UNIVERSITÀ DEGLI STUDI DI MILANO
DIPARTIMENTO DI BIOSCIENZE

Piccoli peptidi: nuovi prodotti per la difesa delle colture

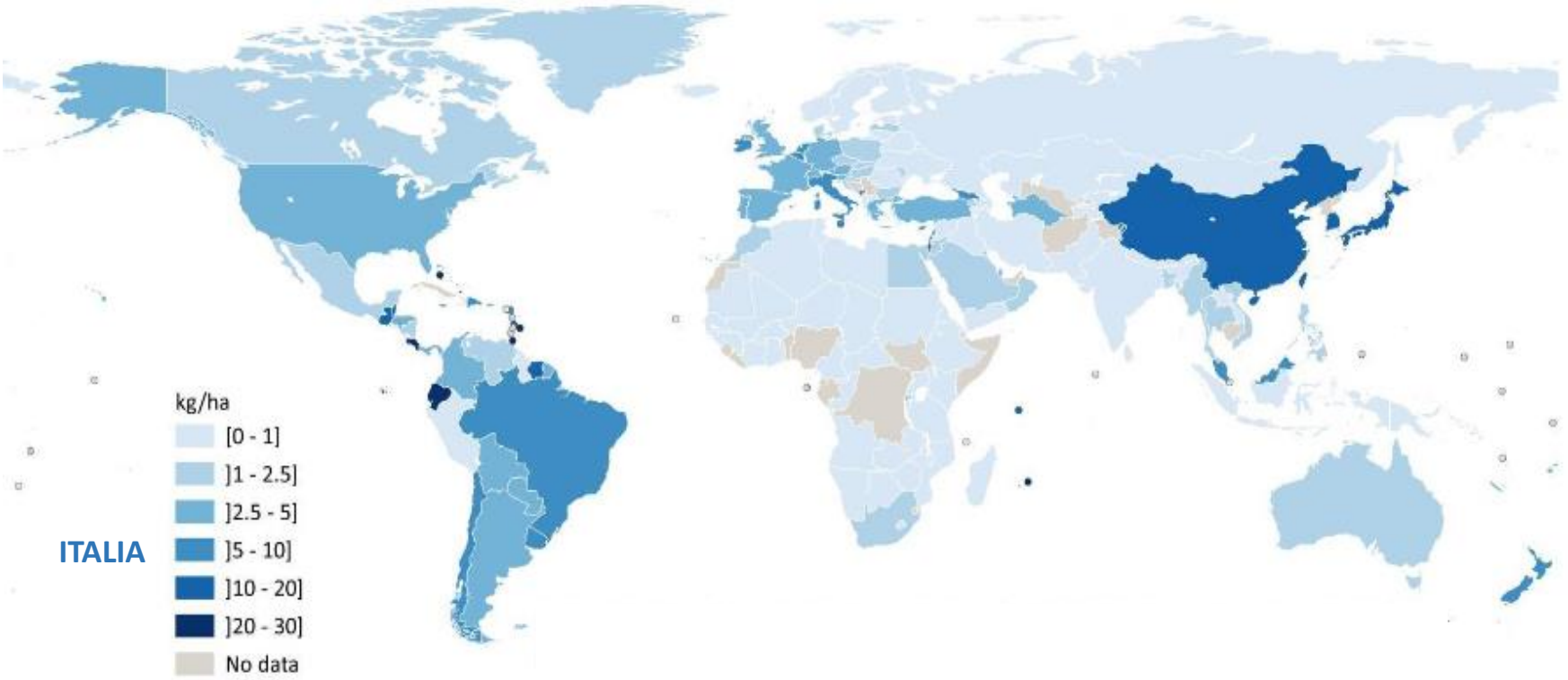
Simona Masiero

The workers

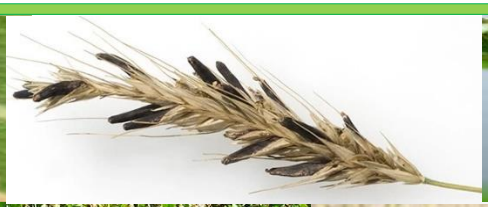
The European green deal

PPPs: prodotti per la protezione delle piante

Uso dei pesticidi (2018)



Abbiamo bisogno di PPPs?

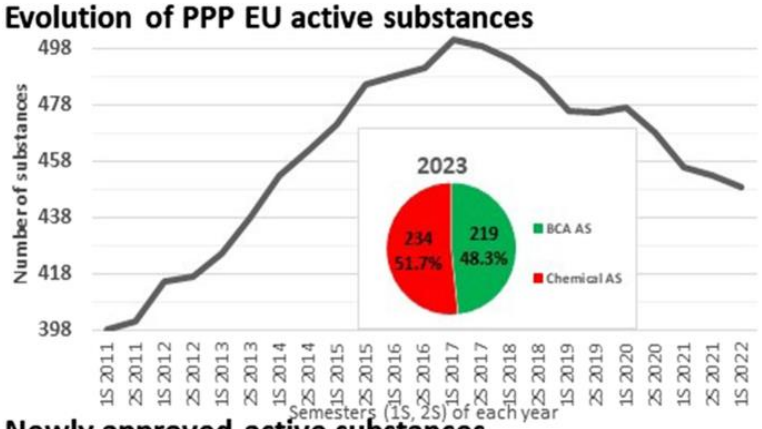



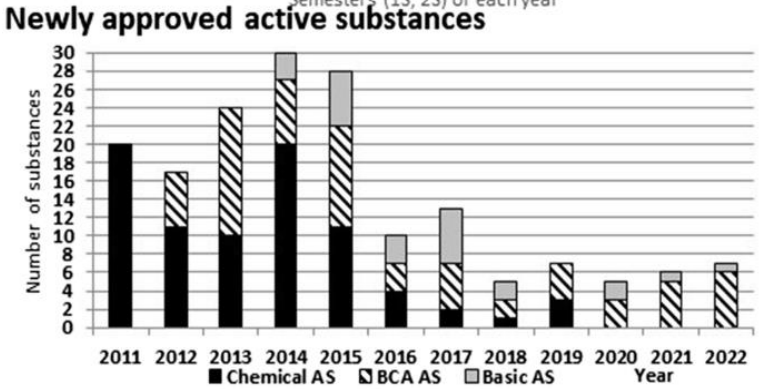


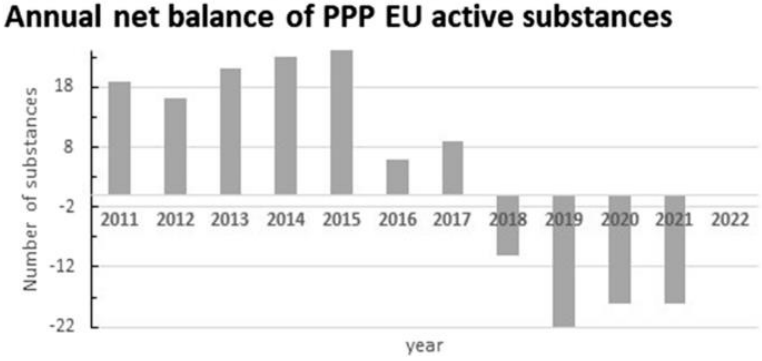




La viticoltura ha bisogno di PPPs!







CfS: Candidates for Substitutions

circa 77 dei 440 PPP approvati in EU

Prodotti rameosi
Difenoconazole
Dimethomorph
Epoxiconazolo
Metalxyl

Mancozeb (ormai vietato)

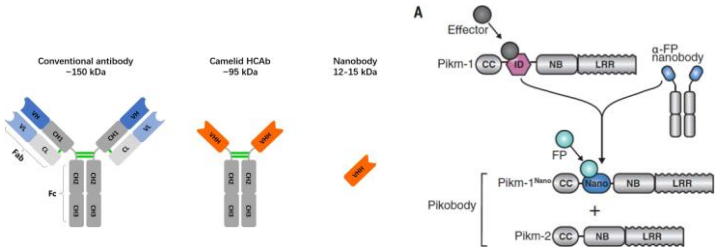
L’Europa ha bisogno di nuovi PPPs

Sostanze a basso rischio (Low PPPS)

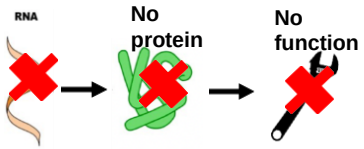
L'Europa ha bisogno di nuovi PPPs

Approcci biotecnologici

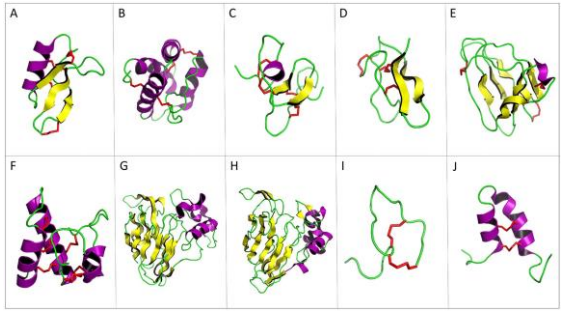
- **nanobodies/picobodies**



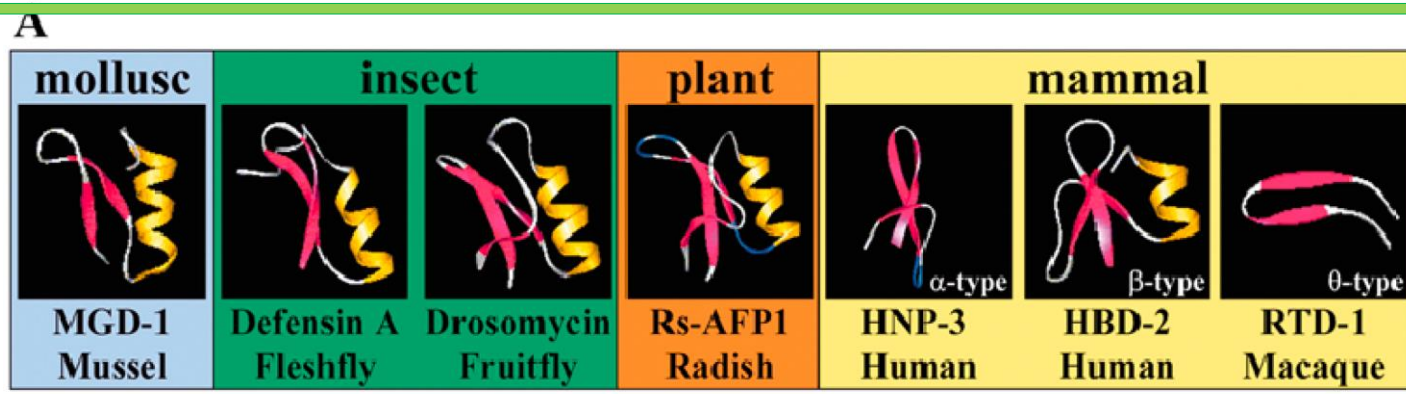
- **RNA interference**

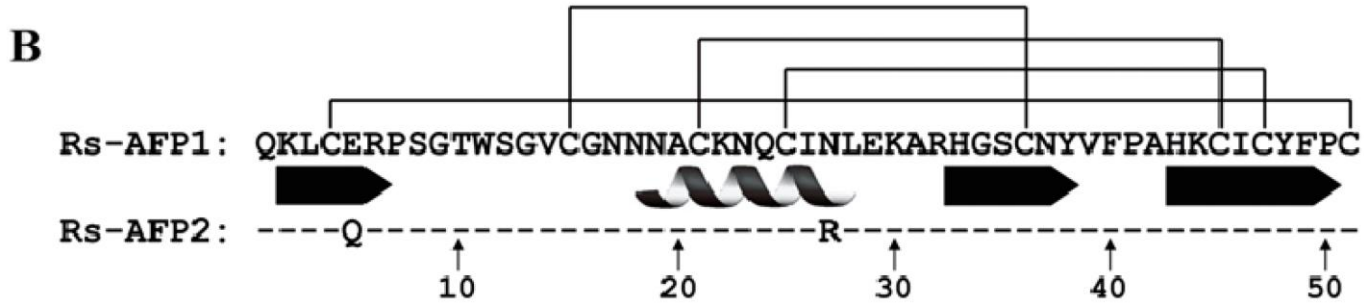


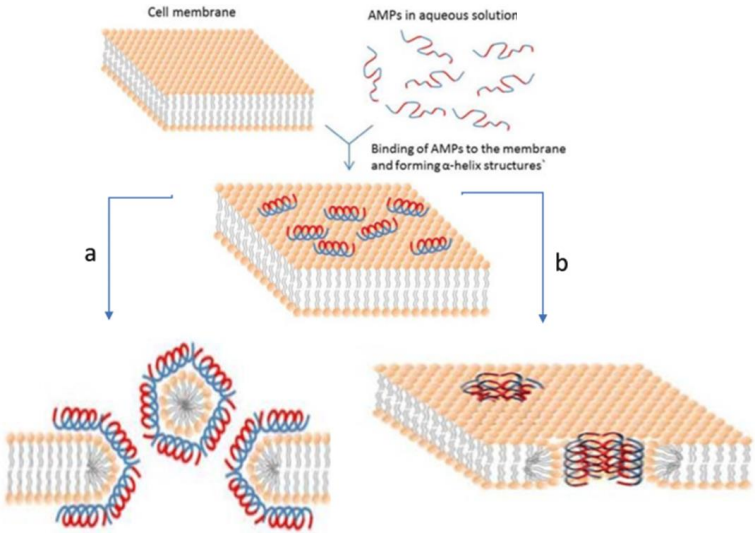
- **Peptidi Anti-microbici**

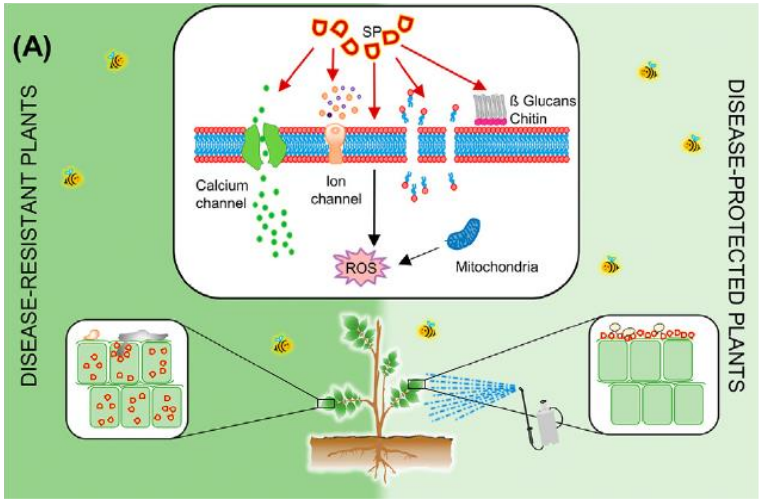


Peptidi antimicrobici









Peptidi antimicrobici: NCR044

Proc Natl Acad Sci U S A. 2020 Jul 7; 117(27): 16043–16054.
Published online 2020 Jun 22. doi: 10.1073/pnas.2003526117
PMCID: PMC7354933
PMID: 32571919

Antifungal symbiotic peptide NCR044 exhibits unique structure and multifaceted mechanisms of action that confer plant protection

Siva L. S. Velivelli,[a] Kirk J. Czymmek,[a,b] Hui Li,[a] Jared B. Shaw,[c] Garry W. Buchko,[c,d] and Dilip M. Shah[a,1]

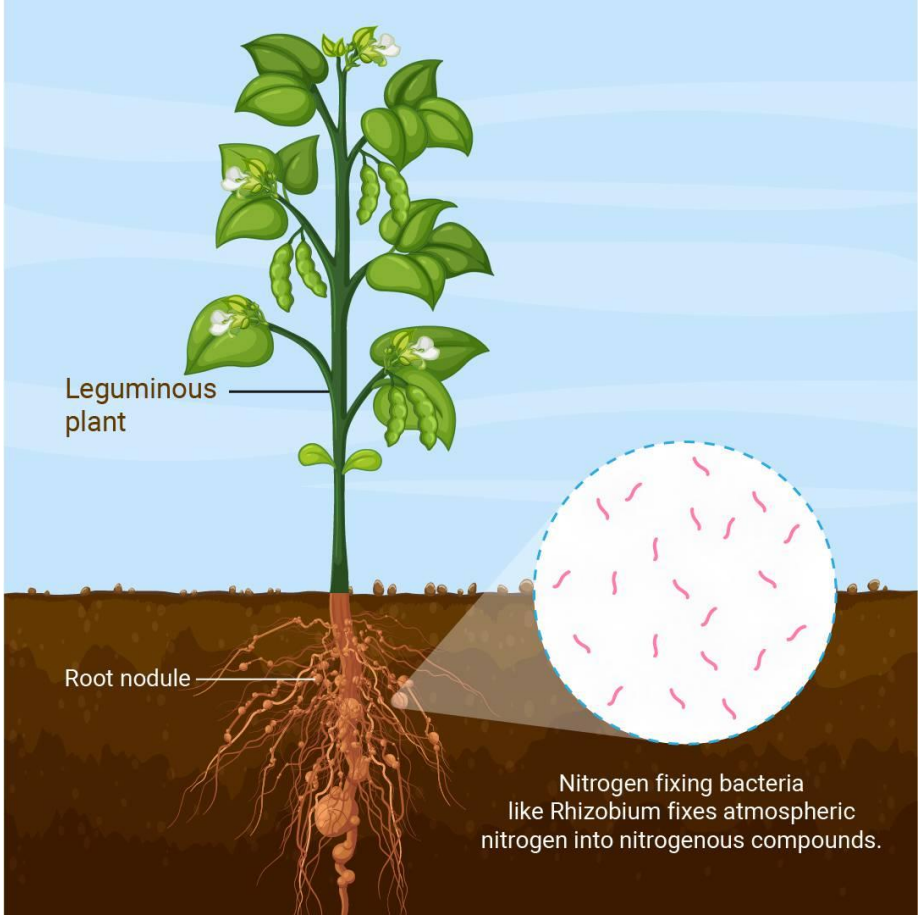
Associated Data

Supplementary Materials

SIGNIFICANCE

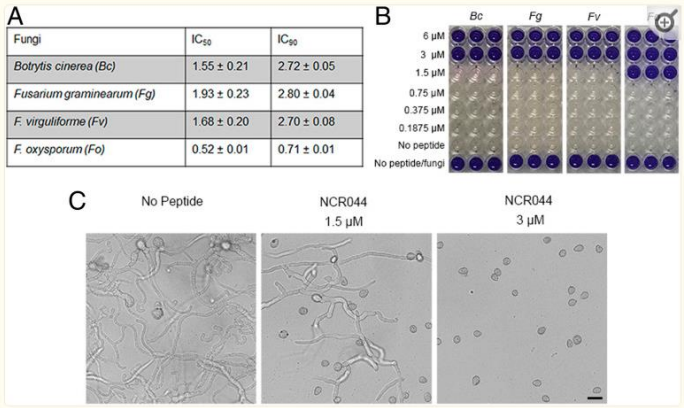
Go to:

Several nodule-specific cysteine-rich (NCR) peptides expressed in a model legume *Medicago truncatula* exhibit potent antimicrobial activity. However, their structure–activity relationships and mechanisms of action against fungal pathogens of plants are still largely unknown. A small highly cationic peptide NCR044 with potent antifungal activity has been identified. This peptide has a unique highly dynamic structure and exhibits multifaceted mechanisms of action against a fungal pathogen *Botrytis cinerea*. Exogenous application of this peptide confers resistance to a gray mold disease caused by *B. cinerea* in tobacco and tomato plants as well as postharvest products. Our work paves the way for future development of NCR peptides as spray-on antifungal agents.



Peptidi antimiocrobici: NCR044

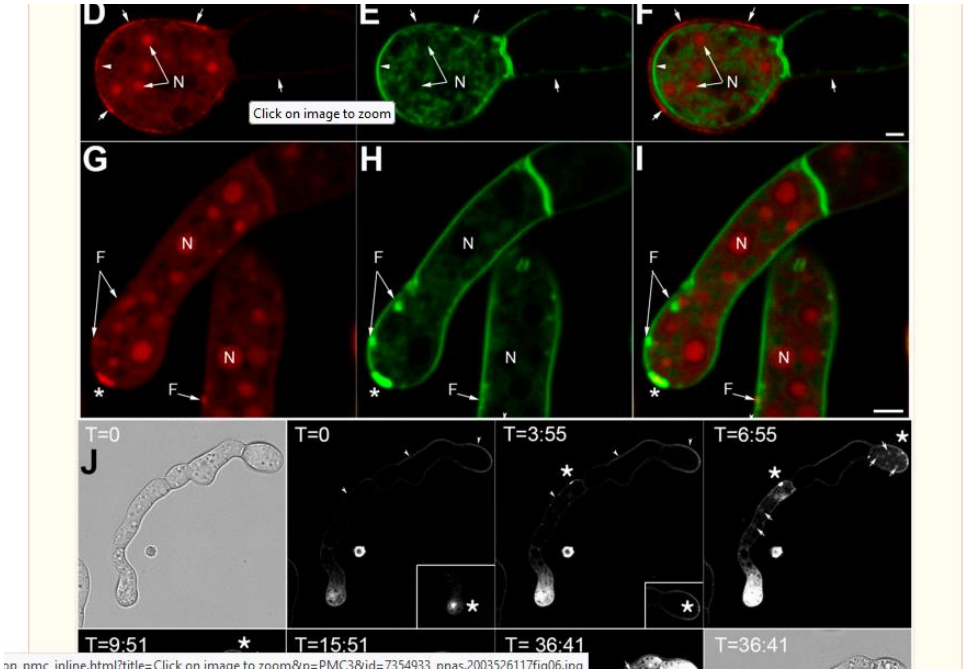
concentration of 3 μM (Fig. 3 *B* and *C*). We also determined the antifungal activity of the chemically synthesized reduced form of the NCR044 peptide against *B. cinerea*. The reduced form of the NCR044 peptide inhibited the growth of *B. cinerea* with IC_{50} value of 4.16 ± 0.18 μM as compared with 1.55 ± 0.21 μM for the native oxidized form of the peptide. The resazurin cell viability assay revealed that *B. cinerea* spores lose their cellular metabolic activity at a concentration of 6 μM as compared with 3 μM for the native oxidized form of the peptide (*SI Appendix*, Fig. S2 *A* and *B*).



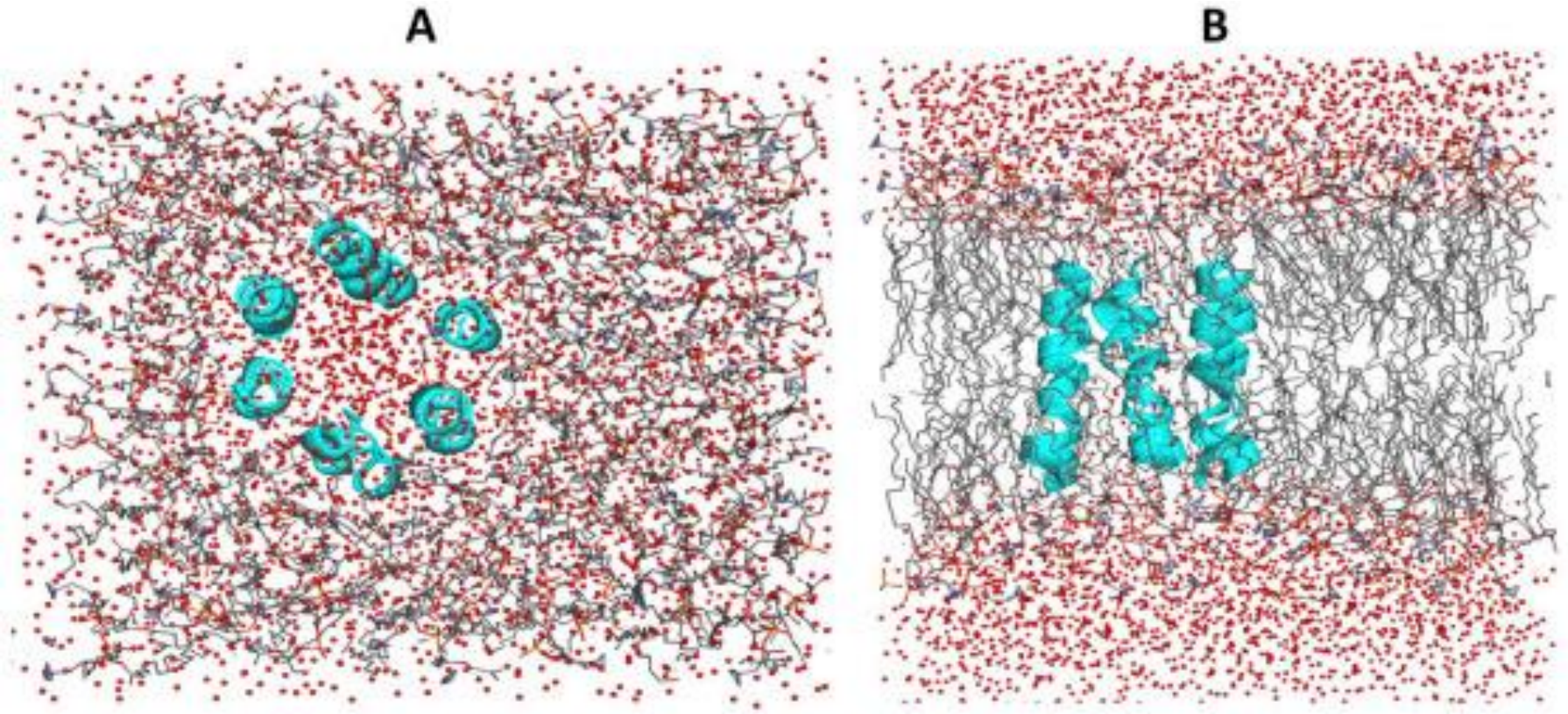
Fungi	IC_{50}	IC_{90}
Botrytis cinerea (Bc)	1.55 ± 0.21	2.72 ± 0.05
Fusarium graminearum (Fg)	1.93 ± 0.23	2.80 ± 0.04
F. virguliforme (Fv)	1.68 ± 0.20	2.70 ± 0.08
F. oxysporum (Fo)	0.52 ± 0.01	0.71 ± 0.01

Fig. 3.

Antifungal activity of NCR044 against *B. cinerea* and *Fusarium* spp. (*A*) IC_{50} and IC_{90} values of NCR044 for each pathogen are shown. Data are means ± SEM of three independent biological replicates ($n = 3$). (*B*) Results of the fungal cell viability assay using resazurin, a metabolic indicator of living cells. A change from blue to pink/colorless signals resazurin reduction and indicates metabolically active fungal spores after 60 h. In the presence of 3 or 6 μM NCR044, fungal cells lost their metabolic capacity and did not reduce resazurin. (*C*) Representative microscopic images showing the inhibition of *B. cinerea* growth 24 to 48 h after treatment with 1.5 or 3 μM of NCR044 (*Right*). *B. cinerea* without peptide added served as a negative control (*Left*). (Scale bar, 20 μm.)

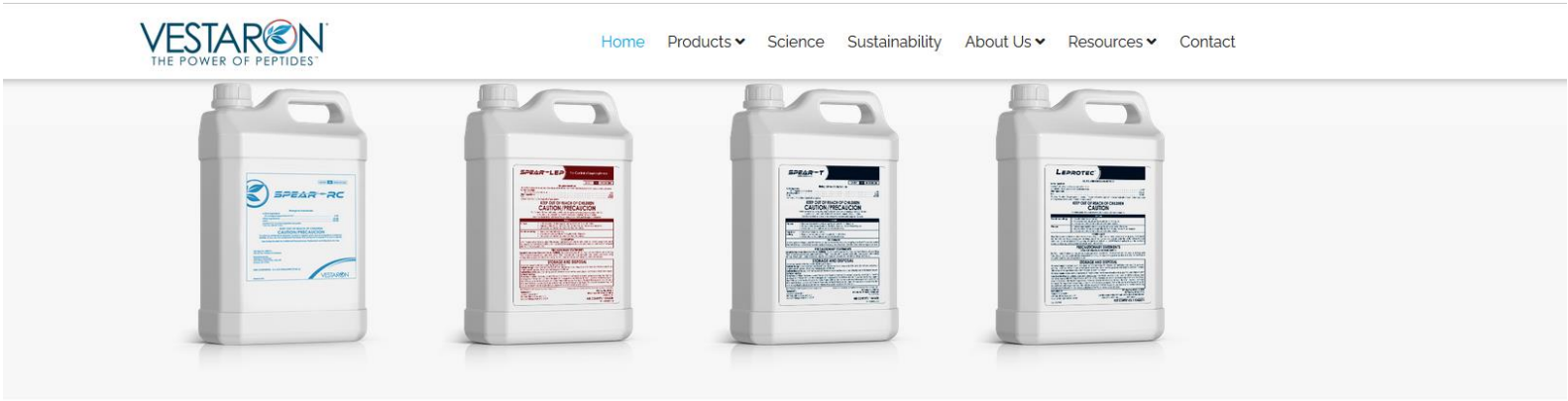


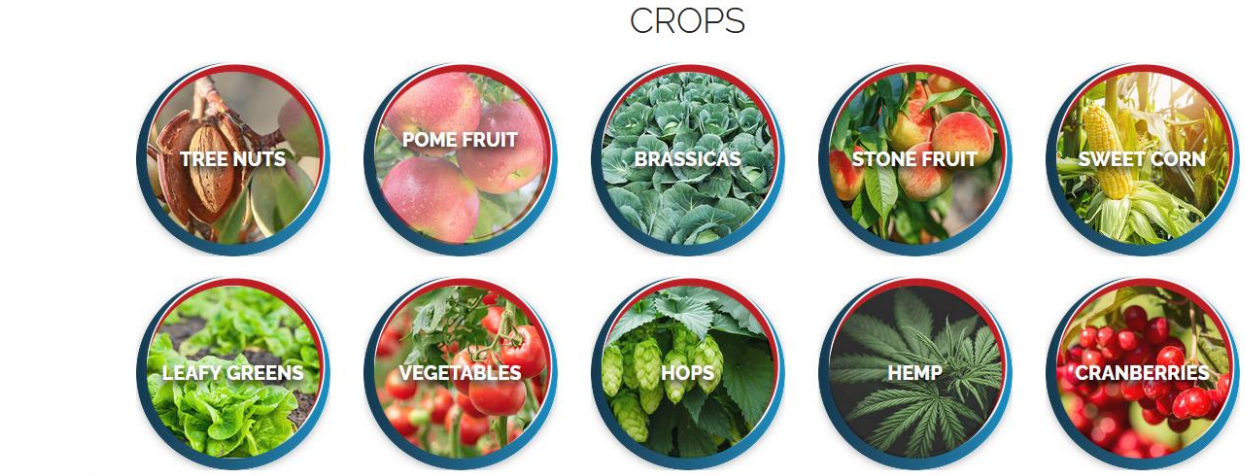
Peptidi antimicrobici: peptaibols



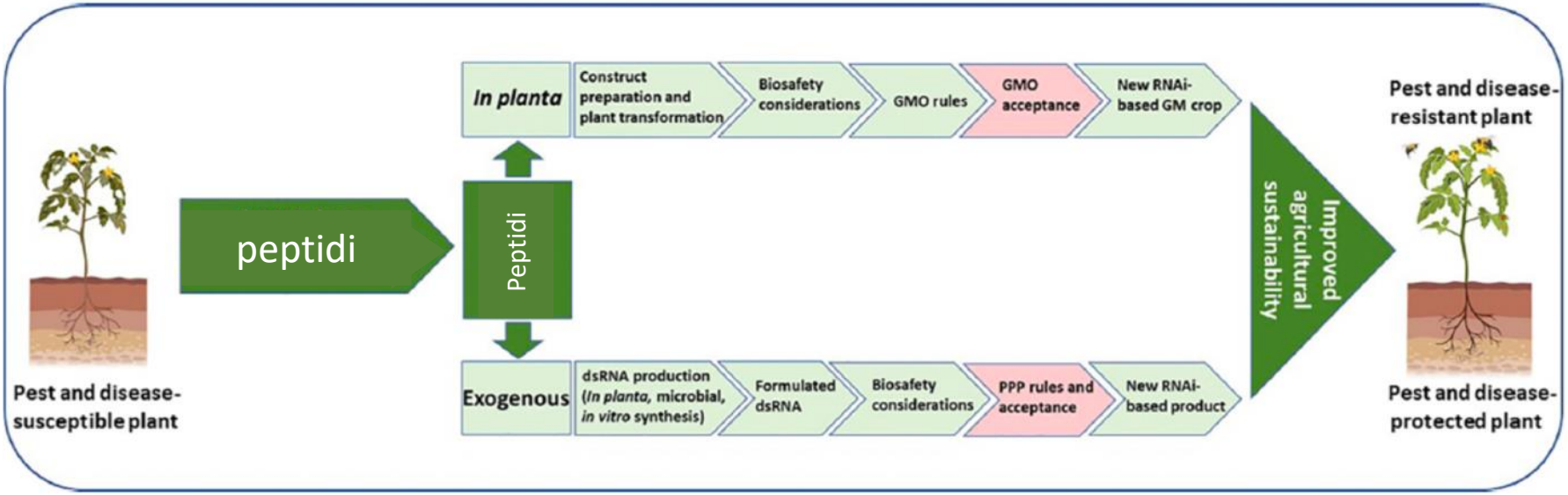
Modello di un canale formato da peptaibols usando membrane artificiali

I peptidi sono commercializzati (USA)

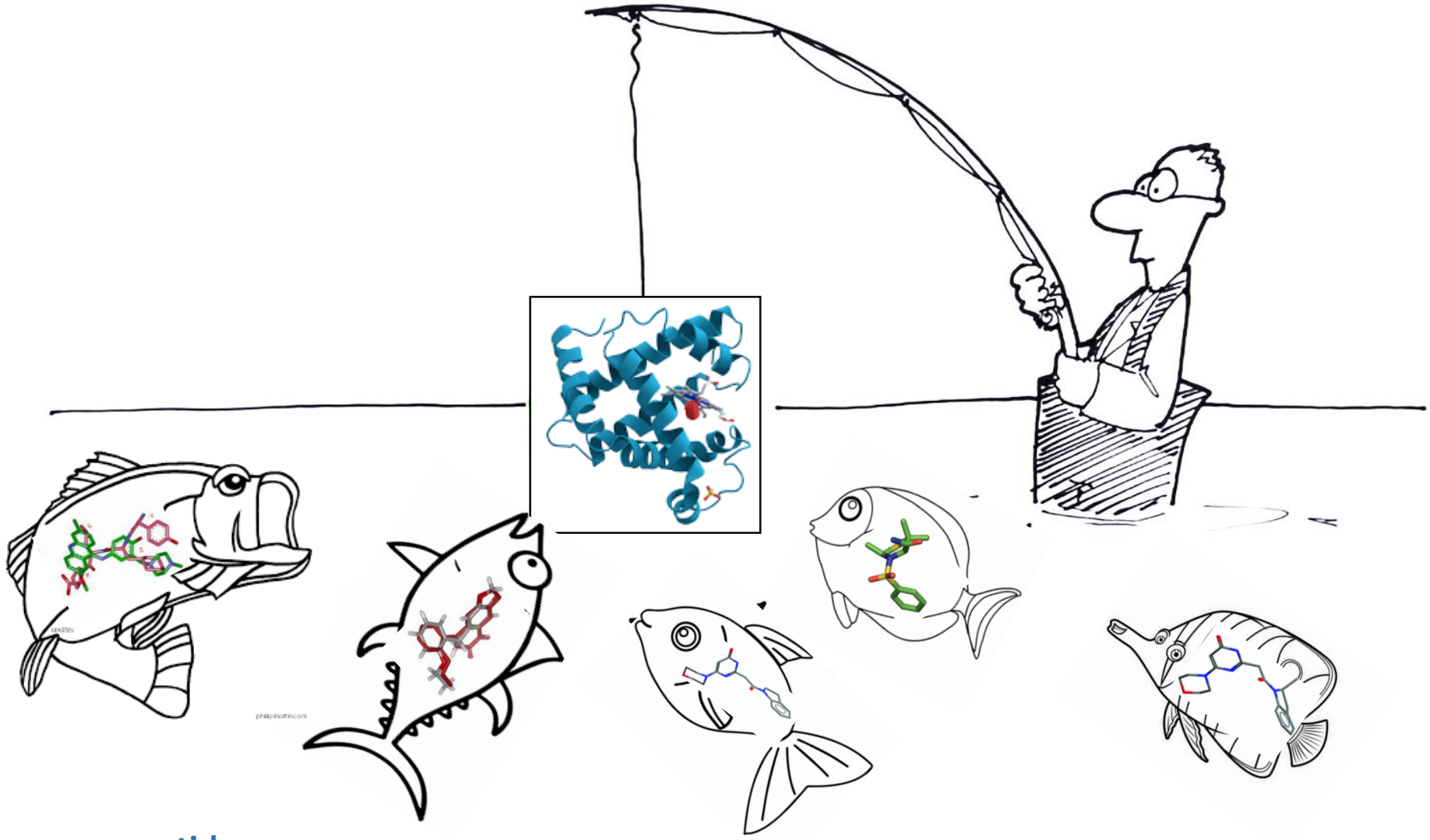




Piccoli-peptidi



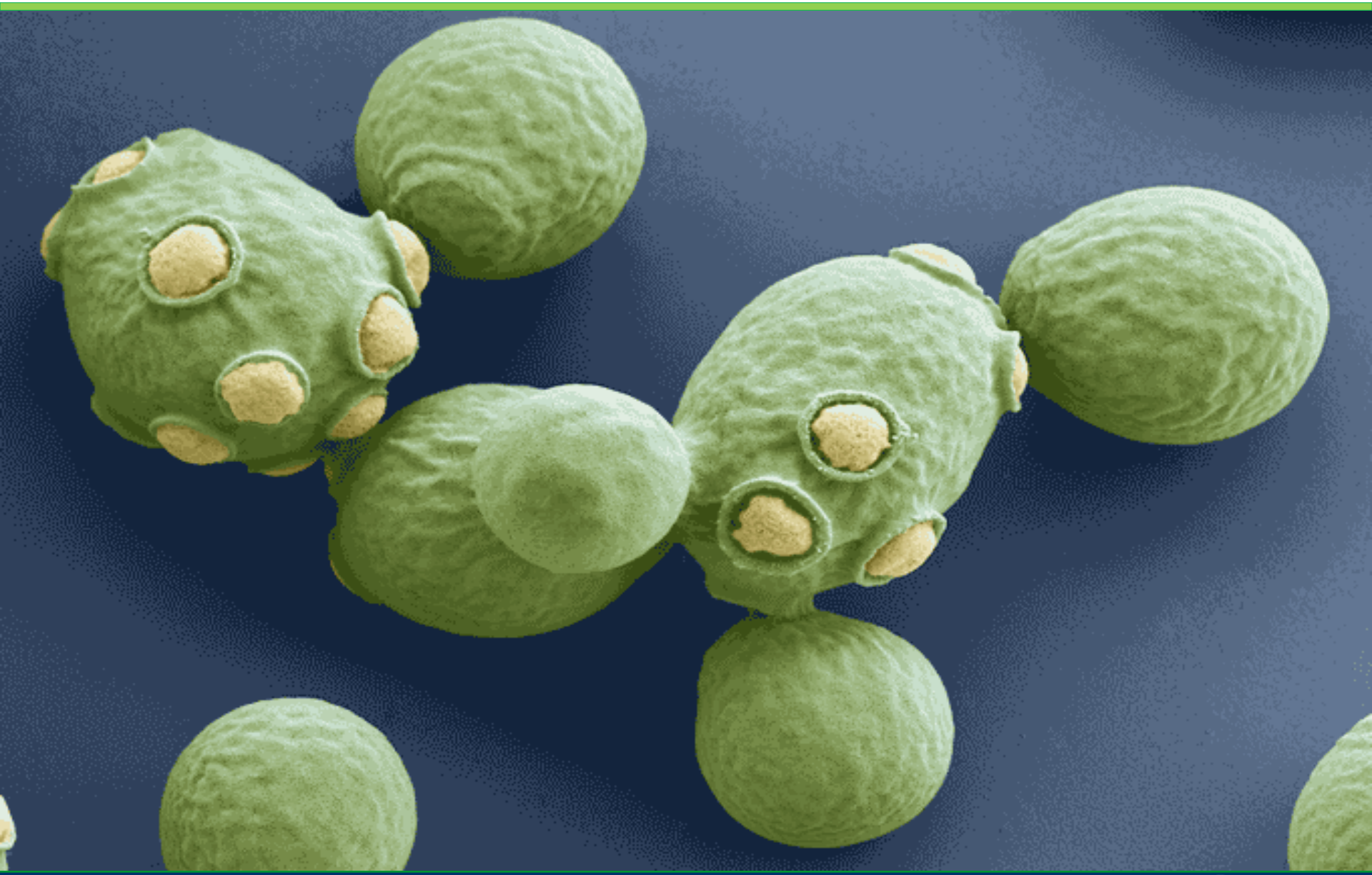
Farmaci peptidici



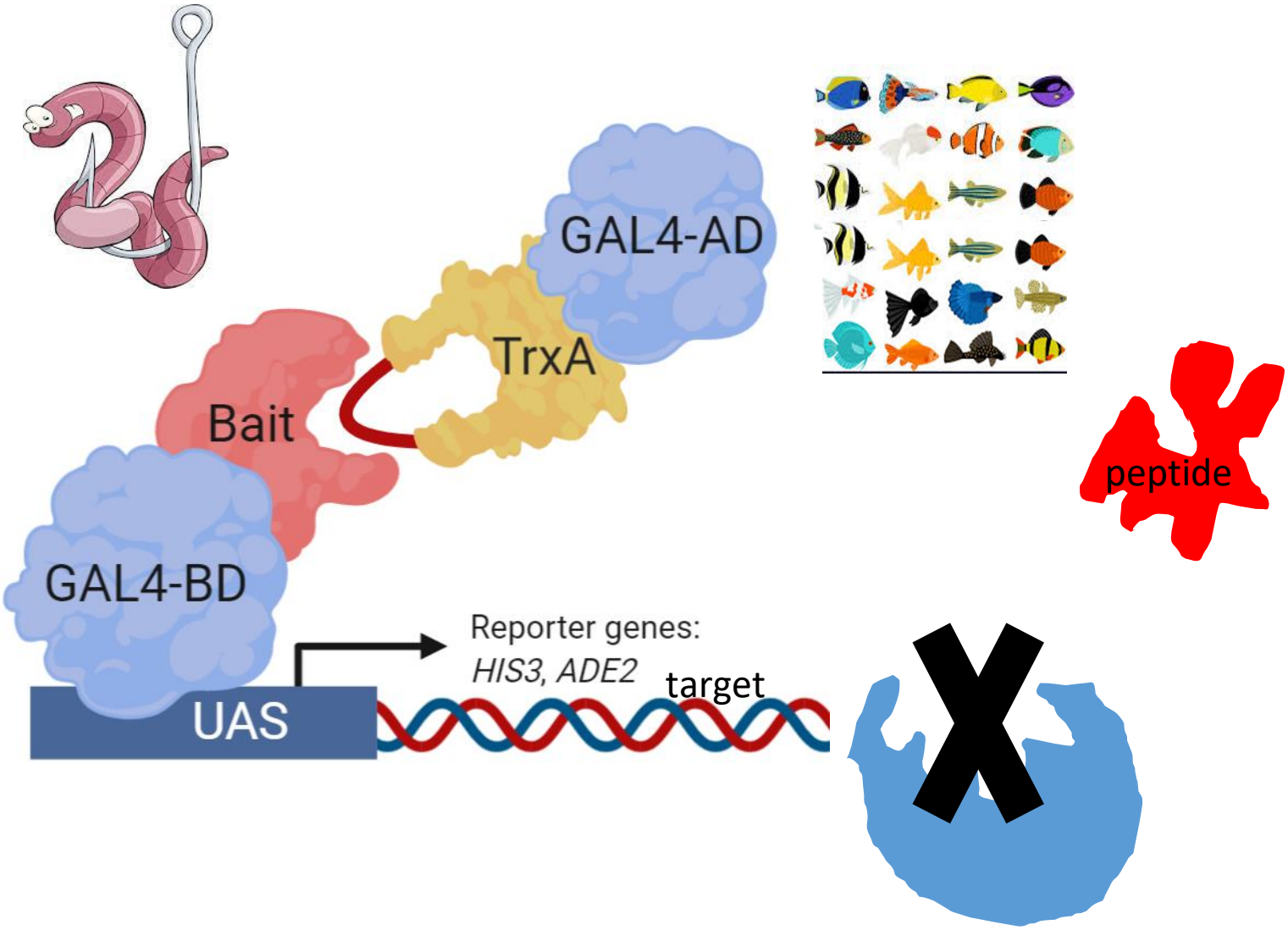
pesce=peptide

Possiamo saggiare milioni di peptidi

Il doppio ibrido per trovare nuove molecole



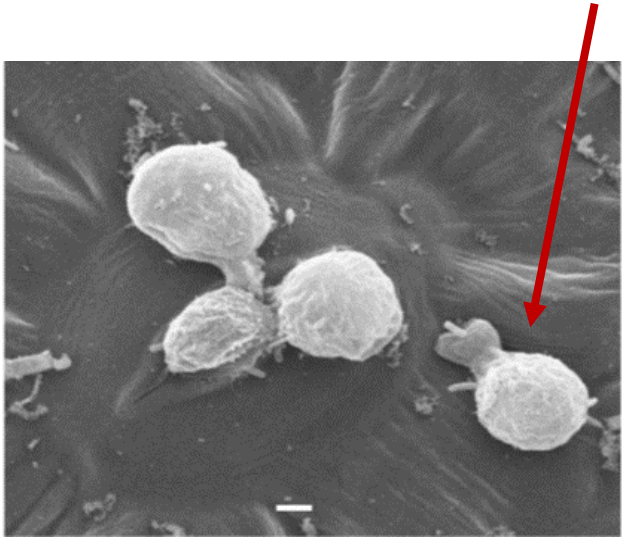
Il nostro test



Il nostro bersaglio: *P. viticola*

Le zoospore di P. viticola necessitano

della parete per attaccare la vite



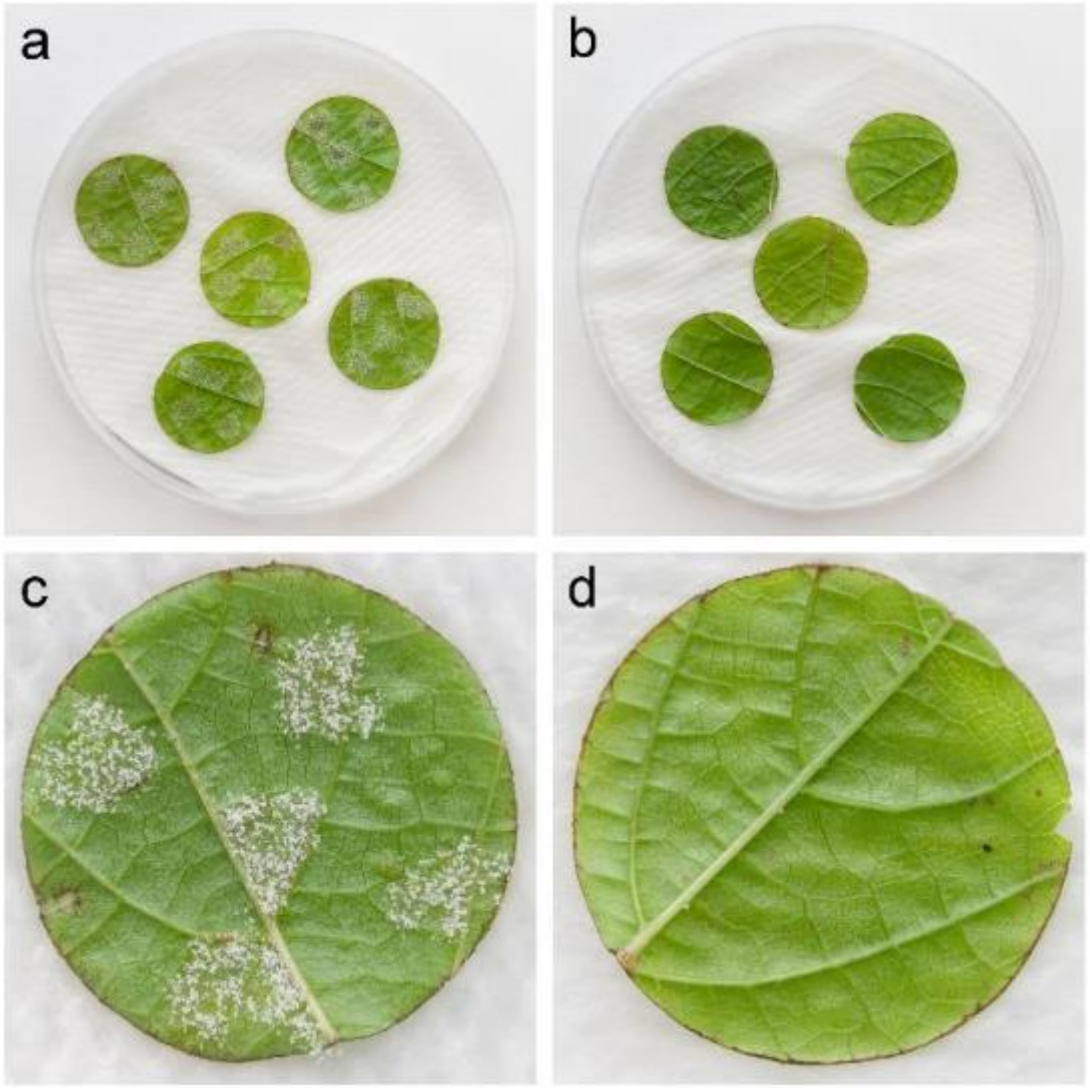
Zoospore di P. viticola

Colombo et al., 2020

Fondazione CARIPLO

TUTE SERVARE MUNIFICE DONARE · 1816

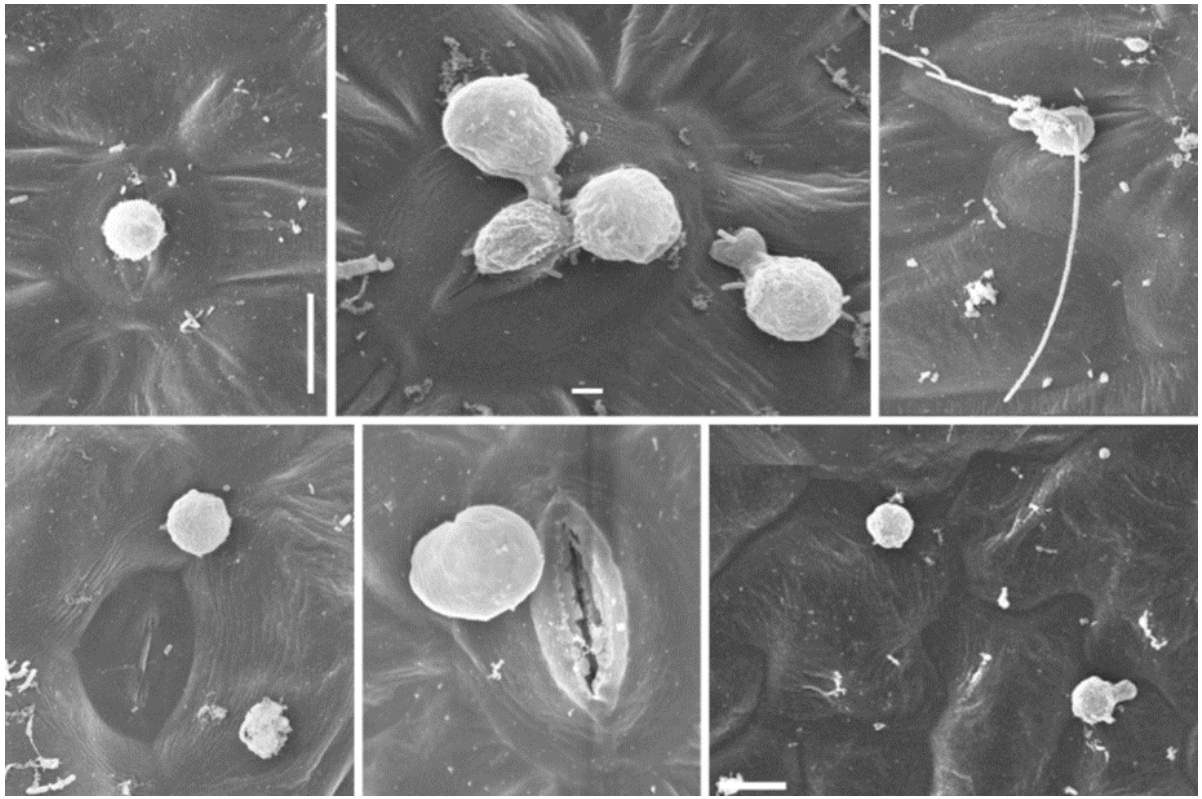
NoPv1 e *P. viticola*



Fondazione CARIPLO

TUTE SERVARE MUNIFICE DONARE • 1816

NoPv1 e *P. viticola*

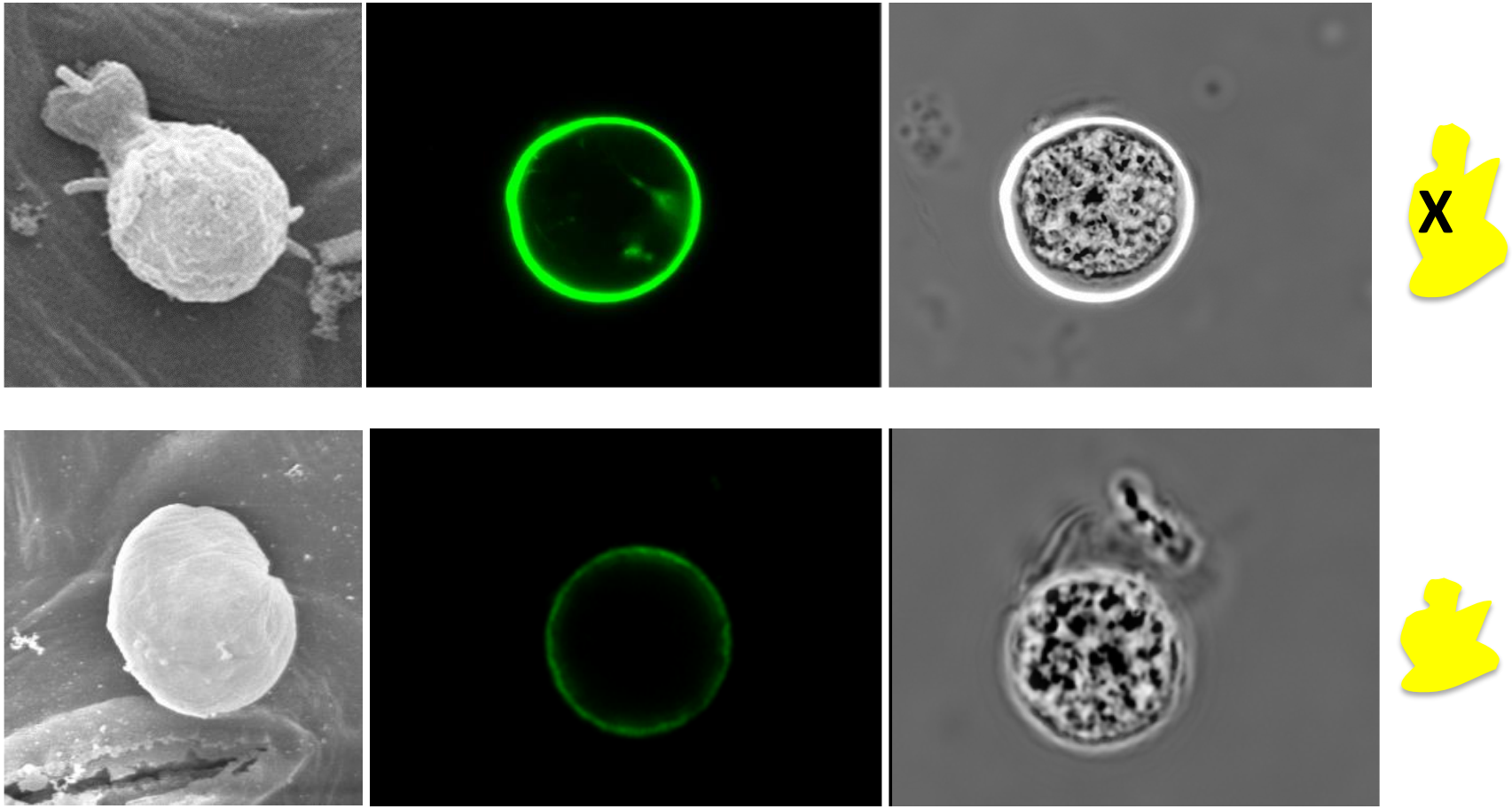


Controll

NoPv1

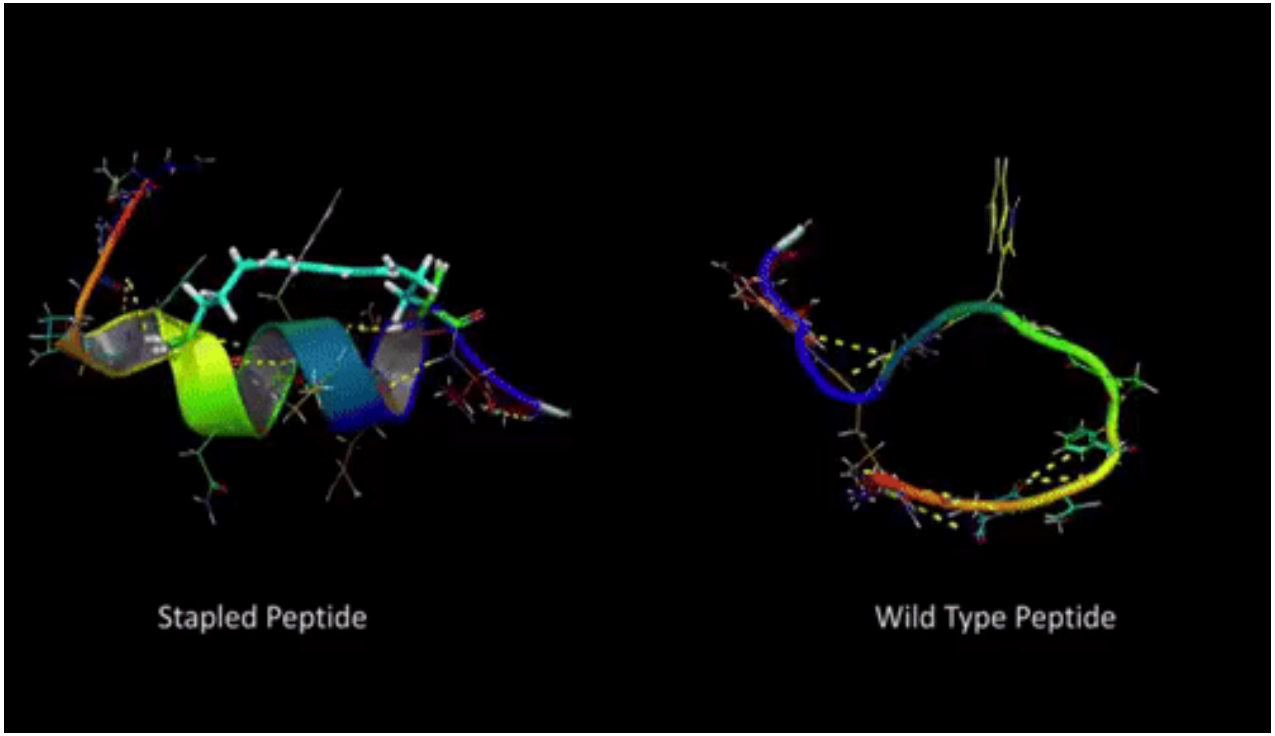
PCT/IB2018/059834
Colombo et al., 2020

NoPv1



Il doppio ibrido e la scoperta di nuovi peptidi

- I peptidi ciclici sono piu' resistenti alle proteasi
- I peptidi ciclici passano piu' facilmente attraverso le membrane.
- Maggiore affinità per i target



Rosa et al., 2023

Bilancio Fitosanitario Toscana 2023

Malattie segnalate

Prod. integrata	Peronospora: 96% Black rot: 46% Oidio: 23% Botrite: 11%	100 aziende
Prod. biologica	Peronospora: 99% Black rot: 39% Oidio: 28% Botrite: 10%	80 aziende

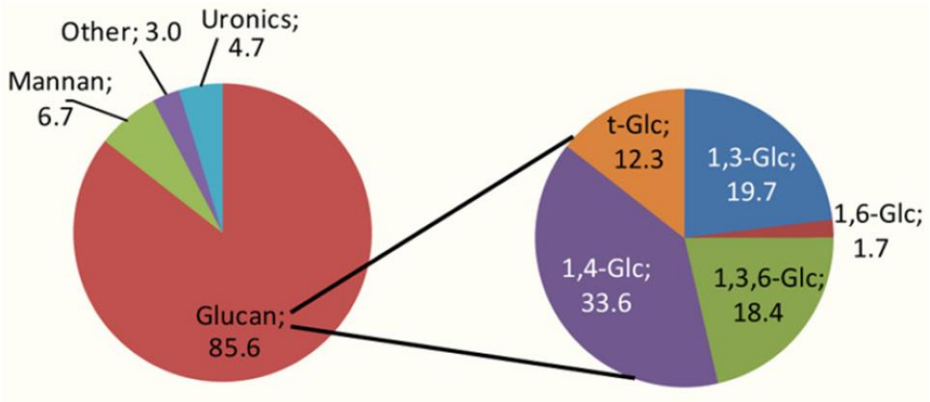
Sintomi peronospora (foto: INRAE)

Sintomi black rot (foto: INRAE)

La parete di *G. bidwellii (black rot)*

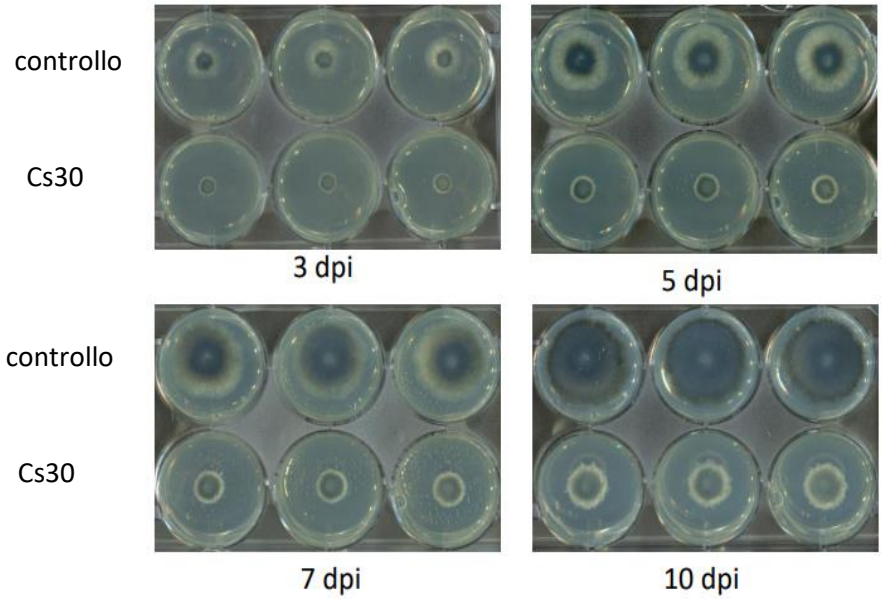
Regione Lombardia

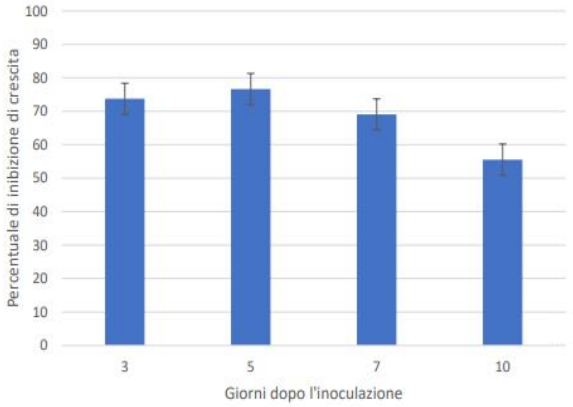
Target: 1,3-beta-glucanosyltransferasi

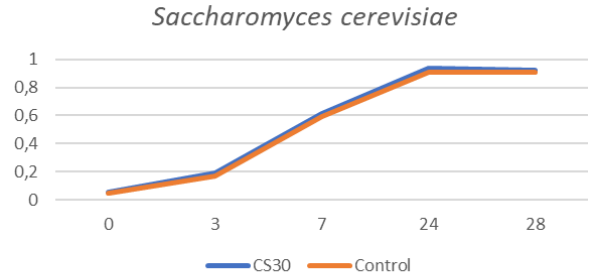


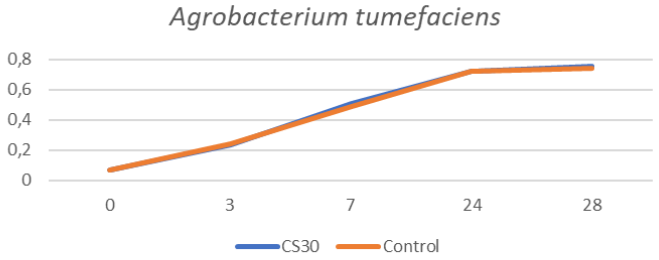
Mélida, Hugo, et al. "Analyses of extracellular carbohydrates in oomycetes unveil the existence of three different cell wall types." *Eukaryotic Cell* 12.2 (2013): 194

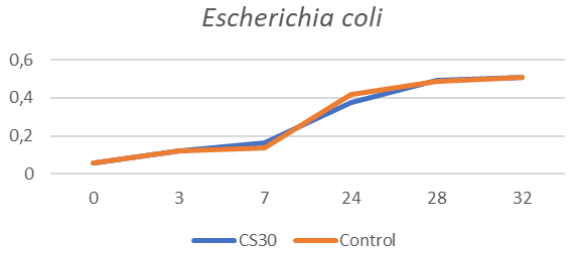
I peptidi sono specifici



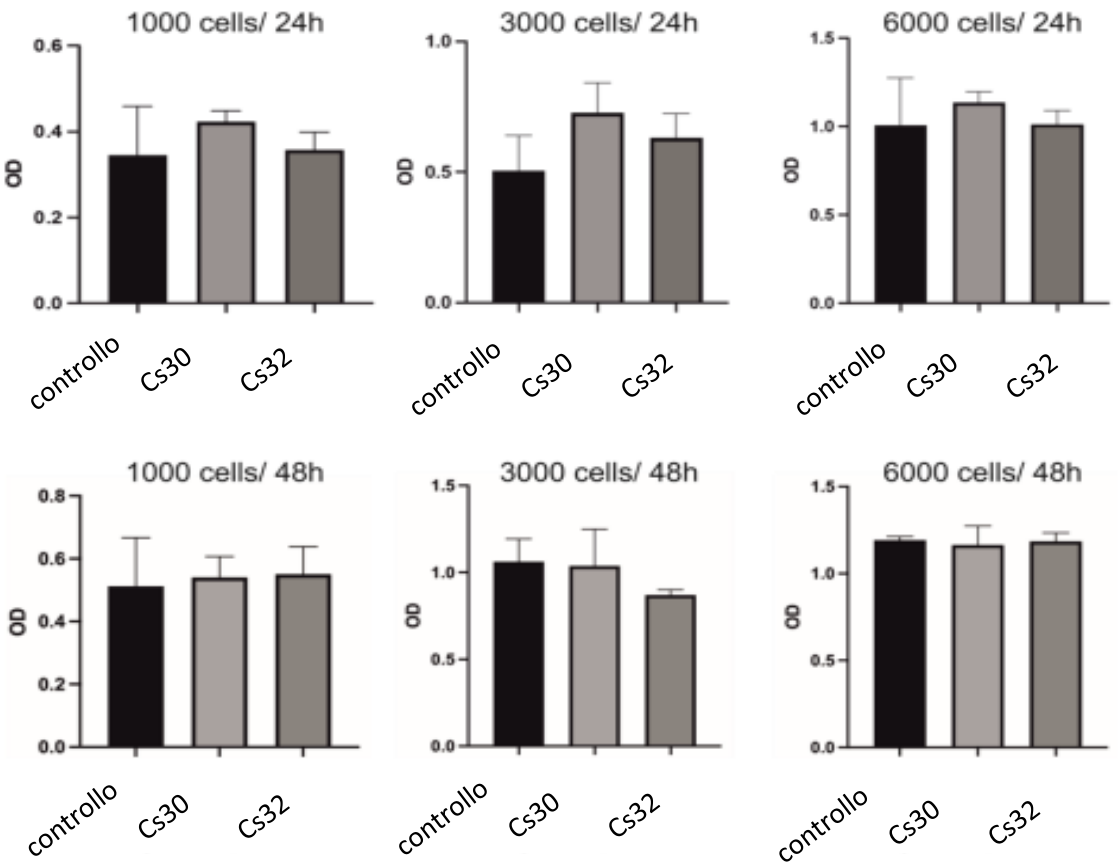








Peptidi anti-BR



Dove siamo

Europäisches Patentamt
European Patent Office
Office européen des brevets

Acknowledgement of receipt

We hereby acknowledge receipt of your request for grant of a European patent as follows:

Submission number	12589019
Application number	EP23207559.8
File No. to be used for priority declarations	EP23207559
Date of receipt	02 November 2023
Your reference	P2367EP
Applicant	Università degli Studi di Milano
Country	IT
Title	ANTIMICROBIAL PEPTIDES FOR THE CONTAINMENT OF OOMYCETES IN AGRICULTURE

Dove intendiamo andare

Il gruppo UNIMI

Produzione su larga scala
- Utilizzando microrganismi
- Messa a punto di protocolli

Test
- Test anti-microbici in serra e in campo

Formulazione
Selezione di formulati

Valutazione dei rischi
- Per l'ambiente (Terrestre acquatico)
- Per l'uomo (operatore e consumatore)

Nuovi PPPs

Grazie a …….

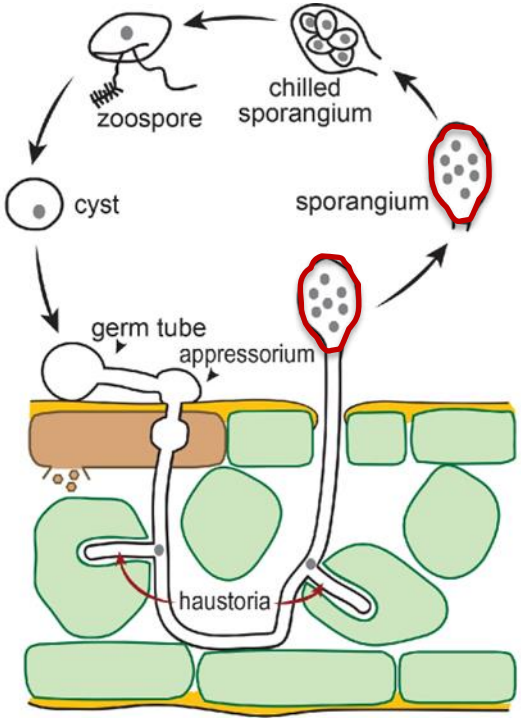
UNIMI
Prof.ssa Silvia L Toffolatti
Prof. Paolo Pesaresi

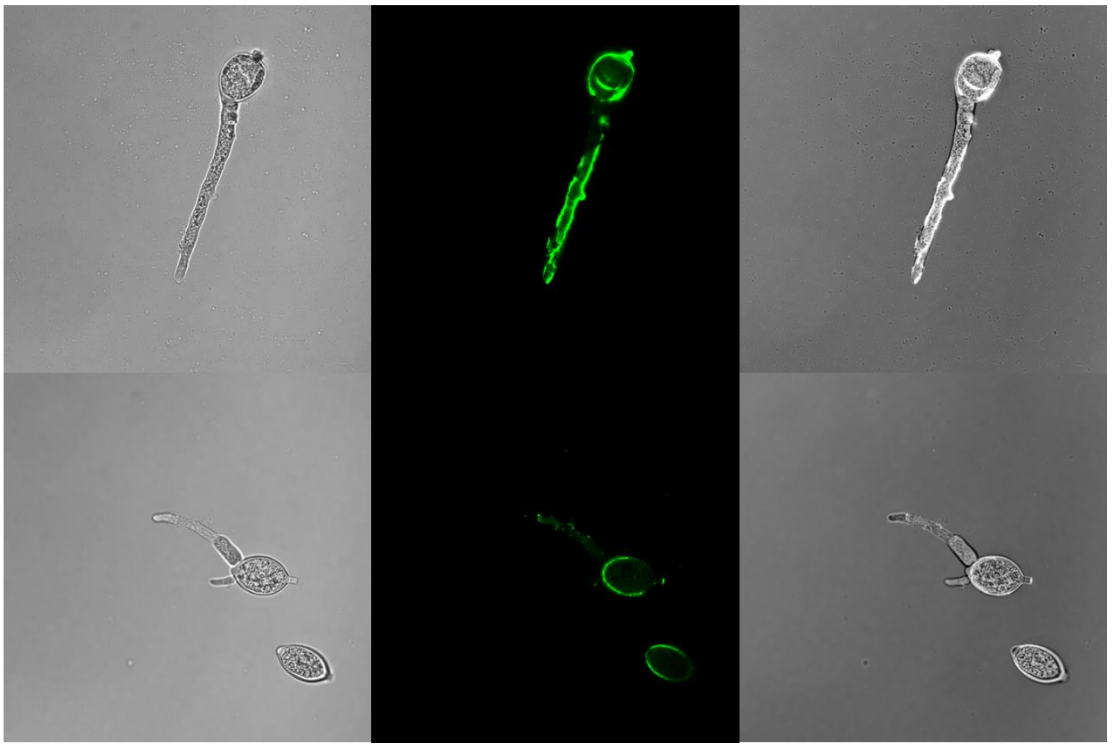
Riverside
Prof Dario Cantu'

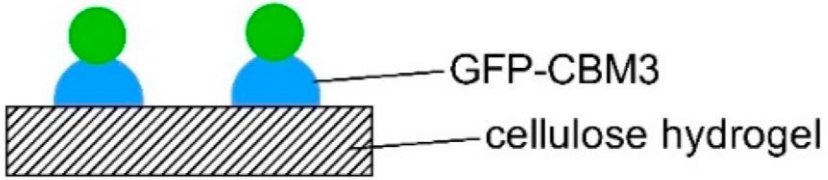
UNIBO
Prof.ssa Elena Baraldi
UNIPM
Prof. Bruno Mezzetti
Prof.ssa Silvia Sabbatini

Fondazione E. Mach
Dr.ssa Silvia Vezzulli
Dr.ssa Paola Bettinelli

Are we able to confirm the interaction?







Are we able to confirm the interaction?