

# 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

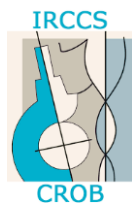


## AAA:SAFE SOS

## PARTNER

### Lead Partner

University of Basilicata - **Referent prof. Patrizia Falabella**



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**

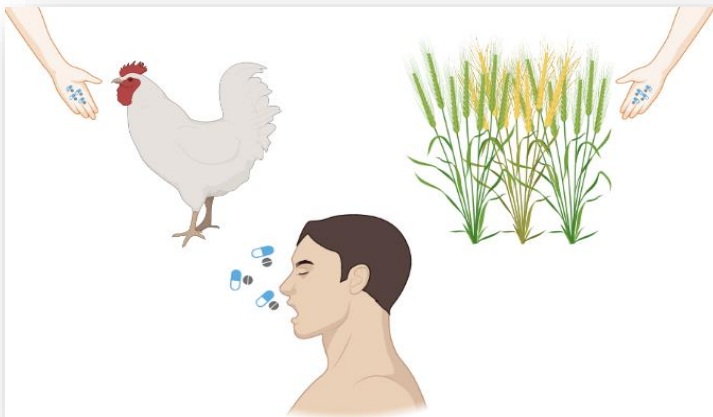


Basilicata Region - Department of Health and Personal Policies

## AAA:SAFE SOS

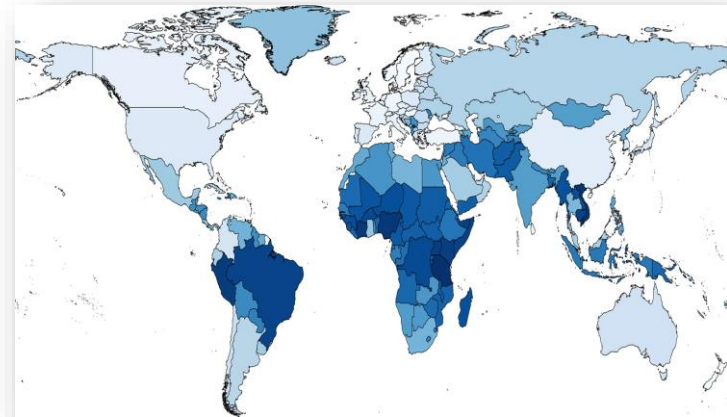
# Antimicrobial Resistance (AMR)

## Antibiotic Resistance



Created by Biorender.com

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



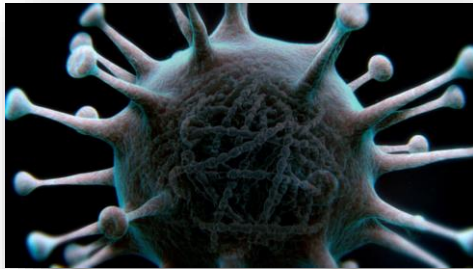
Hendriksen *et al.* 2019. Nature Comm, 10: 1–12

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



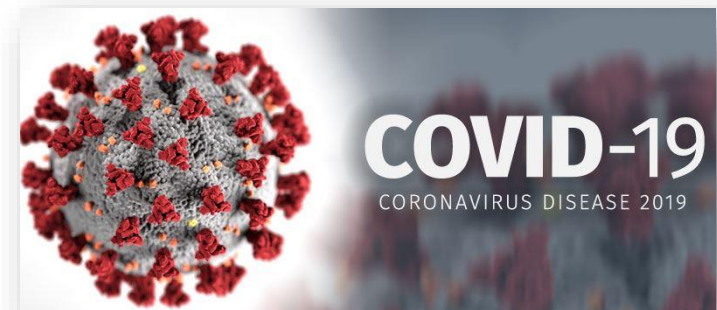
# Antimicrobial Resistance (AMR)

## Antiviral Resistance



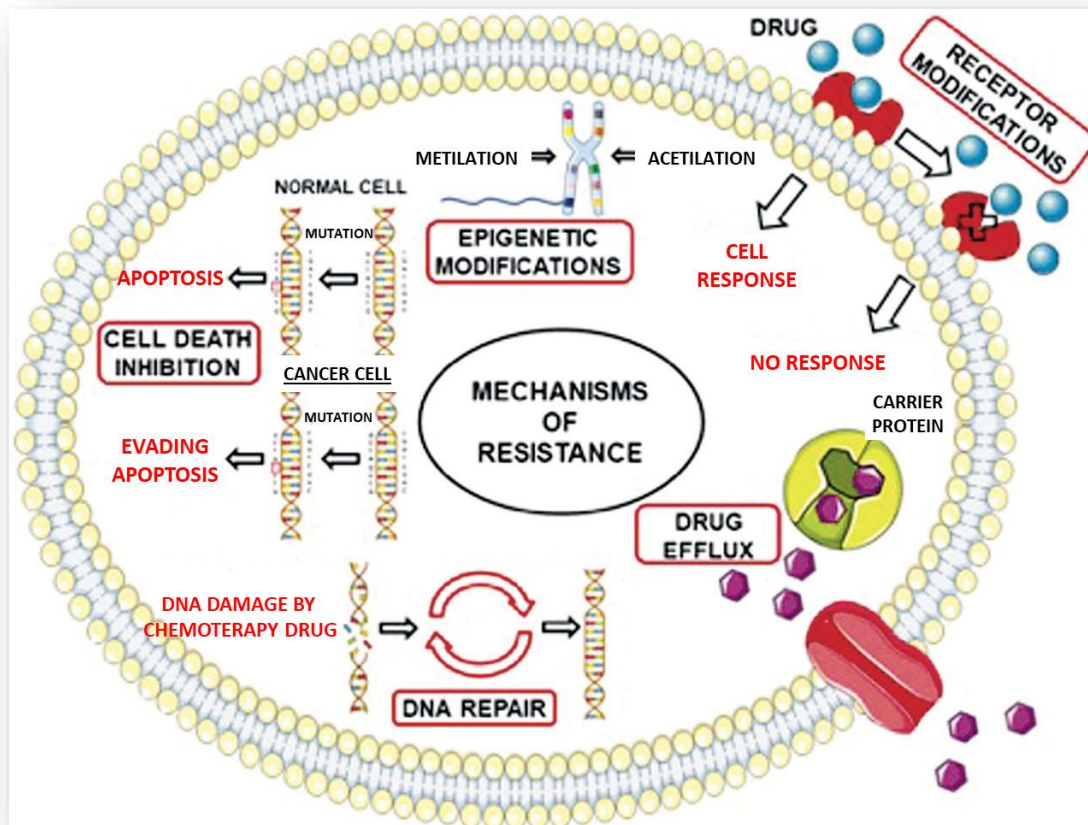
Due to a high constitutive mutability, viruses can increase the infection basin, becoming dangerous also for other species, including humans.

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





# Non-specificity and resistance to chemotherapy drugs

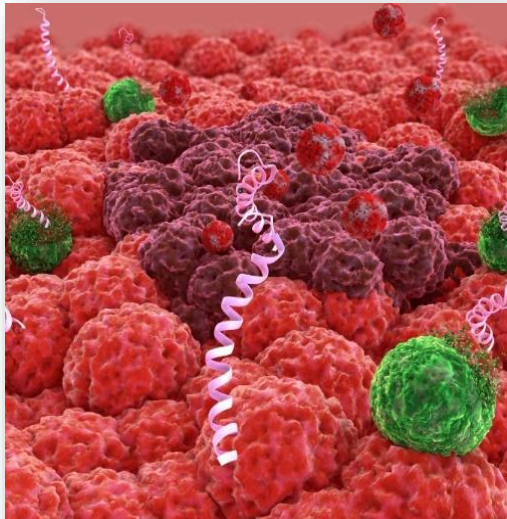


Side effects caused by damage to healthy cells and tissues during the administration of anticancer chemotherapy and resistance to this therapy.



# 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?

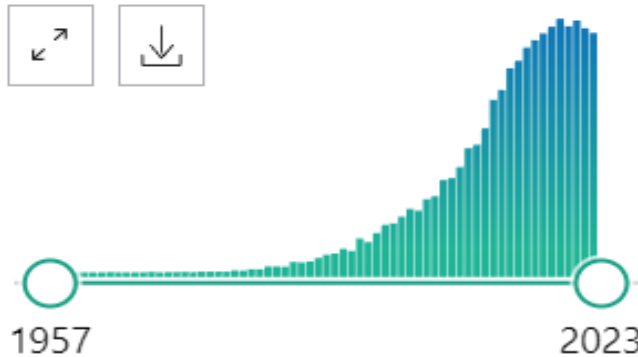
AMPs are able to overcome antimicrobial resistance and have a broad-spectrum action with low toxicity to the host.

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

## RESULTS BY YEAR



STUDIES ON A BACTERICIDAL AGENT EXTRACTED FROM A SOIL BACILLUS  
I. PREPARATION OF THE AGENT. ITS ACTIVITY IN VITRO  
By RENE 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 lyses 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 lyses 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

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



Dubos, 1939. J Experim Med

Discovered for the first time in 1939

# AMP Applications



**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



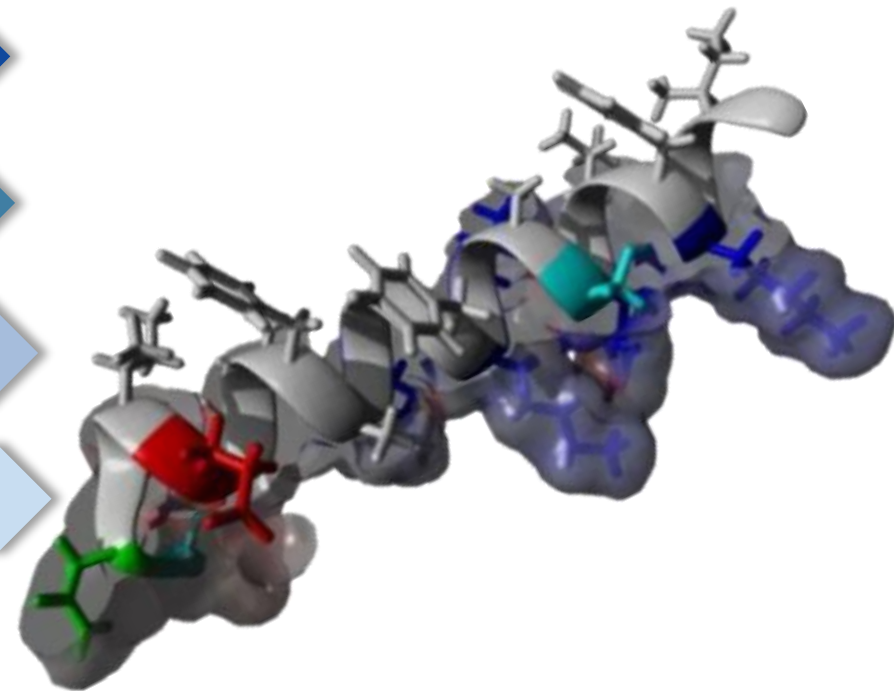
AMPs share some common characteristics:

12-50 amino acids

Positively charged  
(most common)

Amphipathic structure

Negatively charged  
(less common)



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

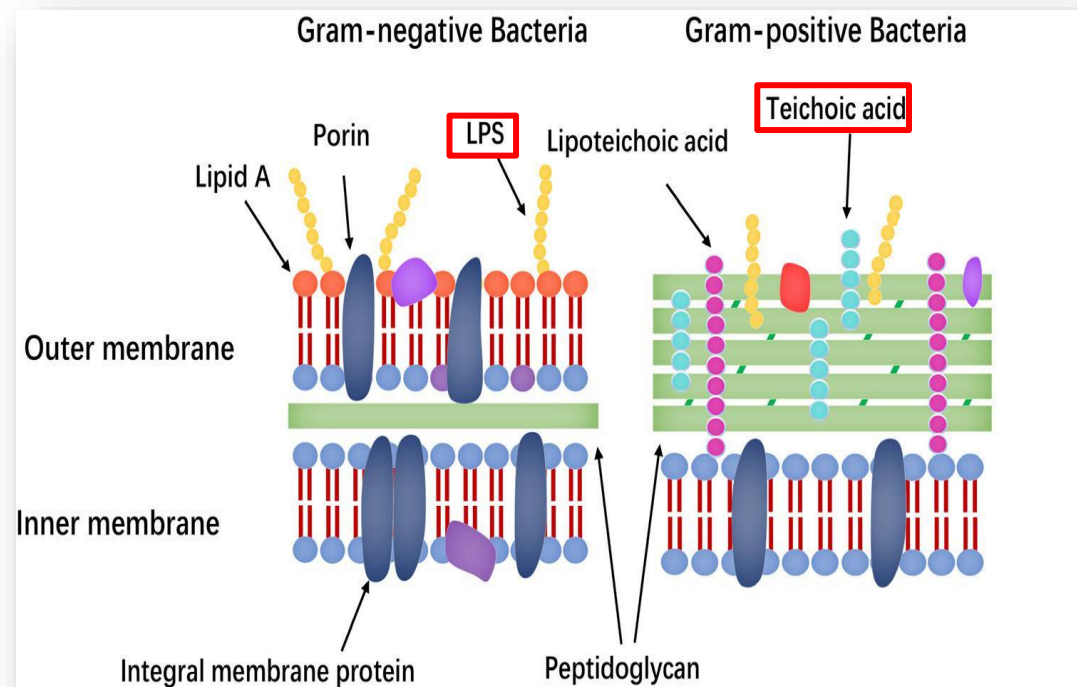


# Antimicrobial Peptide (AMP) Action Mechanism



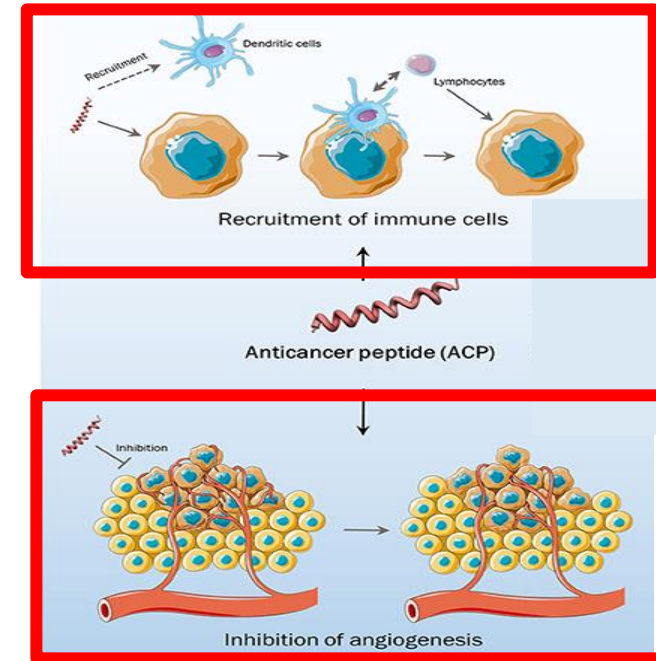
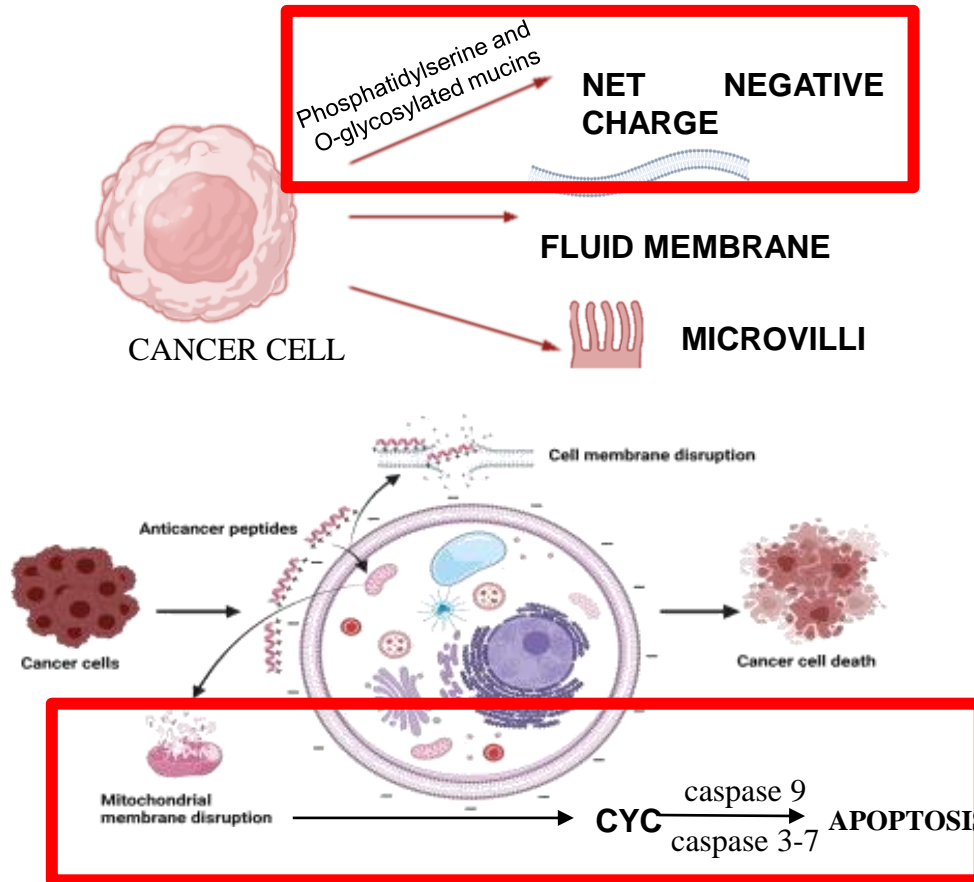
**Membranolytic:**  
destabilize bacterial  
membrane causing their  
disruption

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



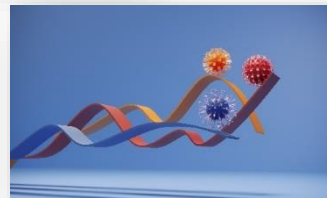
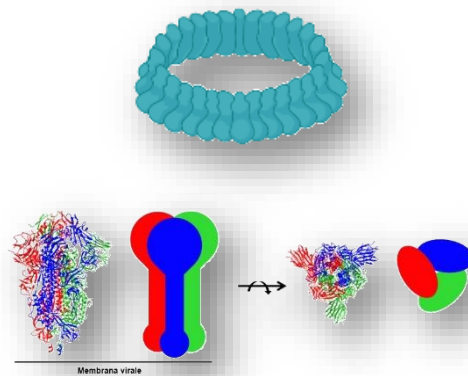
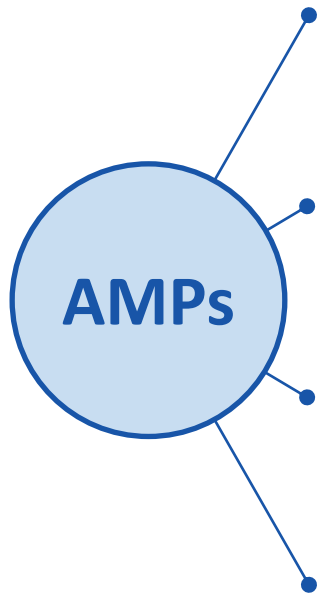


# Anticancer Peptide (ACP) Action Mechanism



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

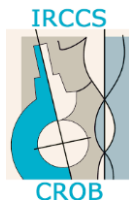


Hsieh e Hartshorn, 2016. *Pharmaceuticals*. 9:53; Ding *et al.*, 2009. *J. Innate Immun*, 1:413–420. Penberthy *et al.*, 2011. *Cell Mol Life Sci*. 68:2231-42.; Bergman *et al.*, 2007. *Curr HIV Res*, 5:410–415. Ahmed *et al.*, 2019. *Viruses*, 1:E704.

# AAA SAFE-SOS

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



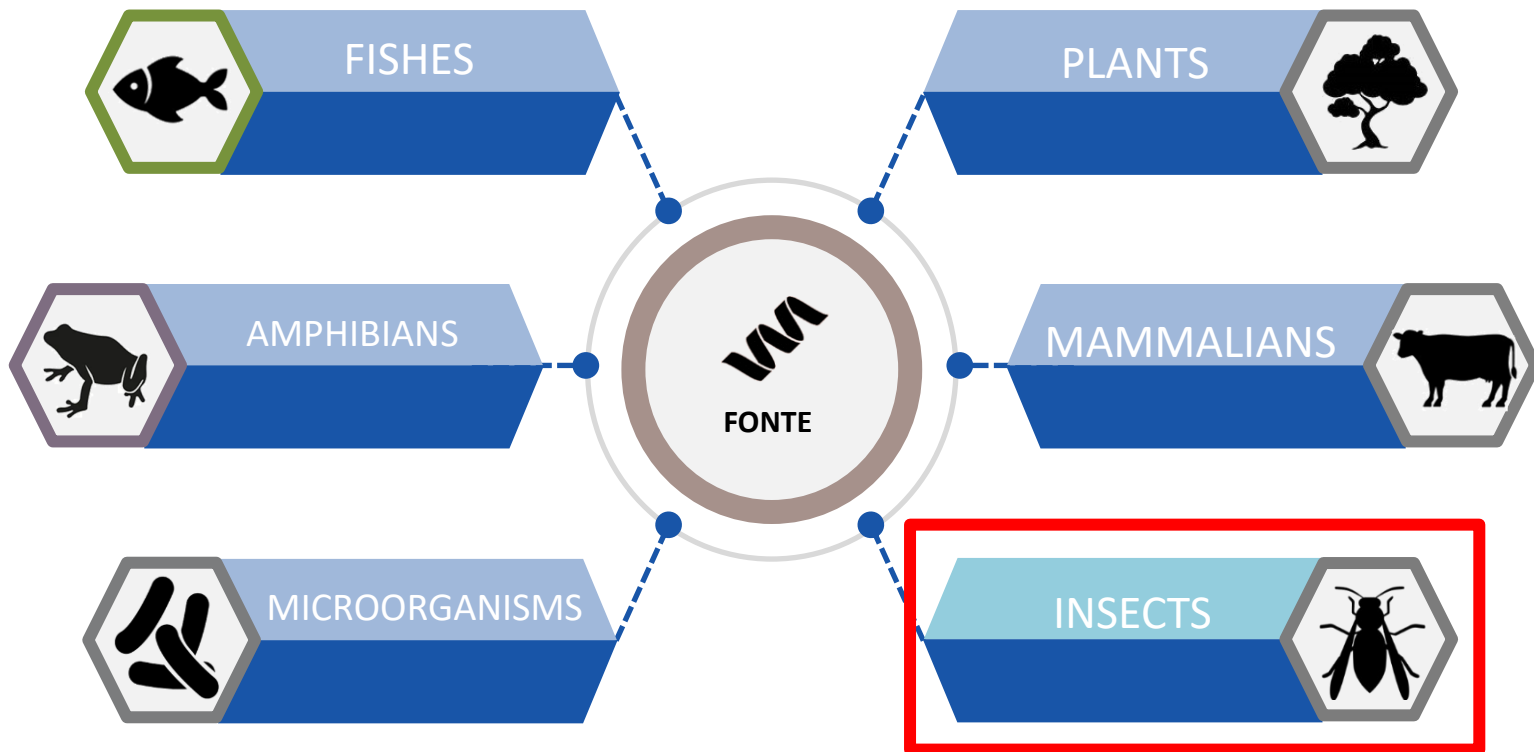
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# AMP Sources

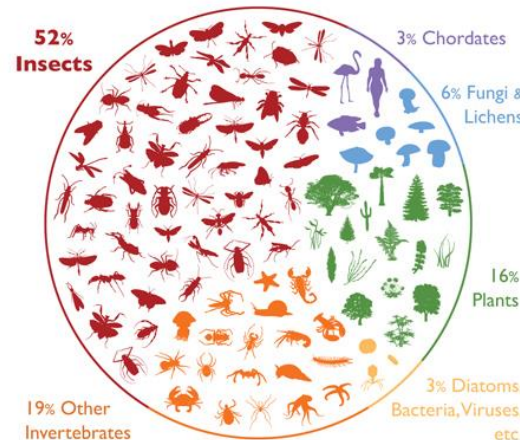


Huan *et al.*, 2020. *Front Microbiol.* 11:582779

# Insects and Biomimetic



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

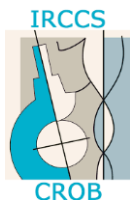


Innovative and inexhaustible source of **USEFUL MOLECULES (R&D)**

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)

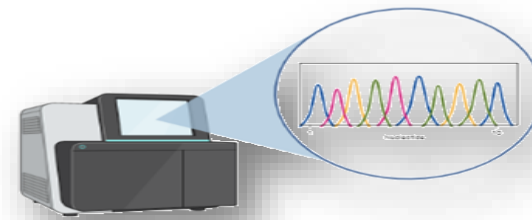
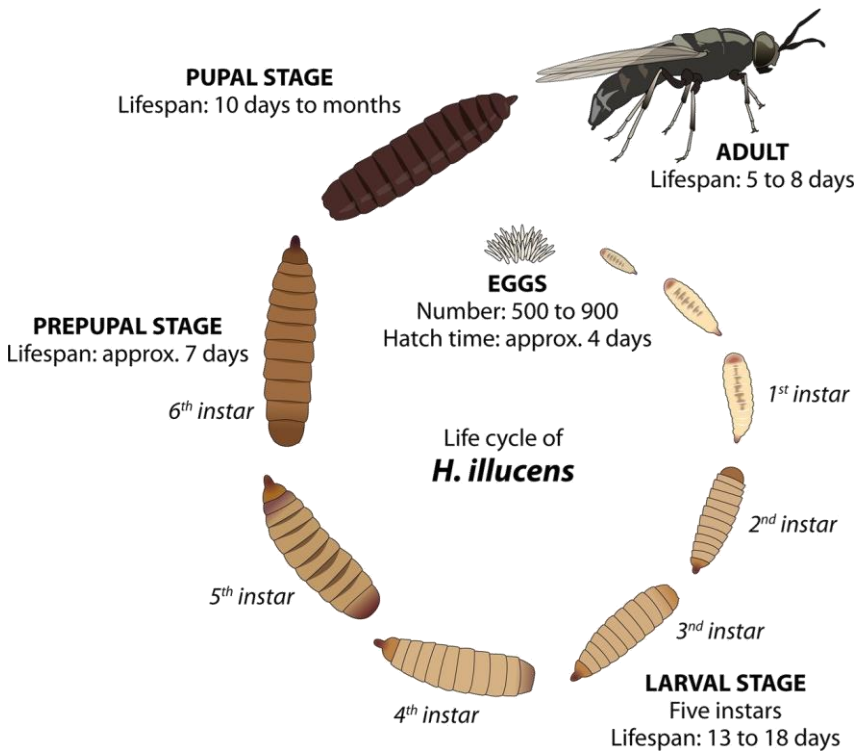




# What has been done

## *Hermetia illucens* breeding

## Reannotation of the transcriptome



34 putative AMPs



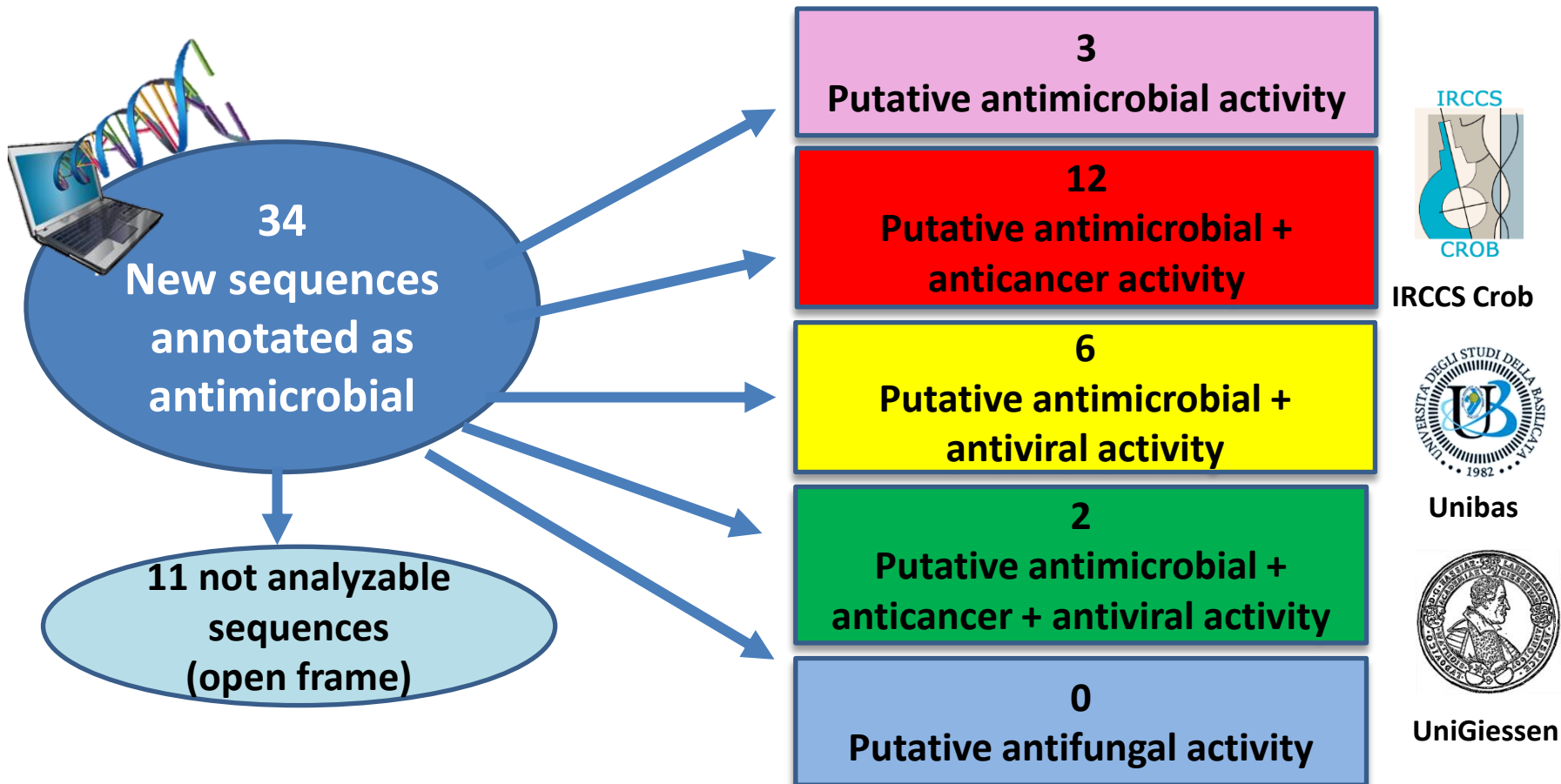
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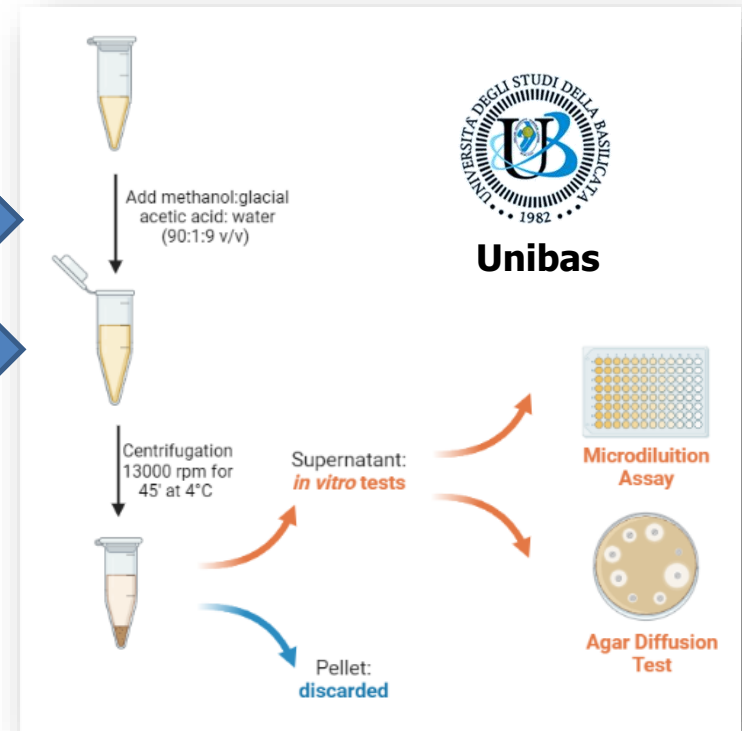
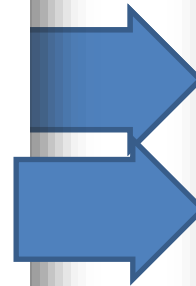
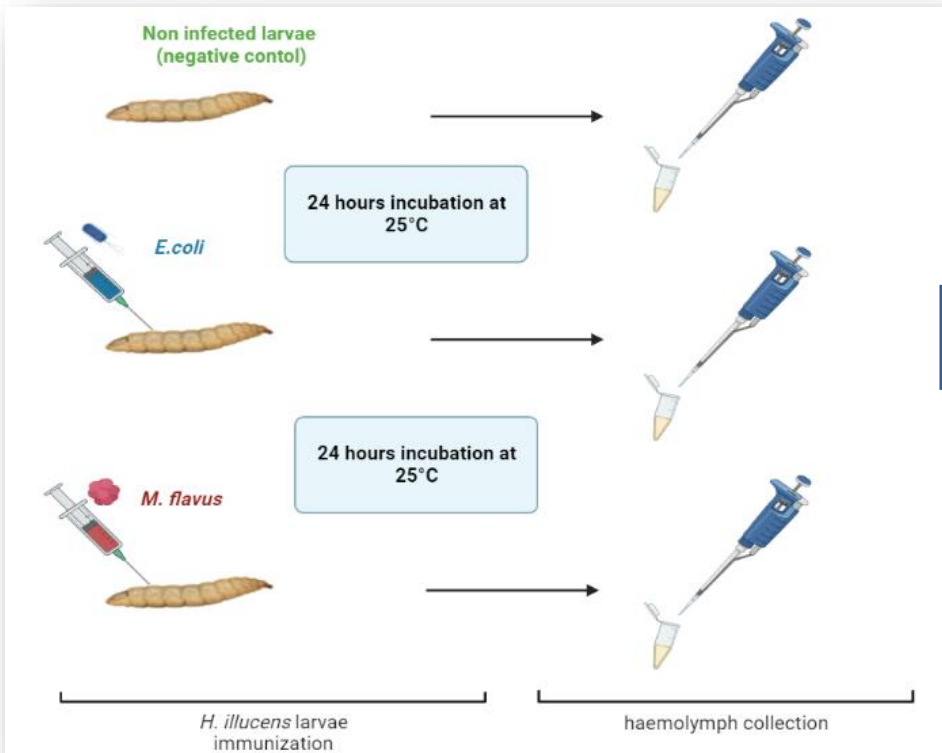
# What has been done

## Bioinformatic analyzes of new AMPs



# 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  
Autobiography

*E. coli*

*M. flavus*

ANTIMICROBIAL  
ACTIVITY  
BY ALL THE TESTED  
SAMPLES  
(hemolymph from  
control larvae,  
stimulated with *E.  
coli*, stimulated with  
*M. flavus*)



Unibas

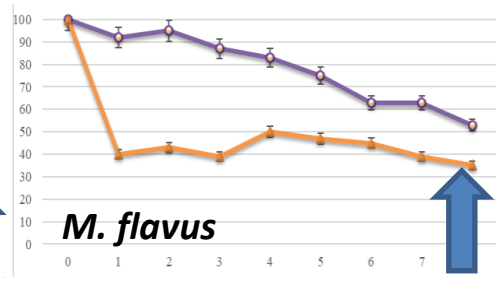
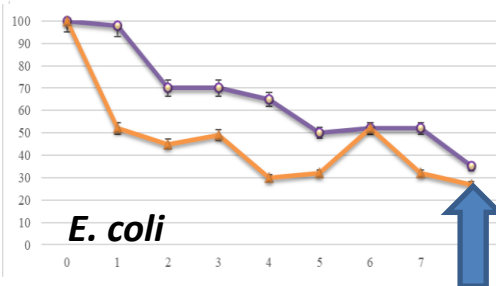
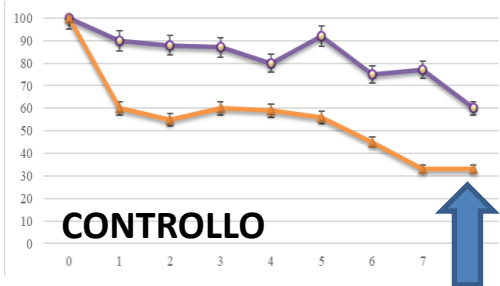
IRCCS Crob

# What has been done

## Cell proliferation tests

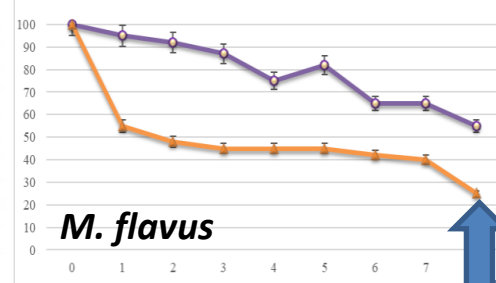
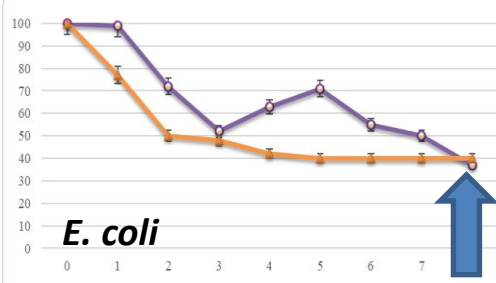
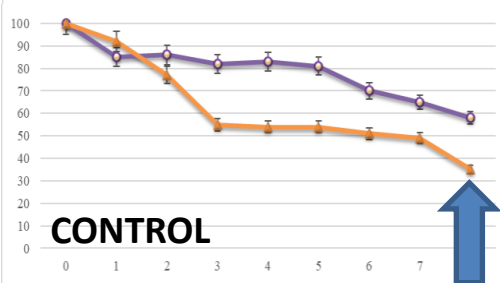
- HT-29 (colorectal adenocarcinoma)
- HCT-116 (colorectal carcinoma)

### HT-29



Legend:  
■ Total hemolymph  
■ Peptide fraction

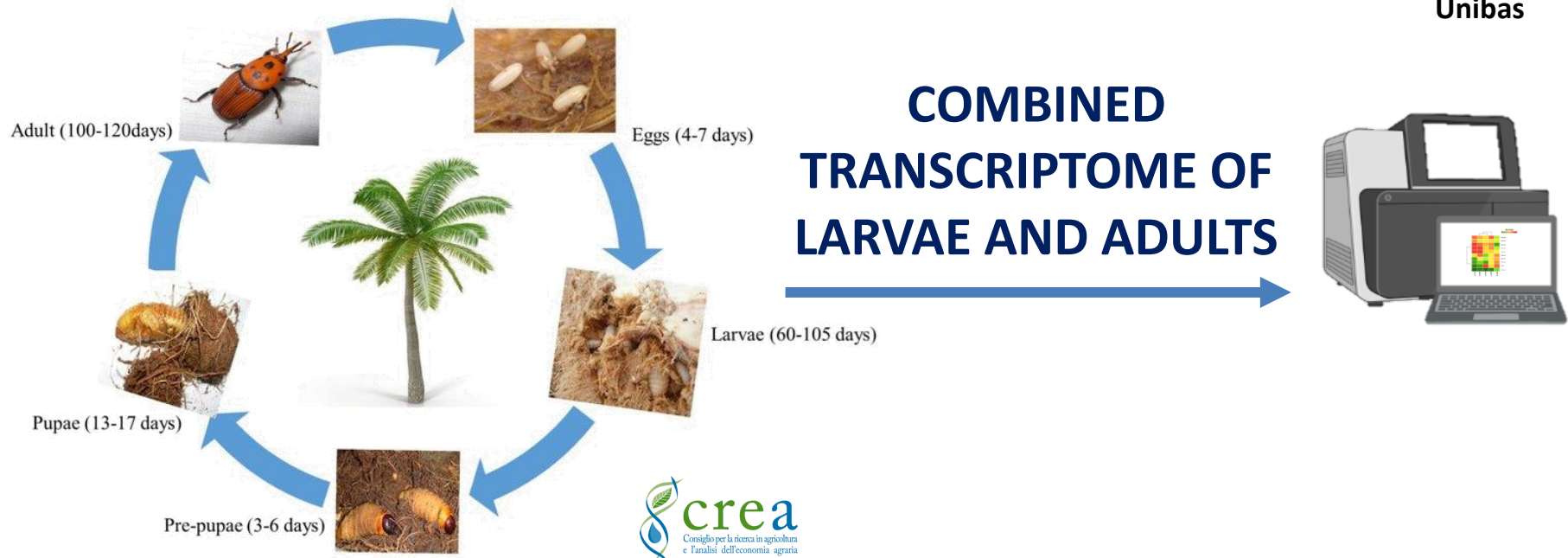
### HCT-116



Serial dilutions from 1:6400 (1) to 1:50 (8)

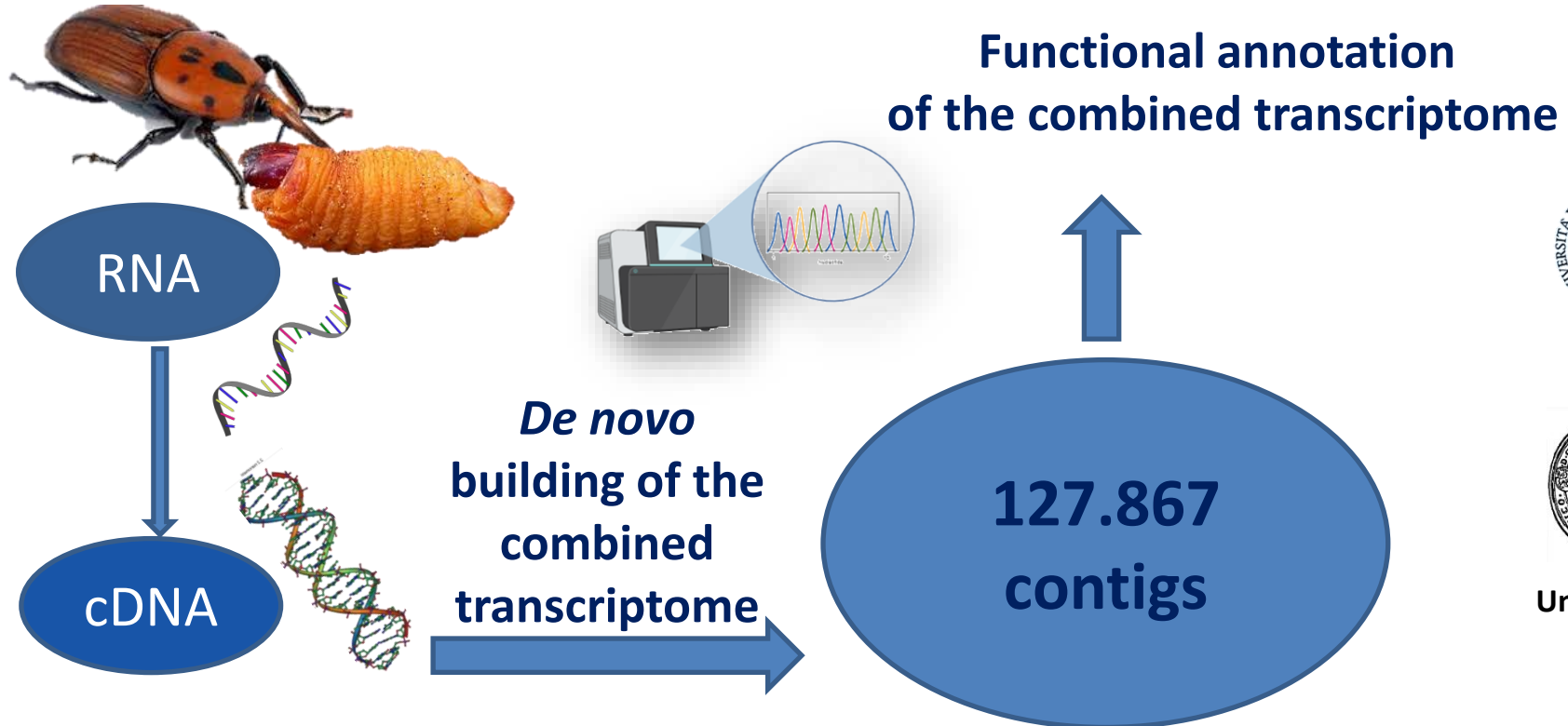
# What has been done

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

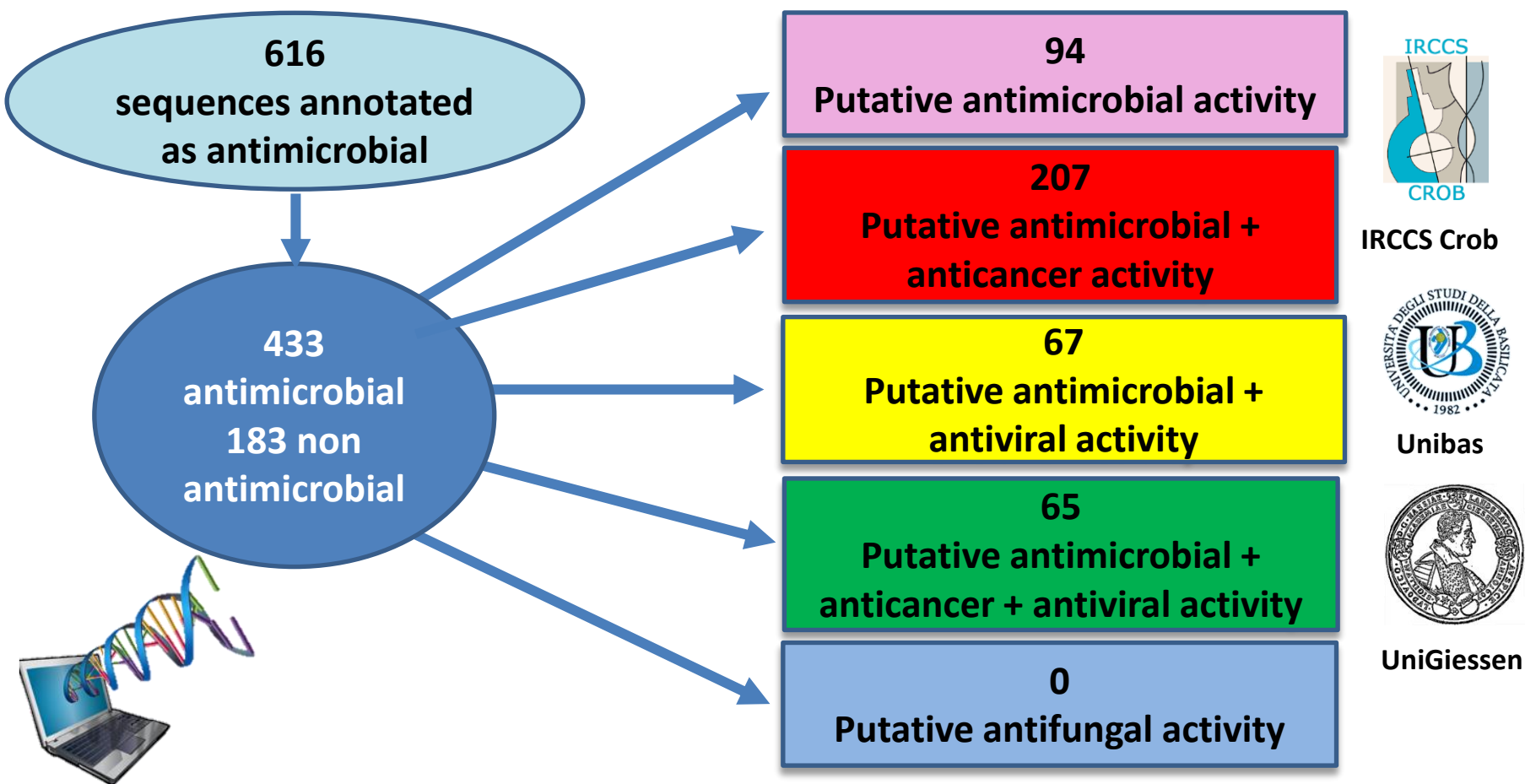


# What has been done

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

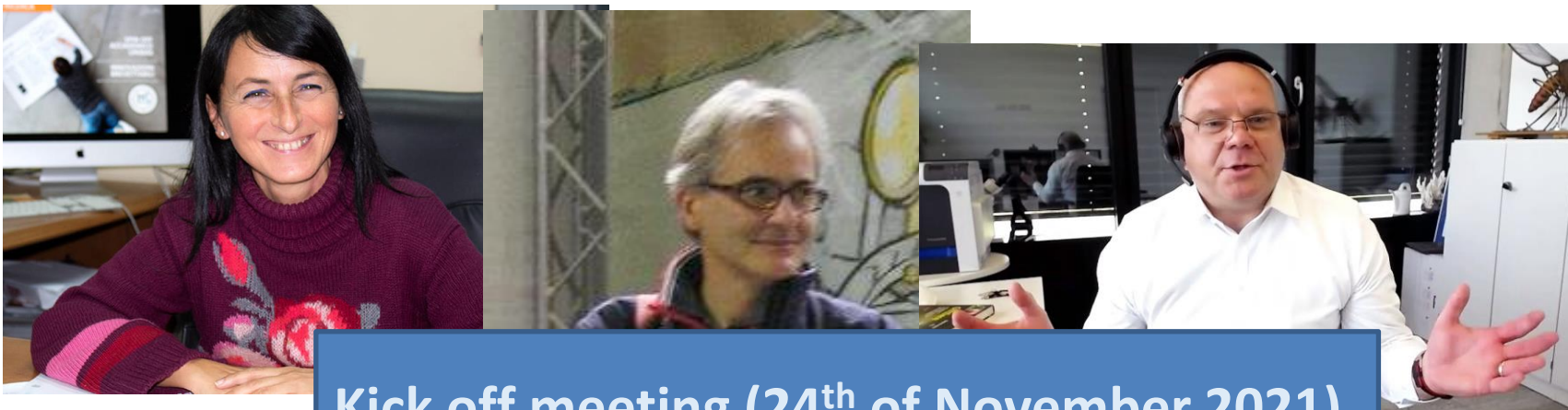


# What has been done Bioinformatic analyzes of AMPs

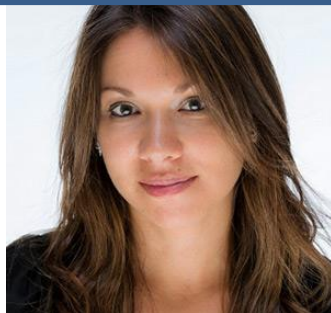




# Coordination and dissemination of results



Kick off meeting (24<sup>th</sup> of November 2021)



# Coordination and dissemination of results

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

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



# Coordination and dissemination of results

## 1<sup>st</sup> semester

### Meetings:

- 28<sup>th</sup> of January 2022
- 21<sup>st</sup> of March 2022
- 10<sup>th</sup> of May 2022



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



Sharing protocols for transcript annotation of *H. illucens*



Activity schedule

## 2<sup>nd</sup> semester

### Meetings:

- 5<sup>th</sup> of July 2022
- 14<sup>th</sup> of September 2022
- 24<sup>th</sup> of November 2022



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



## Coordination and dissemination of results

2<sup>nd</sup> semester

### Meetings:

- 5<sup>th</sup> of July 2022
- 14<sup>th</sup> of September 2022
- 24<sup>th</sup> 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>)

AAA: SAFE SOS

Home page Menu

Benvenuti sul sito del progetto AAA: SAFE SOS!

"Nuove molecole ad attività antimicrobica, anticancro ed antivirale: soluzioni alternative a fronte di tre tra le principali emergenze socio-sanitarie".

Visualizza mappa più grande

Via dell'Ateneo  
Lucano, 10, 85100...

Sileo S.r.l.

Università degli Studi

Via dei Mille

La presente proposta progettuale propone l'impiego di peptidi antimicrobici derivati da insetti come nuova categoria di farmaci sicuri ed efficaci, tali da offrire, per le loro peculiari caratteristiche, soluzioni terapeutiche innovative per la salvaguardia della salute umana.

# Publications

## MINI-REVIEW ARTICLE

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

Antonio Moretta<sup>1,#</sup>, Carmen Scieuzo<sup>1,2,#</sup>, Rosanna Salvia<sup>1,2,#,\*</sup>, Željko D. Popović<sup>3,4</sup>, Alessandro Sgambato<sup>5,6</sup> and Patrizia Falabella<sup>1,2,\*</sup>

<sup>1</sup>Department of Sciences, University of Basilicata, Via dell'Ateneo Lucano 10, 85100, Potenza, Italy; <sup>2</sup>Spinoff XFlies s.r.l, University of Basilicata, Via dell'Ateneo Lucano 10, 85100, Potenza, Italy; <sup>3</sup>Department of Biology and Ecology, Faculty of Sciences, University of Novi Sad, Trg Dositeja Obradovića 2, 21000 Novi Sad, Serbia; <sup>4</sup>GenoLab, Molecular Diagnostic Laboratory, Kosovska 7, 21000 Novi Sad, Serbia; <sup>5</sup>Department of Translational Medicine and Surgery, Università Cattolica del Sacro Cuore, Rome, Italy; <sup>6</sup>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:  
10.2174/1381612828666220817163335



Article

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

1  
2  
3  
4

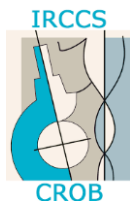
# Ongoing and future activities

In person meetings with partners

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



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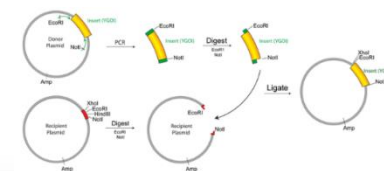


Uni Novi Sad



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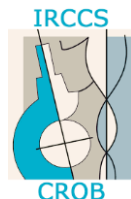
Uni Ioannina



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



Unibas

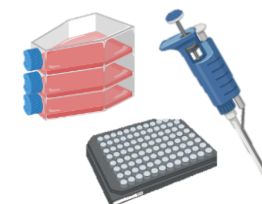
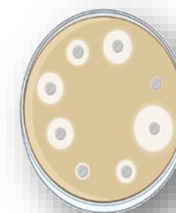


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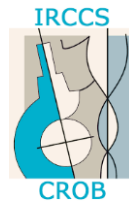


# Ongoing and future activities

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



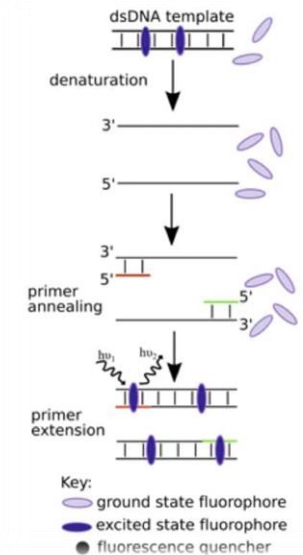
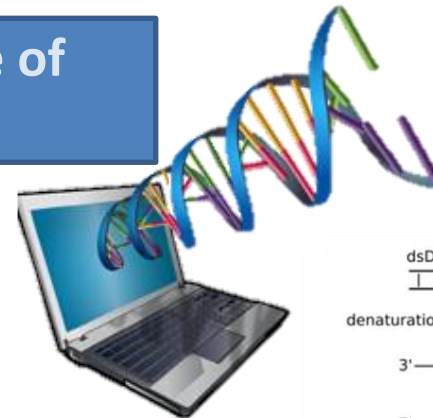
Unibas



IRCCS CroB



UniGiessen



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



Unibas



Uni Novi Sad

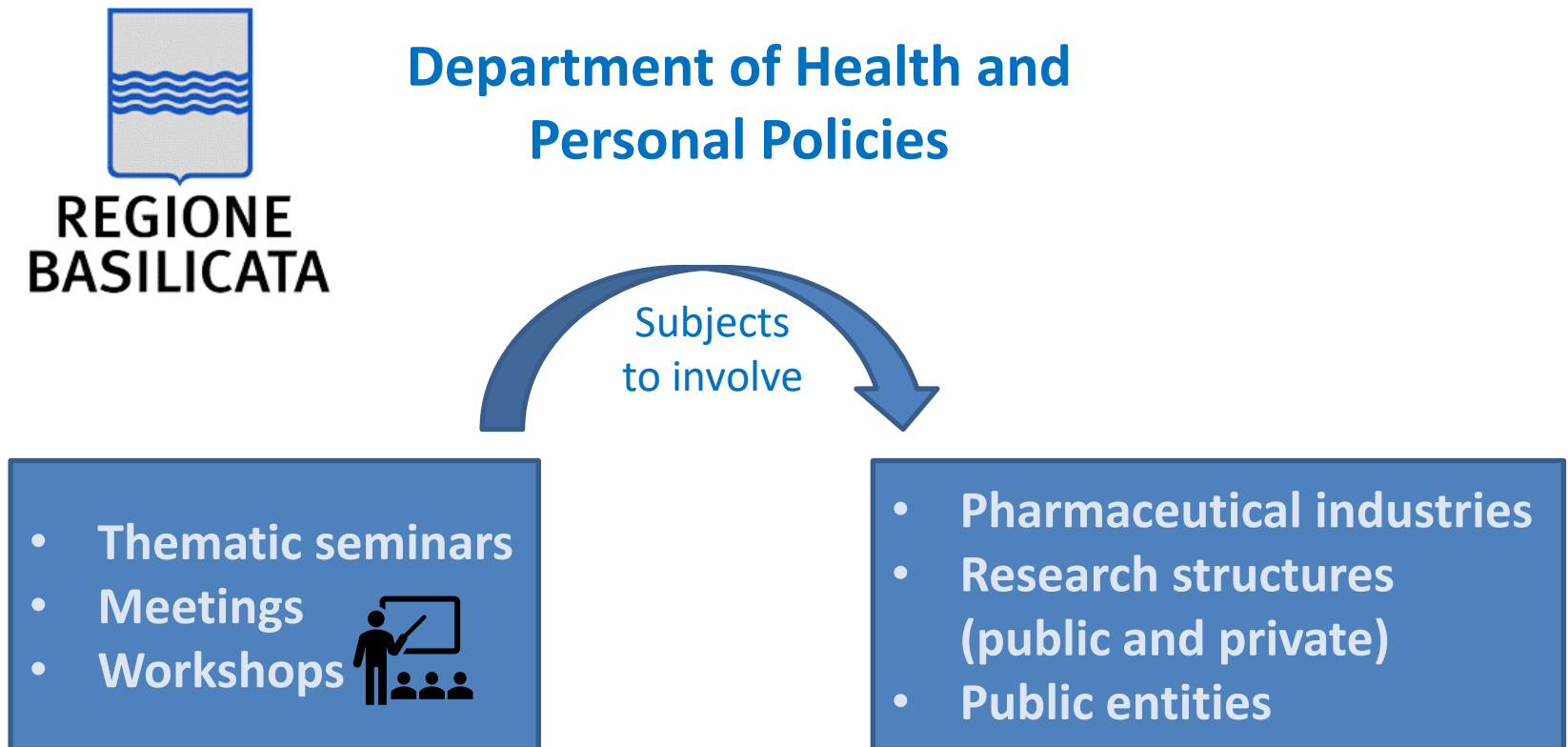


Università di Catania

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# Future dissemination activities



## AAA SAFE-SOS

# *Thank You For Your Attention*



**Unibas**

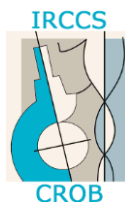


**Uni Novi Sad**



**Uni Catania**

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**IRCCS Crob**



**UniGiessen**



**Uni Ioannina**

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