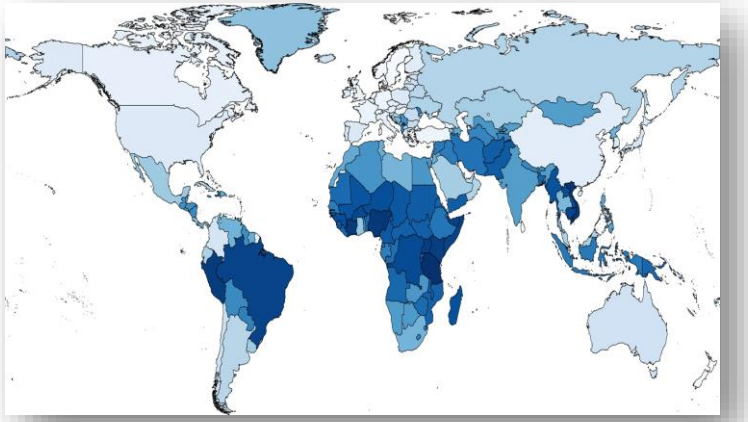
Antimicrobial Resistance (AMR)

Antibiotic Resistance



Created by Biorender.com



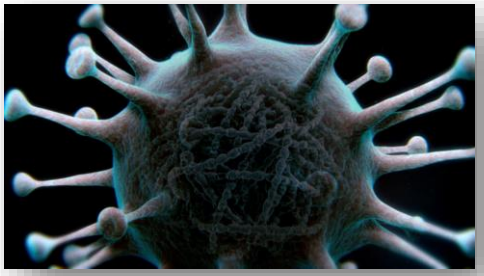
Hendriksen *et al*. 2019. Nature Comm, 10: 1–12

Indiscriminate and prolonged use in human contributed to the development and spread of drug-resistant micro-organisms

AMR is responsible for 700.000 deaths a year and is expected to cause up to 10 Million deaths a year by 2050

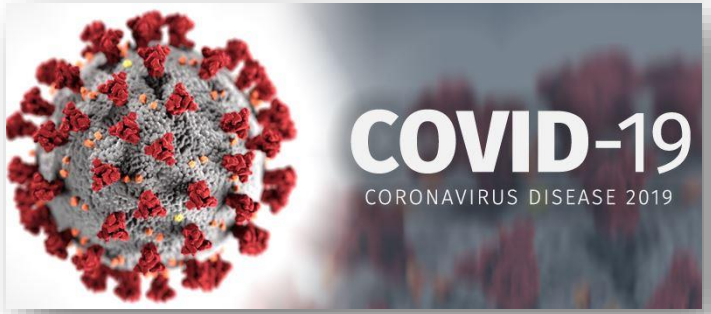
Antimicrobial Resistance (AMR)

Antiviral Resistance



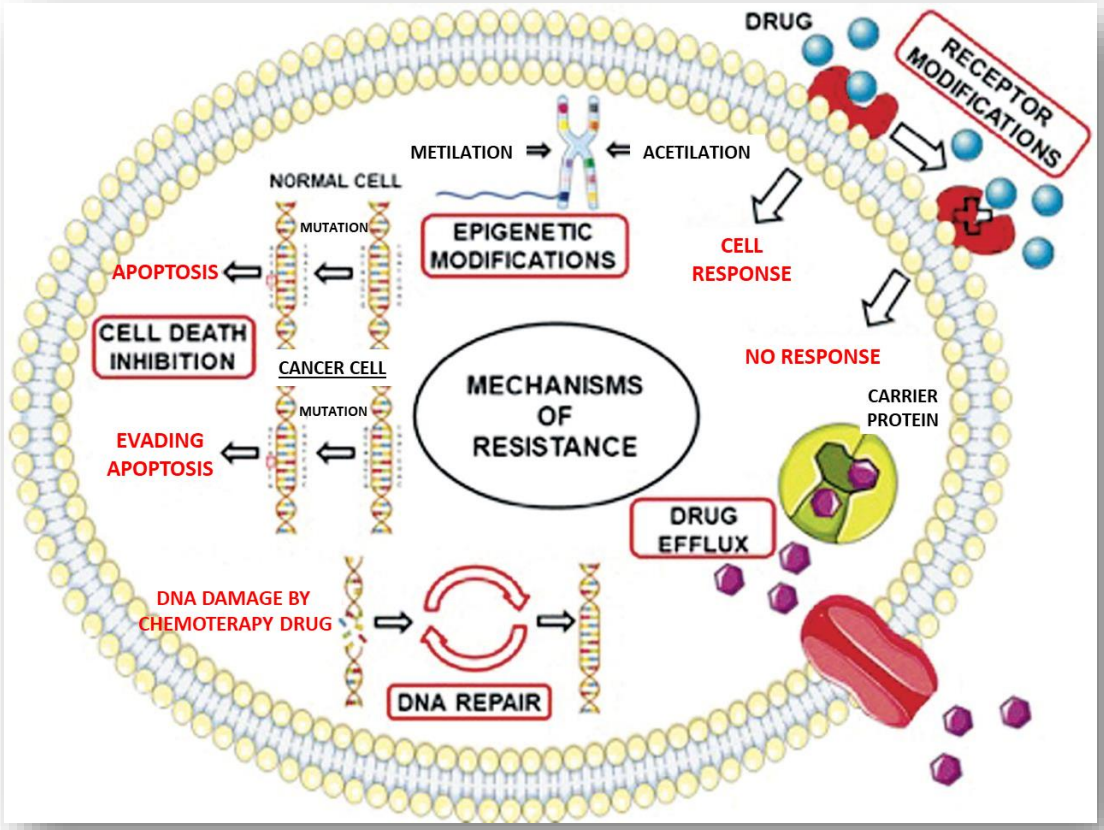
Due to a high constitutive mutability, viruses can increase the infection basin, becoming dangerous also for other species, including humans.

- Host completely lacking specific immune defenses
- Lack of specific vaccines



Geoghegan e Holmes, 2017. Open Biol, 7: 170189; Regoes *et al.*, 2013. Pro Biol Sci, 280:20122047; Lai *et al.*, 2020. Intern J Antimicrob Agents, 55:105924

Non-specificity and resistance to chemotherapy drugs

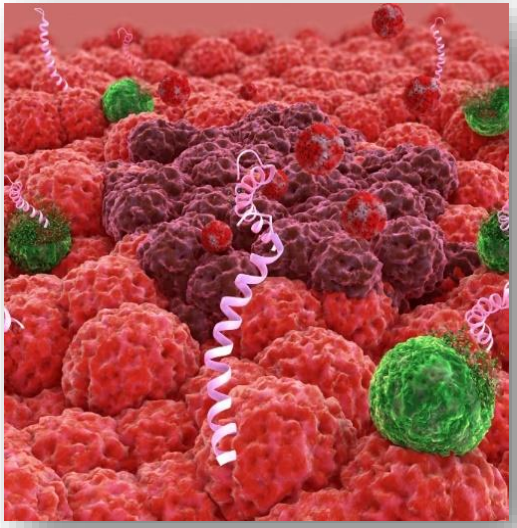


Side effects caused by damage to healthy cells and tissues during the administration of anticancer chemotherapy and resistance to this therapy.

International Institute of Anticancer Research, 2019

Antimicrobial Peptides (AMPs)

AMPs are a class of biologically active compounds produced by all living organisms. They are evolutionarily conserved components of the innate immune response, the first-line defense against microbial attack in eukaryotes or produced as a competitive strategy in prokaryotes to limit the growth of other microorganisms.



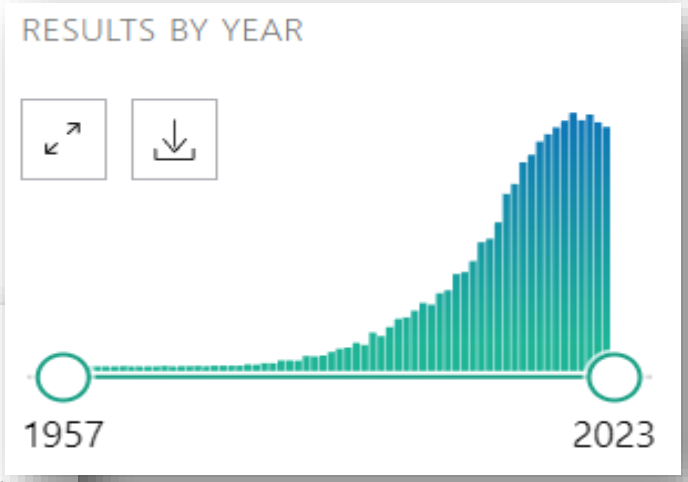
Why AMPs and ACPs?

AMPs are able to overcome antimicrobial resistance and have a broad-spectrum action with low toxicity to the host.

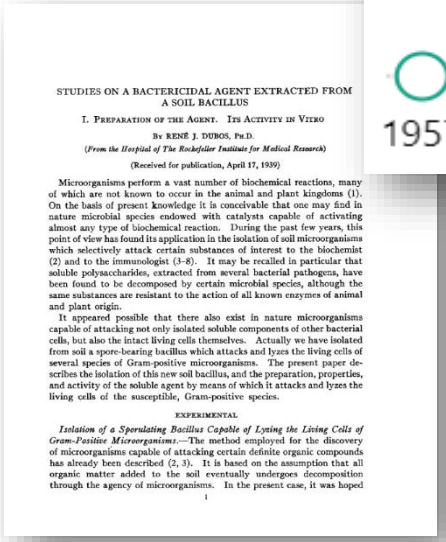
ACPs can overcome resistance to chemotherapies with targeted mechanism of action.

Hollmann *et al.* 2018. Font Chem, 6:204;
Moretta *et al.* 2021, Front Cell Infect Microbiol. 11:668632

AMPs



https://pubmed.ncbi.nlm.nih.gov/



STUDIES ON A BACTERICIDAL AGENT EXTRACTED FROM A SOIL BACILLUS

I. Preparation of the Agent. Its Activity in Vitro

By RENÉ J. DUBOS, Ph.D.

(From the Hospital of The Rockefeller Institute for Medical Research)

(Received for publication, April 17, 1939)

Microorganisms perform a vast number of biochemical reactions, many of which are not known to occur in the animal and plant kingdoms (1). On the basis of present knowledge it is conceivable that one may find in nature microbial species endowed with catalysts capable of activating almost any type of biochemical reaction. During the past few years, this point of view has found its application in the isolation of soil microorganisms which selectively attack certain substances of interest to the biochemist (2) and to the immunologist (3–8). It may be recalled in particular that soluble polysaccharides, extracted from several bacterial pathogens, have been found to be decomposed by certain microbial species, although the same substances are resistant to the action of all known enzymes of animal and plant origin.

It appeared possible that there also exist in nature microorganisms capable of attacking not only isolated soluble components of other bacterial cells, but also the intact living cells themselves. Actually we have isolated from soil a spore-bearing bacillus which attacks and lyzes the living cells of several species of Gram-positive microorganisms. The present paper describes the isolation of this new soil bacillus, and the preparation, properties, and activity of the soluble agent by means of which it attacks and lyzes the living cells of the susceptible, Gram-positive species.

EXPERIMENTAL

Isolation of a Sporulating Bacillus Capable of Lysing the Living Cells of Gram-Positive Microorganisms.—The method employed for the discovery of microorganisms capable of attacking certain definite organic compounds has already been described (2, 3). It is based on the assumption that all organic matter added to the soil eventually undergoes decomposition through the agency of microorganisms. In the present case, it was hoped

1

Dubos, 1939. J Experim Med

Discovered for the first time in 1939

AMP Application

Medicine:
treatment of surgical, dental and ophthalmological infections

Food Market:
substitute of food preservatives

Animal Husbandry and Aquaculture:
improve production

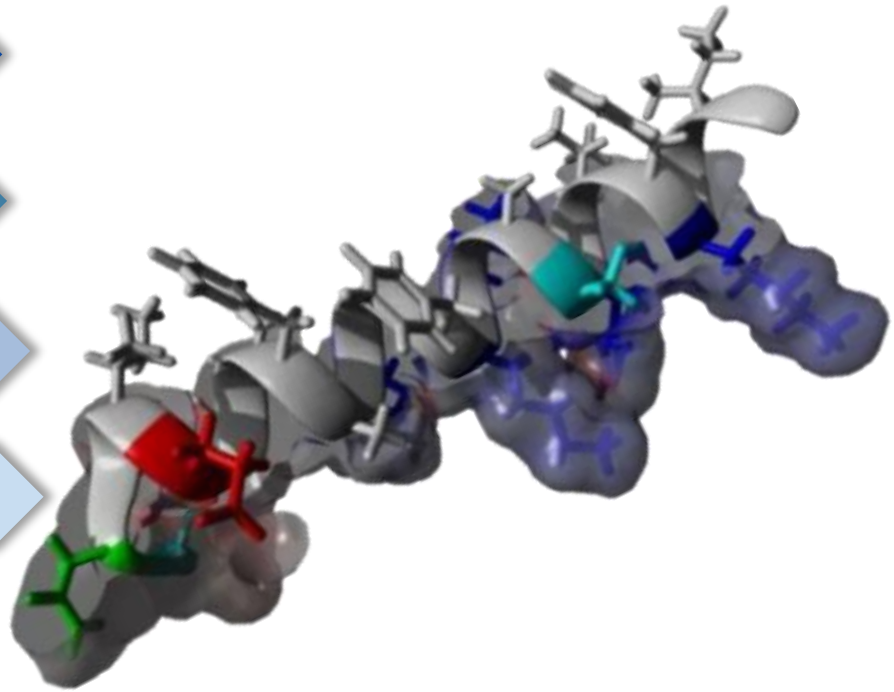
Agriculture:
control of phytopathogens

Huan *et al.*, 2020. Front Microbiol. 11:582779

Antimicrobial Peptides

AMPs share some common characteristics:

- 12-50 amino acids
- Positively charged (most common)
- Amphipathic structure
- Negatively charged (less common)

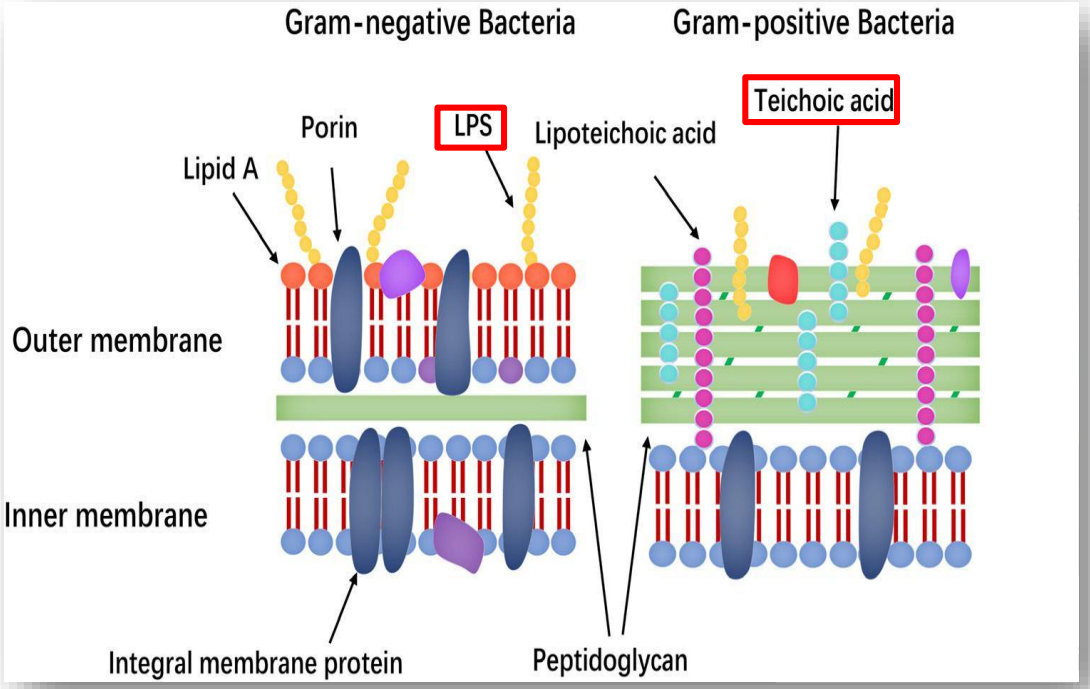


Moretta *et al.* 2021, Front Cell Infect Microbiol. 11:668632

Antimicrobial Peptide (AMP) Action Mechanism

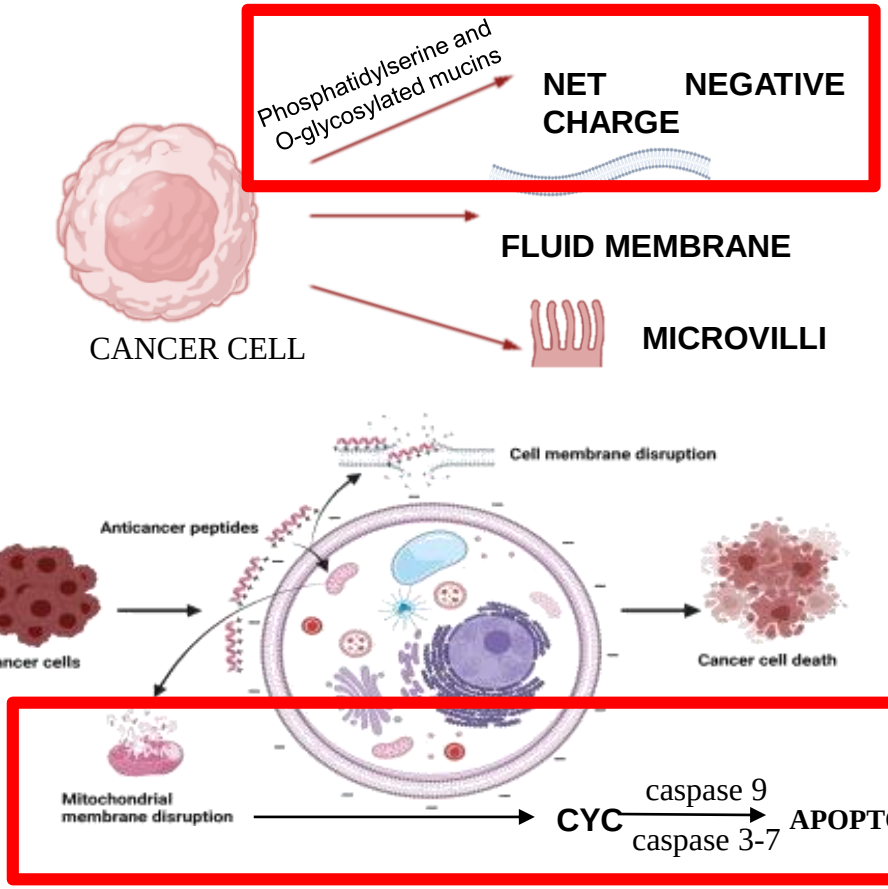
Membranolytic:
destabilize bacterial membrane causing their disruption

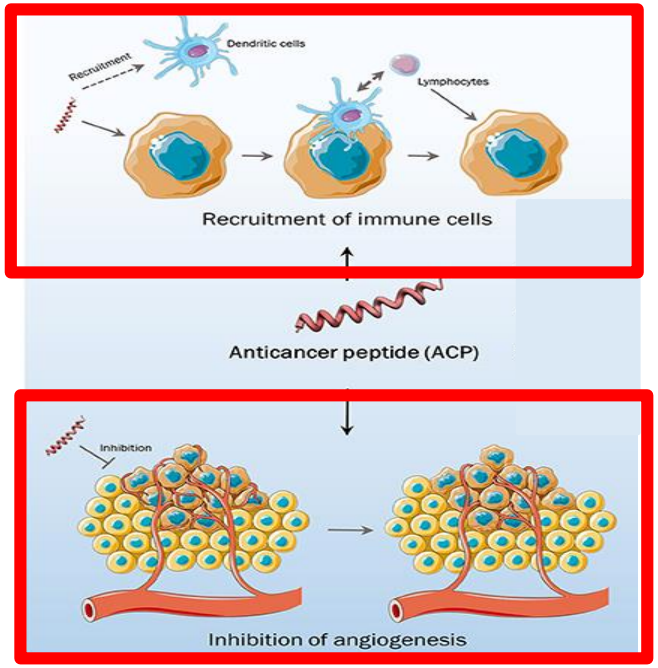
Non-membranolytic:
are able to traslocate across the membrane without damaging them but destabilizing normal cell function



Huan *et al.*, 2020. Front Microbiol. 11:582779

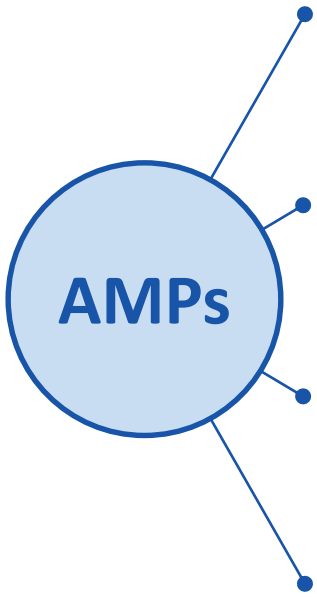
Anticancer Peptide (ACP) Action Mechanism

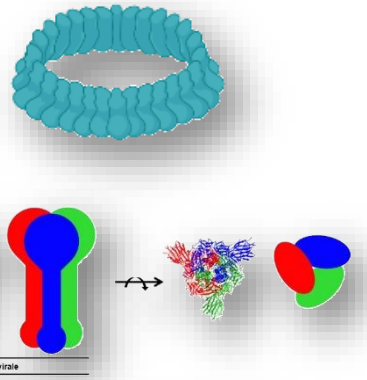


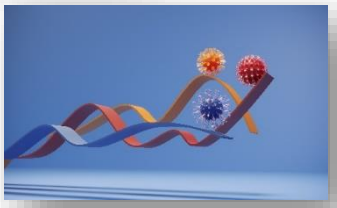


Felicio *et al.*, 2017. Front Chem, 5:5
Papo and Shai, 2005. Cell Mol Life Scie, 62.

Antimicrobial Peptide (AMP) Antiviral Action Mechanism







- FORMATION OF PORES AT THE LEVEL OF VIRAL CAPSID
- BLOCKING OF THE BINDING OF THE VIRUS TO EPARAN SULPHATE
- BLOCKING OF REPLICATION MECHANISMS
- STIMULATION OF HOST IMMUNE RESPONSE

Hsieh e Hartshorn, 2016. Pharmaceuticals. 9:53; Ding *et al.*, 2009. J. Innate Immun, 1:413–420. Penberthy *et al.*, 2011. Cell Mol Life Sci. 68:2231-42.; Bergman *et al.*, 2007. Curr HIV Res, 5:410–415. Ahmed *et al.*, 2019. Viruses, 1:E704.

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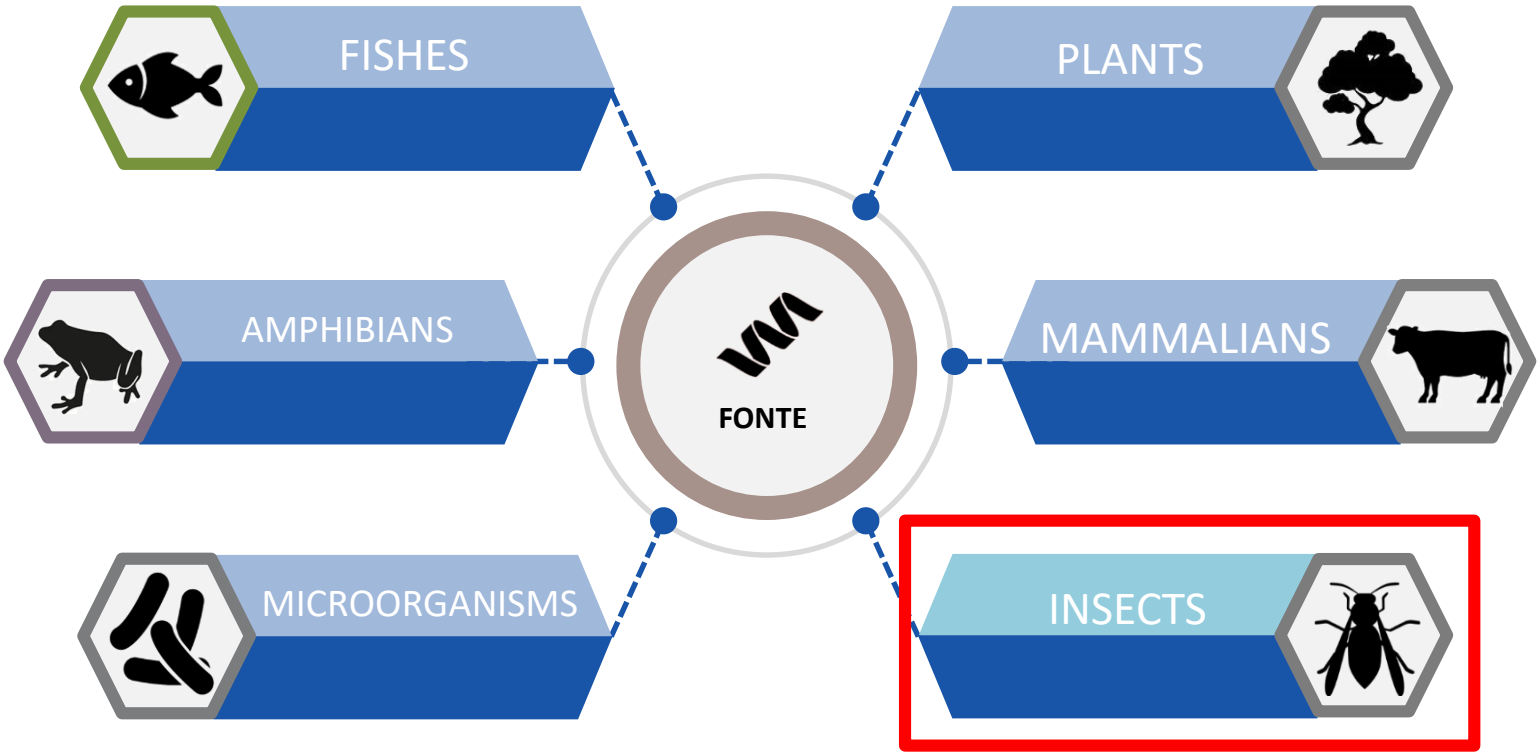
New molecules with antimicrobial, anticancer and antiviral activity: alternative solutions for three of the main social-health emergencies

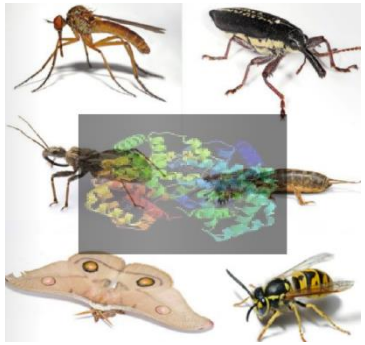
- AMPs as new molecules with antimicrobial, anticancer and antiviral activity
- Innovative source

AMP Sources

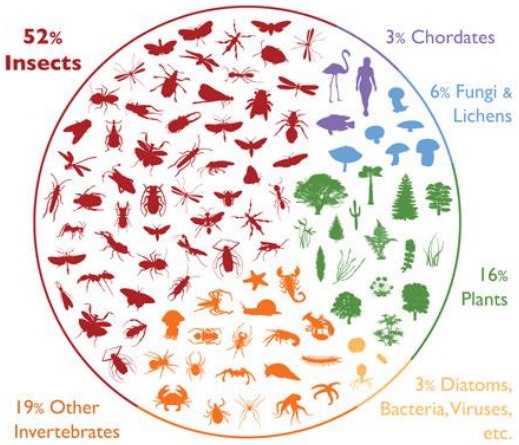


Huan *et al.,* 2020. Front Microbiol. 11:582779

Insects and Biomimetic



Heterogeneous group of organisms with the highest level of ***BIODIVERSITY***



Innovative and inexhaustible source of USEFUL MOLECULES (R&D)

Didham *et al.,* 2015. Insect Conser Diversity, 8:1-2

THE PROJECT: AAA SAFE-SOS

- «Omic, *in silico* and *in vitro*» approach for the identification and functional characterization of insect-derived AMPs as a new category of safe and effective drugs, as an innovative therapeutic solution for the protection of human health.
- Use of AMPs as alternative molecules or in synergy with conventional antibiotics, chemotherapeutics, antivirals and antifungals.

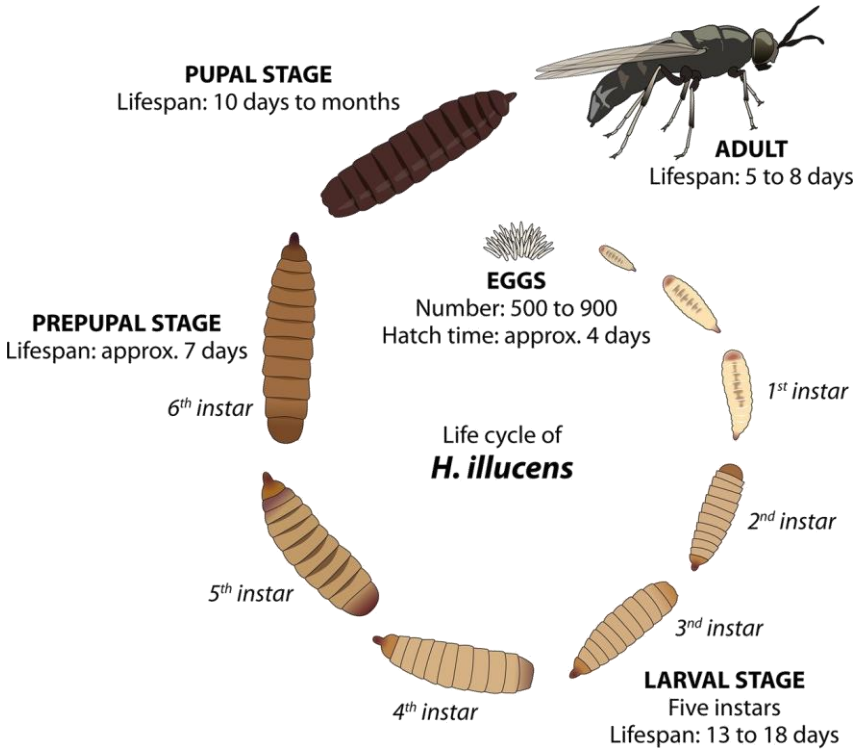
THE PROJECT: AAA SAFE-SOS

Black Soldier Fly
Hermetia illucens
(Diptera: Stratiomyidae)

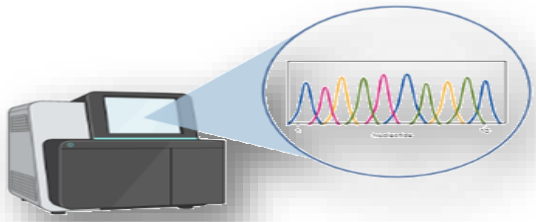
Red Palm Weevil
Rhynchophorus ferrugineus
(Coleoptera: Curculionidea)

What has been done

Hermetia illucens breeding



Reannotation of the transcriptome



b2g blast2go

34 putative AMPs

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What has been done

Bioinformatic analyzes of new AMPs

34
New sequences annotated as antimicrobial

11 not analyzable sequences (open frame)

3
Putative antimicrobial activity

12
Putative antimicrobial + anticancer activity

6
Putative antimicrobial + antiviral activity

2
Putative antimicrobial + anticancer + antiviral activity

0
Putative antifungal activity

IRCCS CROB
IRCCS Crob

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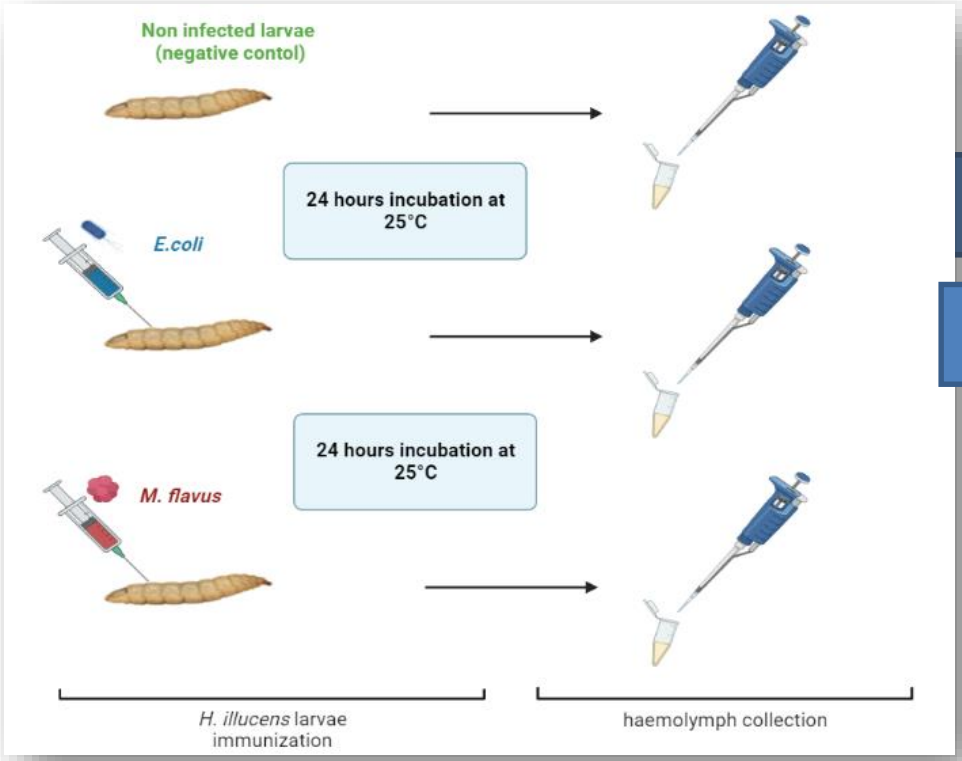
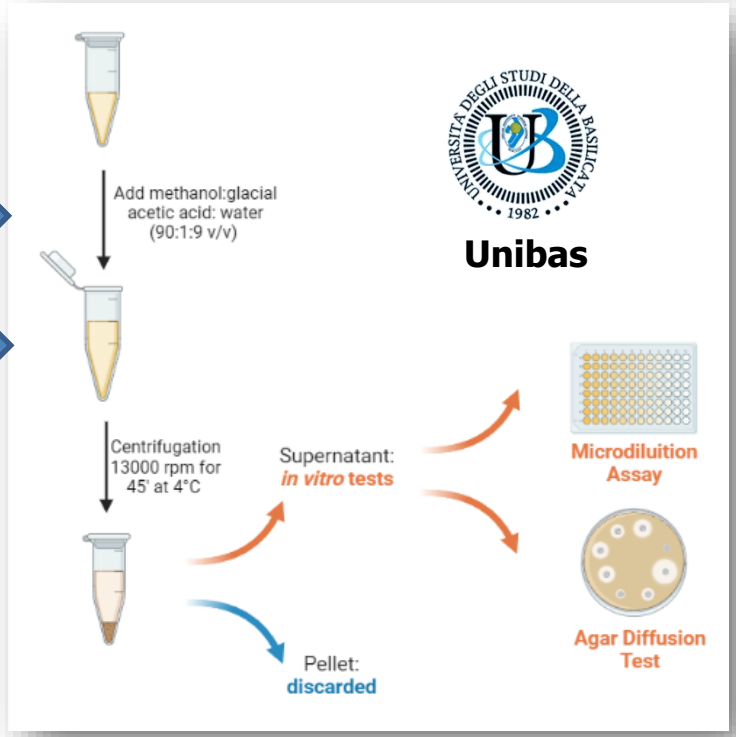
What has been done

Infection of larvae of *H. illucens* with gram+ and gram- bacteria and peptidic fraction extraction



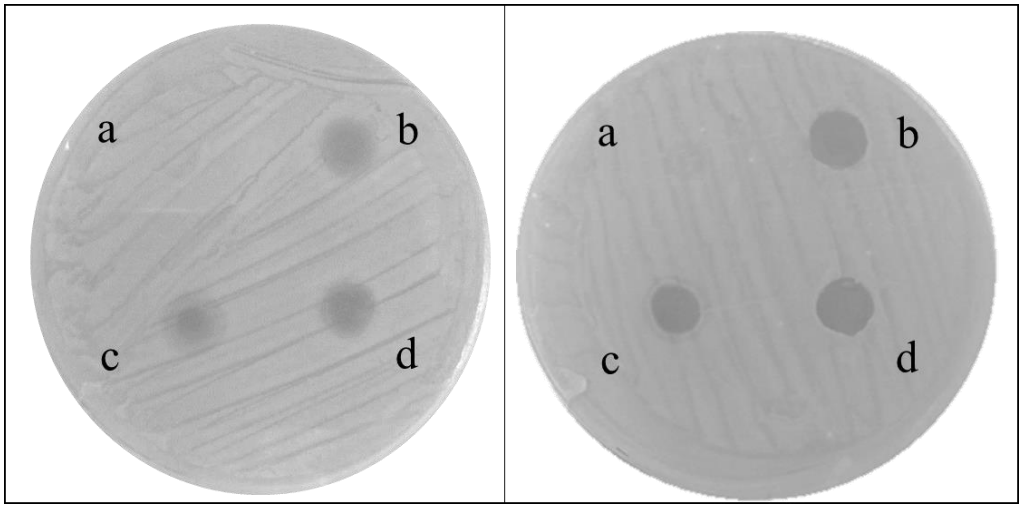


What has been done

Agar diffusion test

Unibas

***E. coli* Gram -**



***M. flavus* Gram +**

a) H_2O, negative control
b) peptide fraction from larvae infected with *E. coli*
c) peptide fraction from control larvae
d) peptide fraction from larvae infected with *M. flavus*

What has been done

Autobiography

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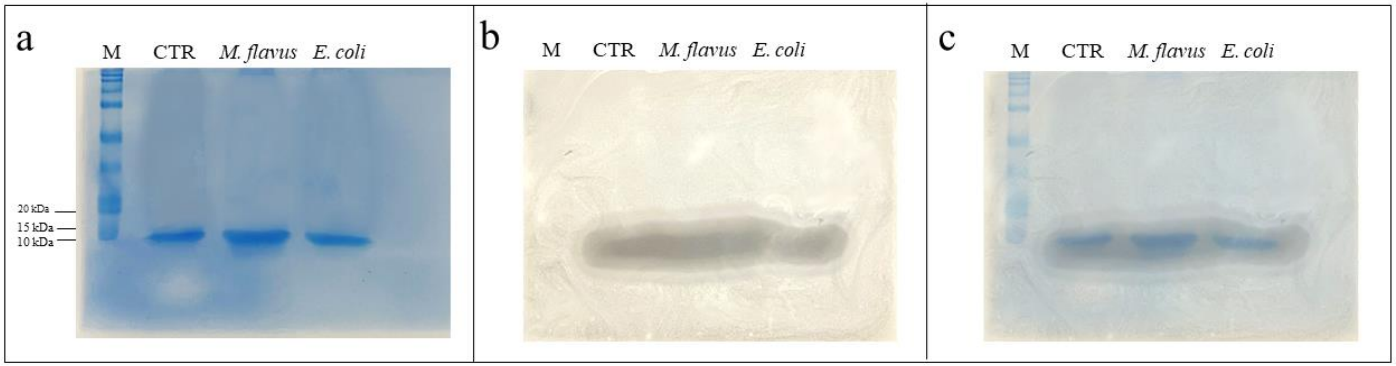
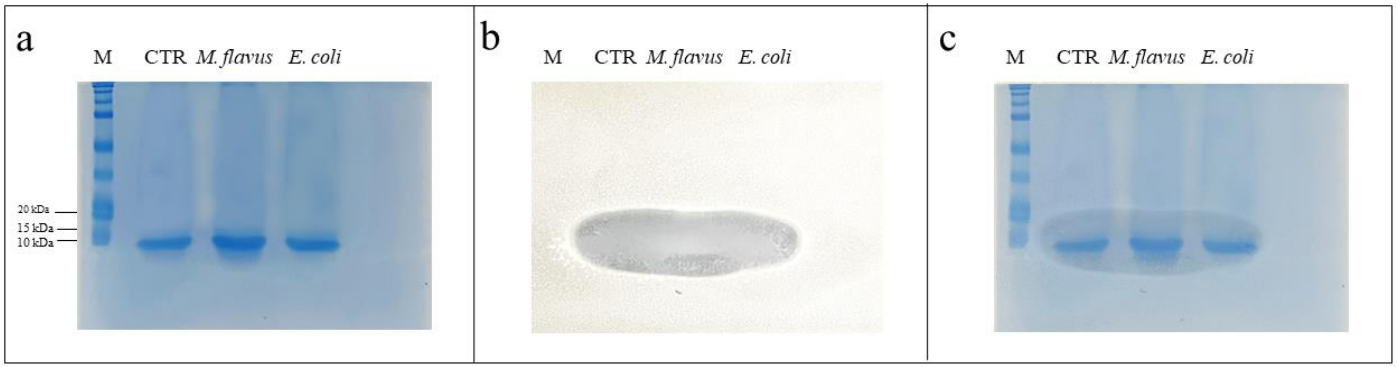
E. coli



M. flavus



a) SDS-PAGE
b) Autobiography
c) Overlap of the previous images

What has been done

Cell proliferation tests

- HT-29 (colorectal adenocarcinoma)
- HCT-116 (colorectal carcinoma)

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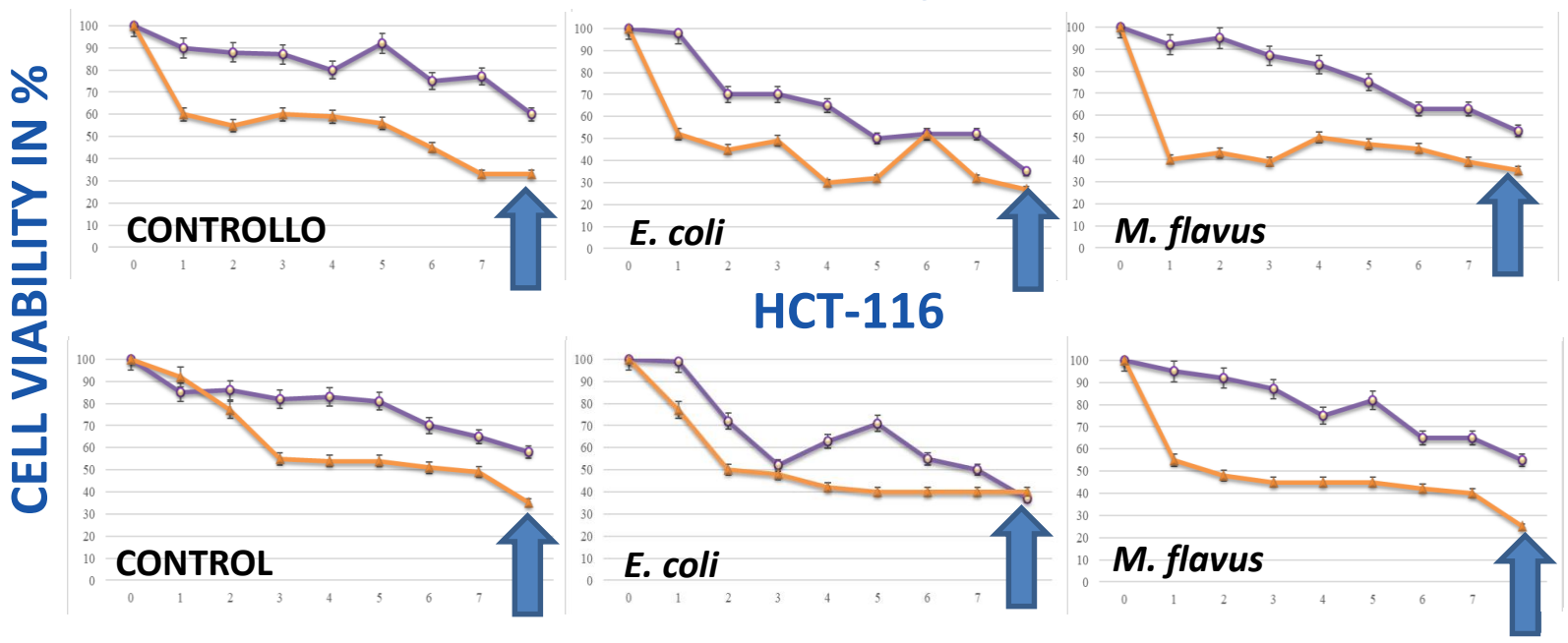

What has been done

New breeding of *Rhynchophorus ferrugineus* and selection of specimens for the *de novo* construction of the transcriptome

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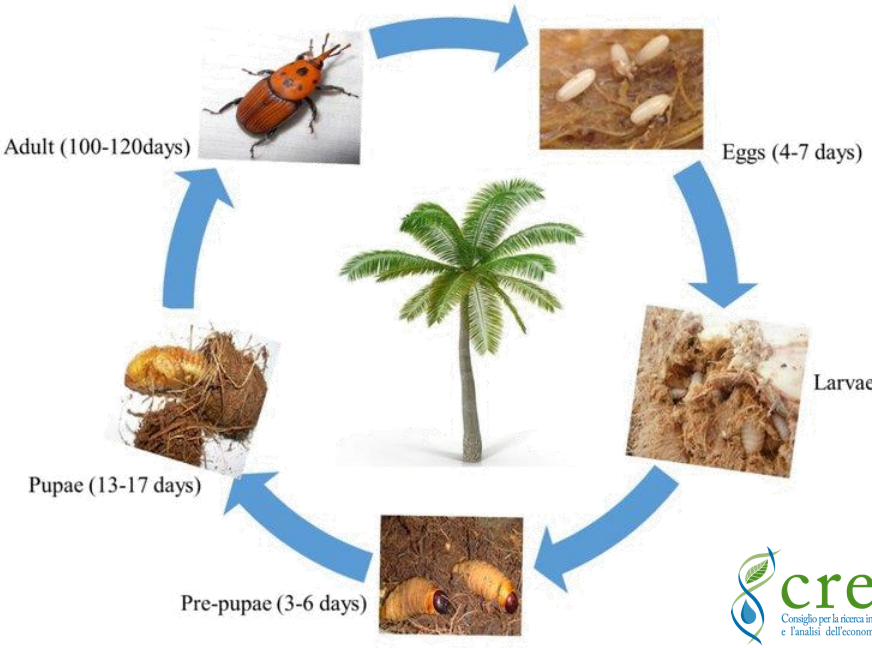
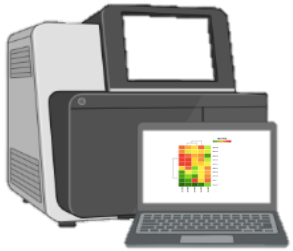


crea
Consiglio per la ricerca in agricoltura e l'analisi dell'economia agraria

COMBINED TRANSCRIPTOME OF LARVAE AND ADULTS

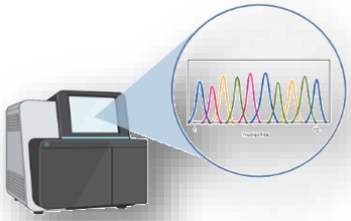


What has been done

De novo building of the combined transcriptome of larvae and adults of *R. ferrugineus*

RNA

cDNA



De novo building of the combined transcriptome

127.867 contigs

Functional annotation of the combined transcriptome

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What has been done

Bioinformatic analyzes of AMPs

616 sequences annotated as antimicrobial

433 antimicrobial
183 non antimicrobial

- 94 Putative antimicrobial activity
- 207 Putative antimicrobial + anticancer activity
- 67 Putative antimicrobial + antiviral activity
- 65 Putative antimicrobial + anticancer + antiviral activity
- 0 Putative antifungal activity

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Coordination and dissemination of results



Kick off meeting (24th of November 2021)



Coordination and dissemination of results

Video conference meetings to monitor the progress of activities, share protocols and results:

- 28th of January 2022
- 21st of March 2022
- 10th of May 2022
- 5th of July 2022
- 14th of September 2022
- 24th of November 2022
- 16th of January 2023

Coordination and dissemination of results

1st semester

Meetings:
- 28th of January 2022
- 21st of March 2022
- 10th of May 2022

→ **Sharing breeding protocols of *H. illucens* and *R. ferrugineus***

→ **Sharing protocols for transcript annotation of *H. illucens***

→ **Activity schedule**

2nd semester

Meetings:
- 5th of July 2022
- 14th of September 2022
- 24th of November 2022

→ **Sharing transcriptomic annotation and bioinformatic analysis results of *R. ferrugineus***

Coordination and dissemination of results

2nd semester

Meetings:
- 5th of July 2022
- 14th of September 2022
- 24th of November 2022

Sharing hemolymph extraction protocols from *H. illucens* larvae and antimicrobial analysis results

Sharing protocols and results anti-cancer analysis on hemolymph extracted from larvae of *H. illucens*

Activity schedule

Coordination and dissemination of results

Creazione di un sito web (https://www.aaa.safesos.com)

Publications

MINI-REVIEW ARTICLE

Tools in the Era of Multidrug Resistance in Bacteria: Applications for New Antimicrobial Peptides Discovery

Antonio Moretta[1,#], Carmen Scieuzo[1,2,#], Rosanna Salvia[1,2,#,*], Željko D. Popović[3,4], Alessandro Sgambato[5,6] and Patrizia Falabella[1,2,*]

[1]Department of Sciences, University of Basilicata, Via dell'Ateneo Lucano 10, 85100, Potenza, Italy; [2]Spinoff XFlies s.r.l, University of Basilicata, Via dell'Ateneo Lucano 10, 85100, Potenza, Italy; [3]Department of Biology and Ecology, Faculty of Sciences, University of Novi Sad, Trg Dositeja Obradovića 2, 21000 Novi Sad, Serbia; [4]GenoLab, Molecular Diagnostic Laboratory, Kosovska 7, 21000 Novi Sad, Serbia; [5]Department of Translational Medicine and Surgery, Università Cattolica del Sacro Cuore, Rome, Italy; [6]Centro di Riferimento Oncologico della Basilicata (IRCCS-CROB), Rionero in Vulture, Italy

Abstract: Antimicrobial peptides (AMPs) are small molecules belonging to innate immunity that act against

ARTICLE HISTORY

Received: April 04, 2022
Accepted: June 14, 2022

DOI:
10.2174/138161282866220817163335

insects

MDPI

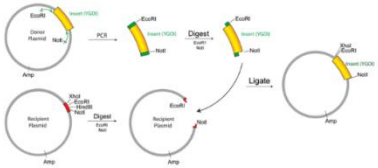
Article

In vitro evaluation of the antibacterial activity of the peptide fractions extracted from the hemolymph of *Hermetia illucens* (Diptera: Stratiomyidae)

Ongoing and future activities

In person meetings with partners

Recombinant production and/or chemical synthesis and purification of 3 antimicrobial peptides of *H. illucens*



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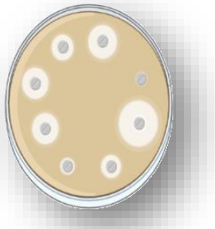
Functional characterization *in vitro* of antibacterial, antiviral and anticancer activity of produced AMPs of *H. illucens*



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Ongoing and future activities

Reanalysis of sequences in the transcriptome of larvae and adults of *R. ferrugineus*

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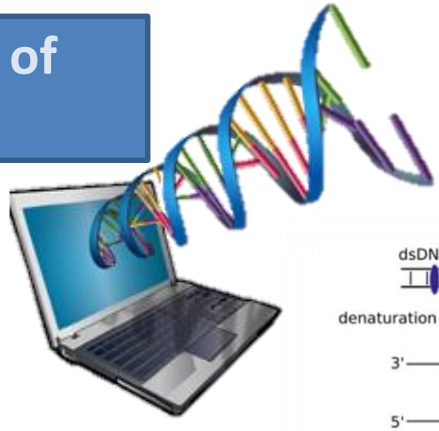

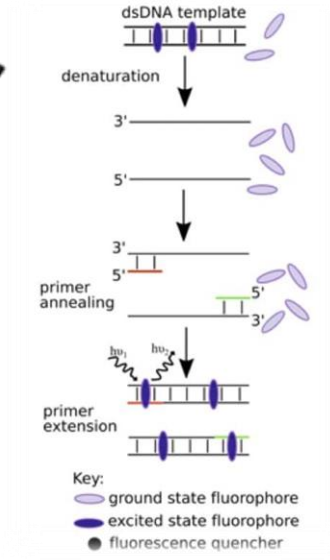

qPCR of 5 AMPs of *R. ferrugineus* larvae stimulated and unstimulated

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Uni Catania

Future dissemination activities

REGIONE BASILICATA

Department of Health and Personal Policies

Subjects to involve

- Thematic seminars
- Meetings
- Workshops

- Pharmaceutical industries
- Research structures (public and private)
- Public entities

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Thank You For Your Attention

Unibas | Uni Novi Sad | Uni Catania | IRCCS Crob | UniGiessen | Uni Ioannina | REGIONE BASILICATA