

Una miscela di oli essenziali quale potenziale rimedio per combattere il cancro del kiwi



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Rino Ragno

Dipartimento di Chimica e Tecnologie del Farmaco

WORKSHOP

**DIFESA INTEGRATA
DELLE COLTURE:
INNOVAZIONE E
DIGITALIZZAZIONE**

EVENTO GRATUITO

20 GENNAIO 2023

ORE 10.00-17.00

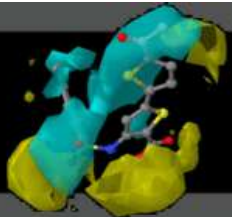
SALA ELSA MORANTE

PIAZZALE FRANCESCO COLAGROSSI

COLONNA (RM)

INFO E PROGRAMMA COMPLETO SU
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Oli Essenziali

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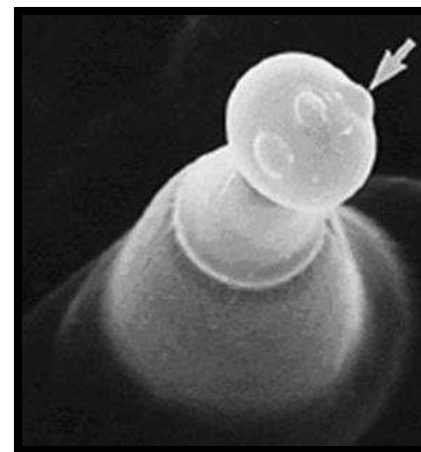
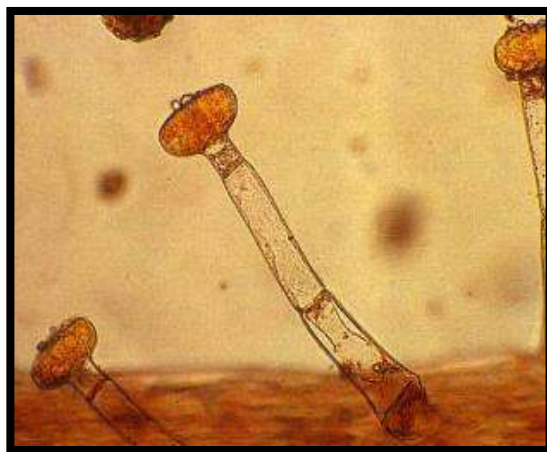
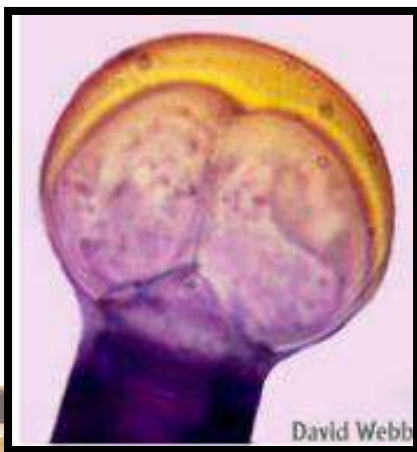


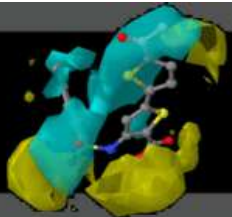
Prodotti odorosi, sostanze volatili, lipofile e generalmente liquide.

Hanno p.e. tra i 160° C e i 260° C.

Generalmente hanno densità minore dell'acqua

Contenuti in organi specifici





Oli Essenziali

by www.RCMD.it

- Miscele Complesse
- Sono studiati in tutto il mondo per numerose applicazioni
- Si possono ottenere mediante diverse tecniche: estrazione con solventi, con fluidi supercritici, **idrodistillazione**, **distillazione in corrente di vapore**, processi accelerate da microonde o ultrasuoni, processi con utilizzo di pressione (Estrattore di Naviglio)
- Differenti procedure producono diversi OE con diversa composizione chimica



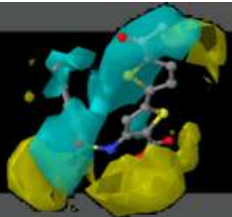
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Oli Essenziali

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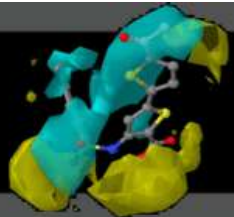
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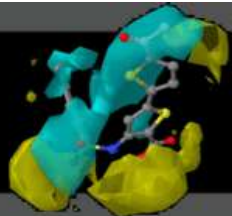


Il Cancro del Kiwi

by www.RCMD.it

Psa è il patogeno batterico responsabile della malattia delle piante pandemia che colpisce sia *A. deliciosa* che *A. chinensis* (Scortichini et al., 2012). Sintomi tipici della malattia sono costituiti da macchie fogliari brune con aloni clorotici, colorazione marrone delle gemme, cancri con essudati tronchi e ramoscelli e frutti crollati (Balestra et al., 2009; Mazzaglia et al., 2012). A seguito delle epidemie di PSA, molti paesi con forti industrie di kiwi, come Nuova Zelanda, Italia, Spagna, Portogallo, Cile, Francia, Giappone, Cina e Corea del Sud, hanno sofferto gravi perdite economiche (Mazzaglia et al., 2012; Scortichini et al., 2012). Il cancro batterico causato da Psa è stato il primo riconosciuto come grave disturbo nella prefettura di Shizuoka, Giappone durante l'inizio della primavera del 1984, dove i sintomi apparso diffuso su tronchi e rami maturi frutteti, con nuovi germogli emergenti durante la tarda primavera e l'estate presenta anche sintomi di malattia (Serizawa et al., 1989). I focolai di malattie proliferarono rapidamente dopo forti venti e periodi umidi, causando gravi perdite di produttività o morte delle viti colpite e perdita di tutto frutteti (Serizawa et al., 1989). La stessa malattia era descritto anche nella provincia di Hunan in Cina durante 1984 e 1985, diffondendosi presto in altre regioni (Mazzaglia et al., 2012).





Natural Product Research, 2015

<http://dx.doi.org/10.1080/14786419.2015.1022543>



Taylor & Francis

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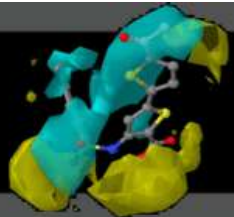
Antibacterial activity of essential oils mixture against PSA

Elisabetta Vavala^a, Claudio Passariello^a, Federico Pepi^b, Marisa Colone^c, Stefania Garzoli^b, Rino Ragno^d, Adele Pirolli^d, Annarita Stringaro^c and Letizia Angiolella^{a*}

^aDepartment of Public Health and Infectious Diseases, University of Rome 'Sapienza', Piazzale Aldo Moro, 5, 00161 Rome, Italy; ^bDepartment of Drugs Chemistry and Technology, University of Rome 'Sapienza', Piazzale Aldo Moro, 5, 00161 Rome, Italy; ^cDepartment of Technology and Health, Italian National Institute of Health, Viale Regina Elena 299, 00161 Rome, Italy; ^dDepartment of Drugs Chemistry and Technology, Rome Center for Molecular Design, University of Rome 'Sapienza', Rome, Italy

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Isolamento di PSA

by www.RCMD.it

Sono stati eseguiti tre isolamenti uno dalla corteccia (COR), uno dall'essudato bianco (EB) ed uno dall'essudato rosso (ER), prelevati in campo di actinidia ad Aprilia a febbraio 2011



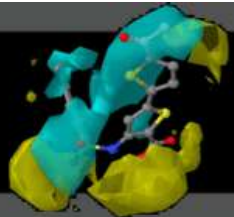
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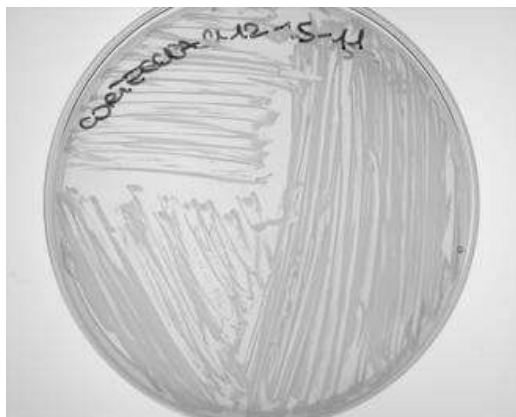
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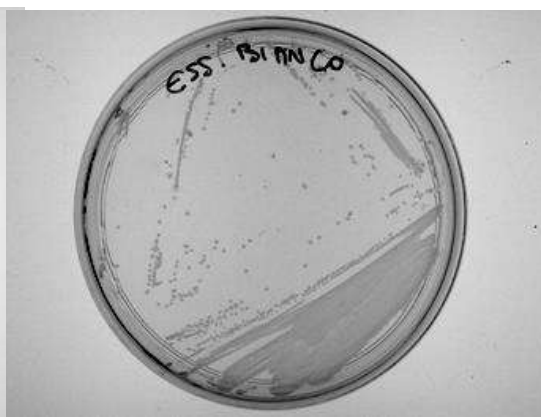
Isolamento di Psa

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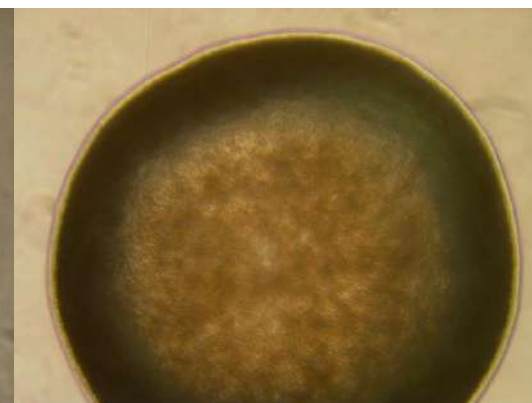
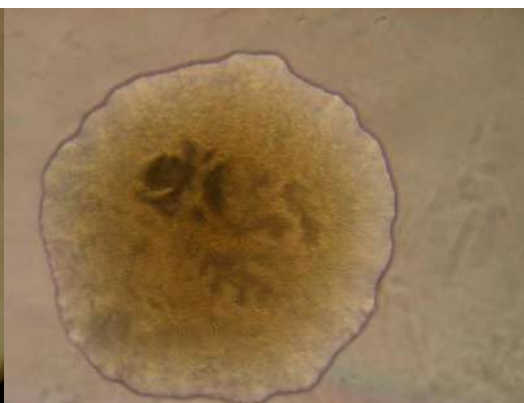
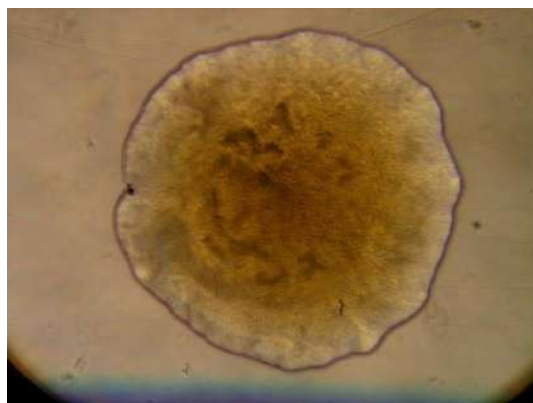
Corteccia (COR)



Essudato Bianco (EB)



Essudato Rosso (ER)



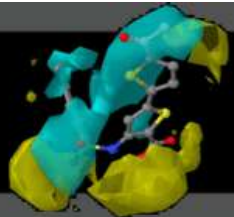
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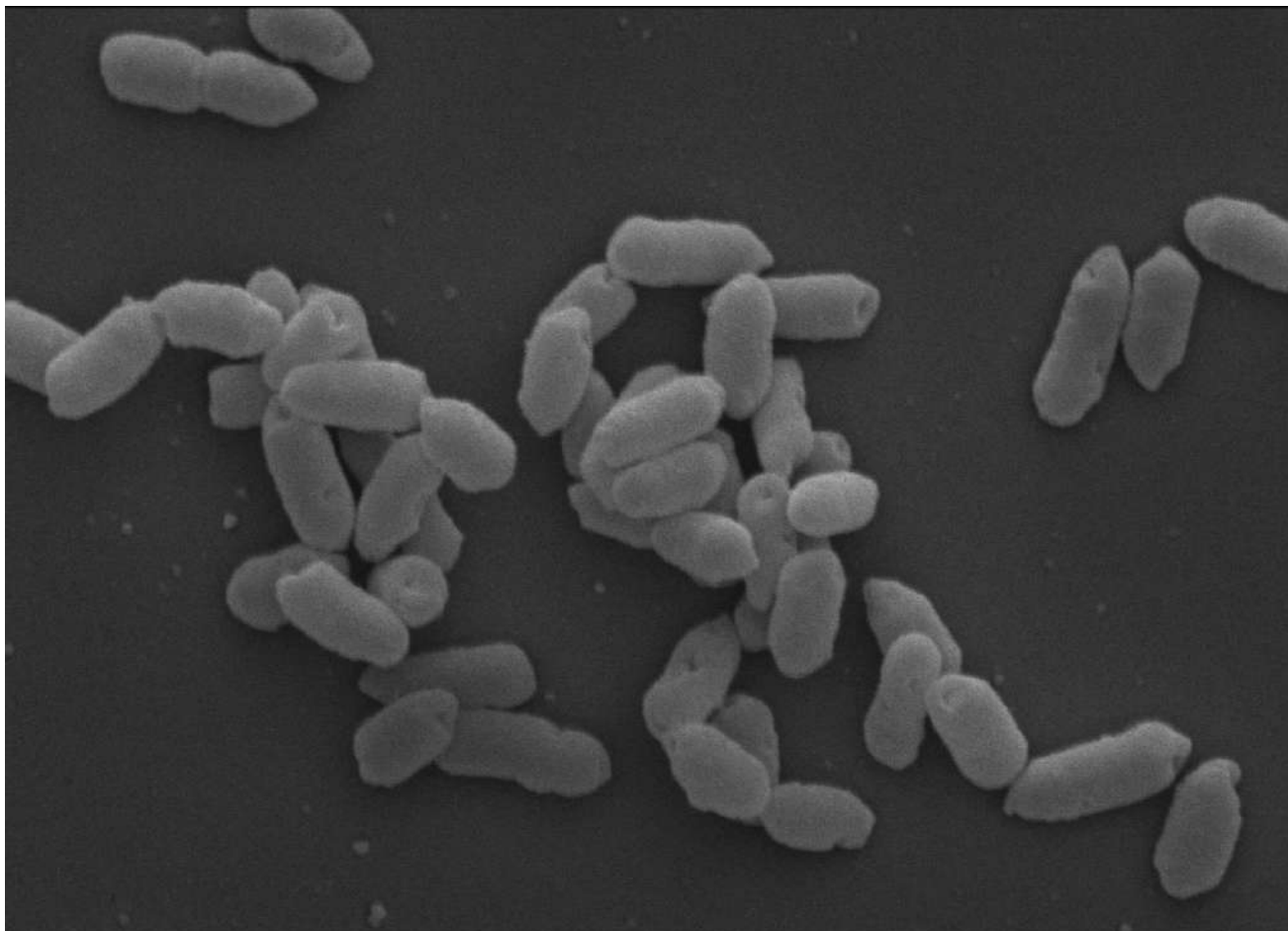


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SEM di Psa da COR

by www.RCMD.it



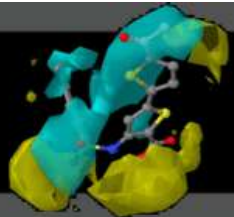
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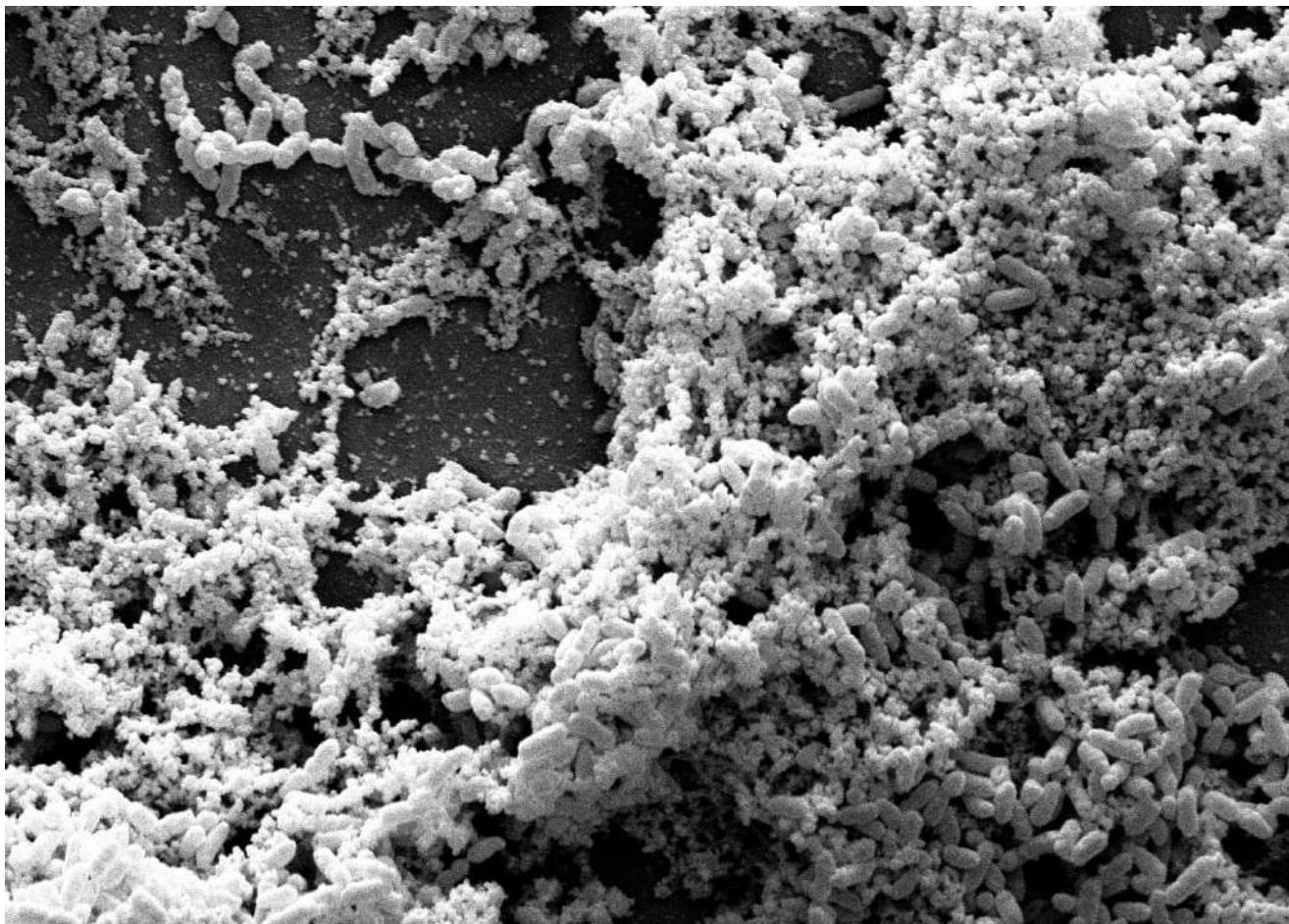


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SEM di Psa da ER

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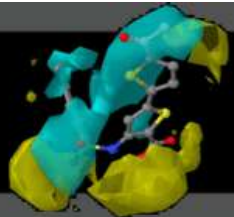
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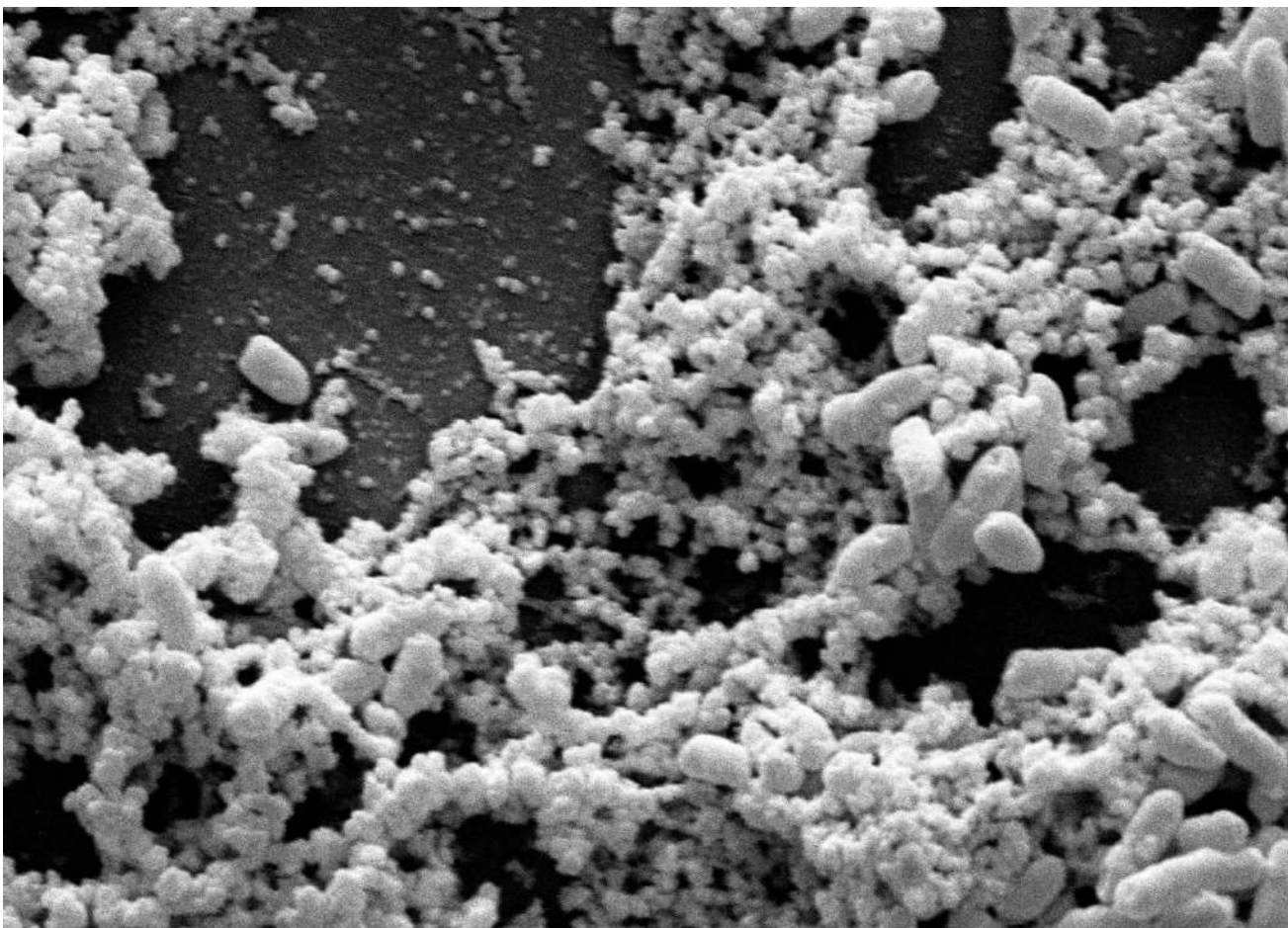


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Material e Esopolisaccaridico

by www.RCMD.it



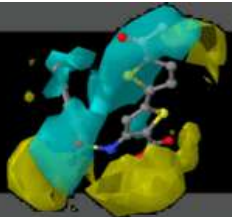
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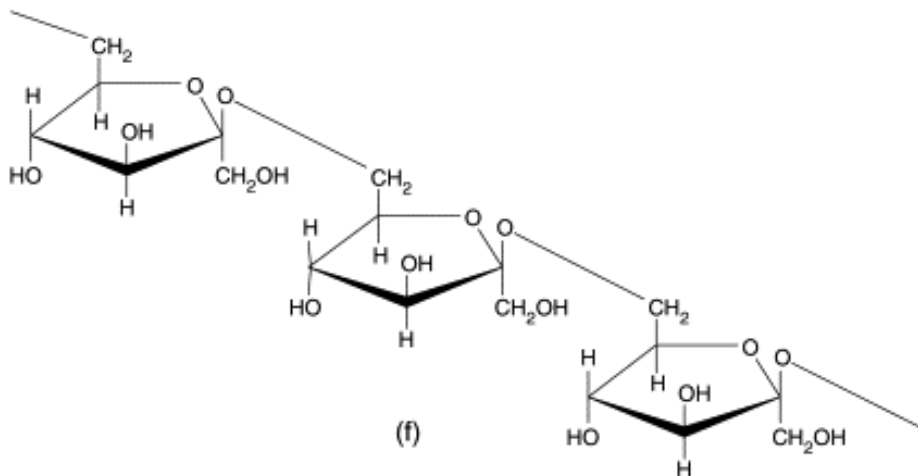


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Esopolisaccaridi

by www.RCMD.it



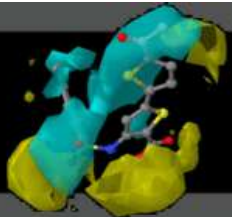
Levano: unità D-fruttosidiche β -2,6

I batteri che producono questa tipologia di polisaccaridi devono possedere l'enzima levansucrase che catalizza il trasferimento di residui D-fruttosidici

Ruolo fisiologico

Gli EPS prodotti dai batteri svolgono diversi ruoli fisiologici in quanto **proteggono la cellula dalla disidratazione, degli stress osmotici**, dall'attacco di batteriofagi, dall'attacco di protozoi, **attenuano o bloccano l'effetto di sostanze tossiche e/o di antibiotici** e consentono la formazione di biofilms e conferiscono la capacità di aderire alle superfici solide.

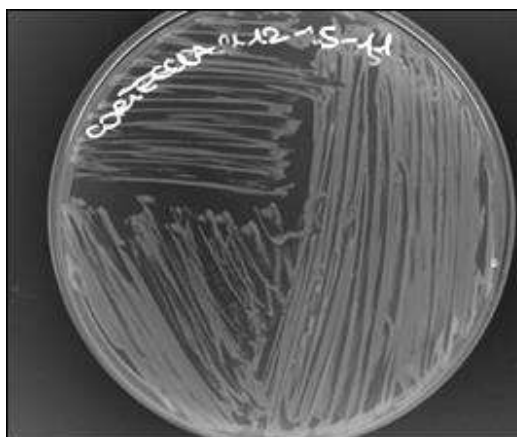




Diverse condizioni ambientali

by www.RCMD.it

Psa COR



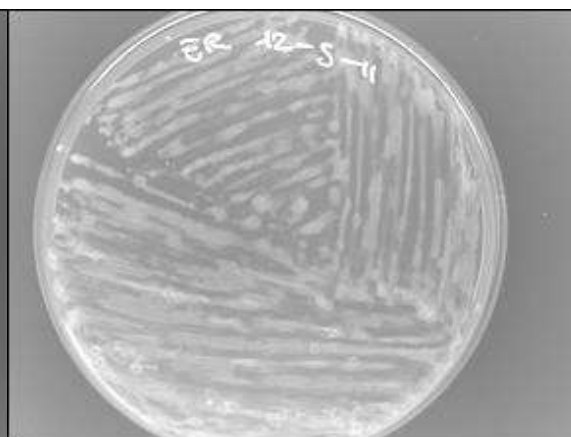
**Senza Materiale
Esopolimerico**

Psa COR



**Con Materiale
Esopolimerico**

Psa ER



**Con Materiale
Esopolimerico**



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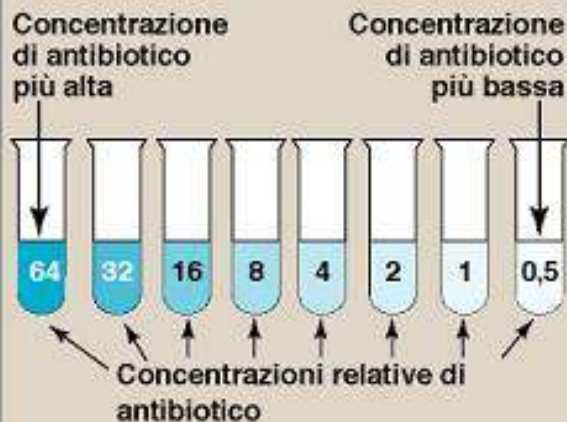
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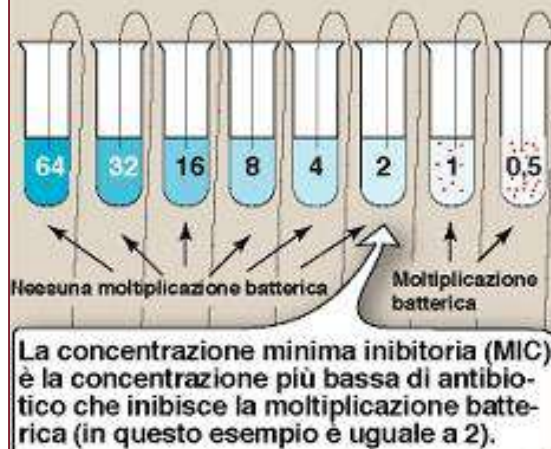


Attività antimicrobica MIC ed MBC *RCMD*.it

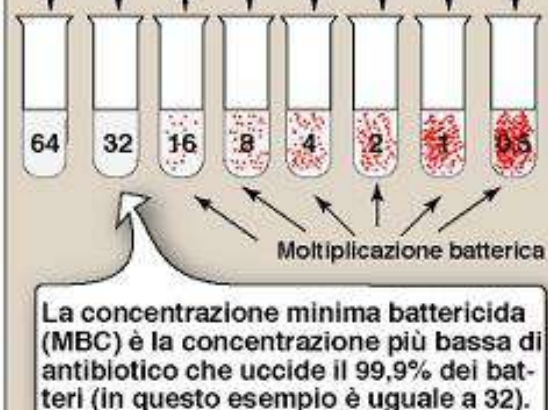
- 1** Provette contenenti varie concentrazioni di antibiotico sono inoculate con il microrganismo di studio.

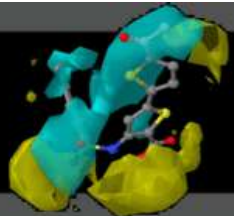


- 2** Si misura la moltiplicazione dei microrganismi dopo 24 ore di incubazione.



- 3** Subculture in mezzo privo di antibiotico e misurazione della moltiplicazione batterica dopo 24 ore di incubazione.





Attività antimicrobica di sostanze di origine naturale

by www.RCMD.it

Isolato	Sostanza	Temperatura 25°C		Temperatura ambiente 18-20 °C	
		MIC mg/ml	MBC mg/ml	MIC mg/ml	MBC mg/ml
Psa ER	EOMS	0,097	6,25	0,097	6,25
	REO	12,5	12,5	12,5	12,5
	TTO	6,25	>12,5	>12,5	>12,5
	SEO	12,5	12,5	12,5	12,5
	LEO	6,25	6,25	6,25	6,25
	Ampicillina	12,5 µg/ml	0,400	12,5 µg/ml	0,100
Psa COR	EOMS	3,12	3,12	0,39	3,12
	REO	12,5	12,5	12,5	12,5
	TTO	>12,5	12,5	>12,5	>12,5
	SEO	12,5	12,5	12,5	12,5
	LEO	6,25	6,25	6,25	6,25
	Ampicillina	0,200 µg/ml	0.400 µg/ml	0,100 µg/ml	0.400 µg/ml

EOMS: Menta suaveolens essential oil

REO: Rosmarino essential oil

TTO: Tea Tree oil

SEO: Salvia essential oil

LEO: Lauro essential oil





Attività antimicrobica di combinazione di sostanze

by www.RCMD.it

Isolato	Sostanza	Temperatura 25°C	
		MIC mg/ml	MBC mg/ml
Psa ER	S1+S4	1.56+1.56	3,12+3,12
	S1+S4+S5	1.56+1.56+3,12	<u>1.56+1.56+3,12</u>
	S1+S5	1,56+3,12	3,12+6,25
Psa COR	S1+S4	1,56+1,56	1.56+1.56
	S1+S4+S5	0,78 +0,78+1,56	<u>0,78+0,78+1,56</u>
	S1+S5	1,56+3,12	1,56 + 3.12

EOMS: Mentha suaveolens essential oil
(S1)

REO: Rosmarinus essential oil (S2)

TTO: Tea Tree oil (S3)

SEO: Salvia essential oil (S4)

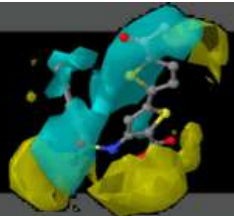
LEO: Lauro essential oil (S5)

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Crescita a temperatura elevata

by **www.RCMD.it**

Isolato	Sostanza	Temperatura 35°C	
		MIC mg/ml	MBC mg/ml
Psa ER	S1+S4	NC	1,56+1,56
	S1+S4+S5	NC	1,56+1,56+3,125
	S1+S5	NC	1,56+3,125
Psa COR	S1+S4	NC	0,78+0,78
	S1+S4+S5	NC	0,78+0,78+1,56
	S1+S5	NC	0,78+1,56

EOMS: Mentha suaveolens essential oil
(S1)

REO: Rosmarinus essential oil (S2)

TTO: Tea Tree oil (S3)

SEO: Salvia essential oil (S4)

LEO: Lauro essential oil (S5)



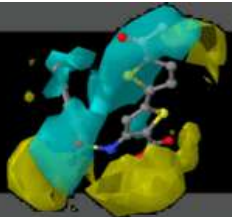
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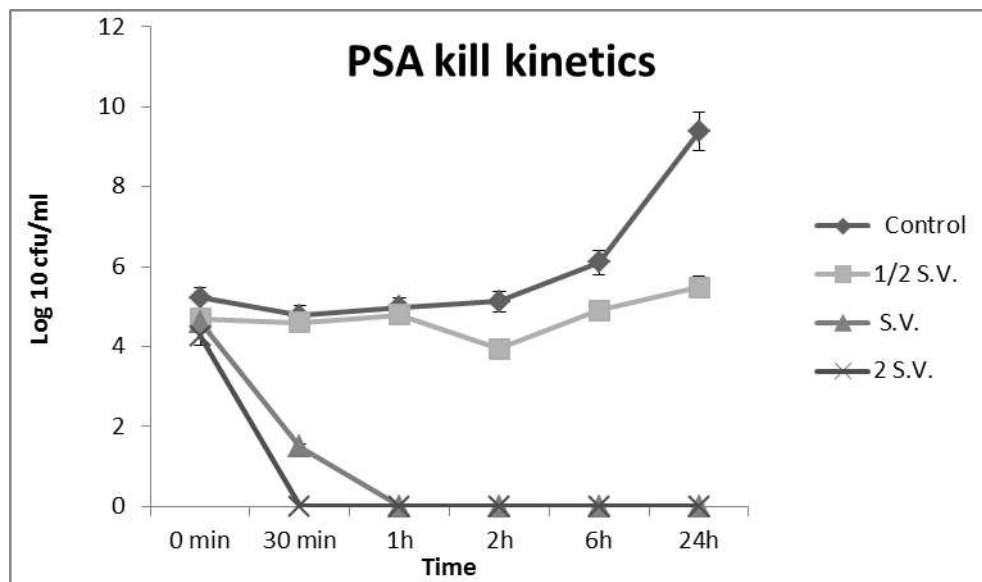
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Time-killing

by www.RCMD.it

Per confermare l'effetto sinergico degli EO combinati e valutare la cinetica dell'effetto letale, l'esperimento cinetico time-kill in vitro è stato condotto con PSA con concentrazioni equivalenti a 1/2, 1, 2 e 4 volte i loro valori sinergici (s.v). Come riportato in Figura, ad una concentrazione di 1/2; v.s. Il microrganismo PSA non è stato ucciso, mentre a concentrazione di 1 x s.v. (rispettivamente 0,78, 1,56 e 0,78 g/L di EOMS, REO e TTO), il numero di colonie (unità formanti colonie) è stato significativamente ridotto da 4,6 a 1,5 log dopo 30 minuti di incubazione e l'effetto battericida totale è stato osservato entro 1 ora dal contatto. Alla concentrazione di 2 x s.v. (rispettivamente 1,56, 3,12 e 1,56 g/L di EOMS, REO e TTO) il l'effetto battericida totale è stato osservato entro soli 30 minuti dal contatto. I risultati hanno dimostrato che il Psa era altamente suscettibile alla combinazione sinergica di concentrazione sub-inibitoria di EOMS, REO e TTO. Ciò suggerisce che i trattamenti combinati hanno esercitato un effetto più forte azione battericida.



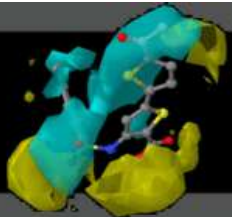
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Note Open Access

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Antibacterial Activity of Cinnamaldehyde and Estragole Extracted from Plant Essential Oils against *Pseudomonas syringae* pv. *actinidiae* Causing Bacterial Canker Disease in Kiwifruit

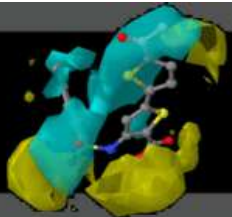
Yu-Rim Song¹, Min-Seon Choi^{1†}, Geun-Won Choi¹, Il-Kwon Park², and Chang-Sik Oh^{1*}

¹Department of Horticultural Biotechnology and Institute of Life Sciences & Resources, Kyung Hee University, Yongin 17104, Korea

²Department of Forest Sciences, Seoul National University, Seoul 08826, Korea

Pseudomonas syringae pv. *actinidiae* (Psa) causes bacterial canker disease in kiwifruit. Antibacterial activity of plant essential oils (PEOs) originating from 49 plant species were tested against Psa by a vapor diffusion and a liquid culture assays. The five PEOs from *Pimenta racemosa*, *P. dioica*, *Melaleuca linariifolia*, *M. cajuputii*, and *Cinnamomum cassia* efficiently inhibited Psa growth by either assays. Among their major components, estragole, eugenol, and methyl eugenol showed significant antibacterial activity by only the liquid culture assay, while cinnamaldehyde exhibited antibacterial activity by both assays. The minimum inhibitory concentrations (MICs) of estragole and cinnamaldehyde by the liquid culture assay were 1,250 and 2,500 ppm, respectively. The MIC of cinnamaldehyde by the vapor diffusion assay was 5,000 ppm. Based on the formation of clear zones or the decrease of optical density caused by these compounds, they might kill the bacterial cells and this feature might be useful for managing the bacterial canker disease in kiwifruit.





Research Article

Essential Oils with Inhibitory Capacities on *Pseudomonas syringae* pv. *actinidiae*, the Causal Agent of Kiwifruit Bacterial Canker

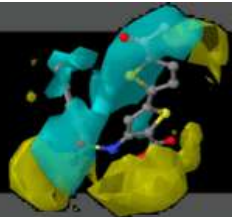
Pucci Nicoletta, Orzali Laura, Modesti Vanessa, Lumia Valentina, Brunetti Angela, Pilotti Massimo and Loreti Stefania

Consiglio per la Ricerca in agricoltura e l'analisi dell'Economia Agraria-Centro di ricerca Difesa e Certificazione, Sede di Roma-CREA-DC, Via C. G. Bertero 22, 00156, Roma, Italy

Abstract

Background and Objective: Bacterial canker of kiwifruit caused by *Pseudomonas syringae* pv. *actinidiae* (Psa) is one of the most severe bacterial disease of tree species and has been defined as a real pandemic. The Psa aggressiveness has made it very difficult to control with the use of single products, thus an integrated pest management seems to be key to successful control. The aim of this study was to evaluate the inhibitory capacity of 30 plant essential oils (EOs) against Psa. **Methodology:** The antimicrobial activity of EOs was performed by an *in vitro* assay based on an evaluation of the bacterial growth in a large volume of nutrient broth supplemented with EOs at different concentrations. The EOs that showed the strongest inhibitive capacities were further tested using a standardized broth microdilution method. Matching the results obtained with both tests, led to the selection of those EOs showing the strongest capacity to inhibit bacterial growth at the lowest concentrations. **Results:** This study shows that the most effective EOs against Psa were from clove bud (*Syzygium aromaticum*), thyme (*Thymus vulgaris*), oregano (*Origanum vulgare*), cinnamon (*Cinnamomum zeylanicum*) and to a lesser extent, garlic (*Allium sativum*). **Conclusion:** This large screening highlight the effectiveness of several EOs to be used for their antibacterial activity against Psa.





In vitro antimicrobial activity of plant extracts against *Pseudomonas syringae* pv. *actinidiae* causal agent of bacterial canker in kiwifruit

Giovanna Simonetti^{a*}, Nicoletta Pucci^{b*}, Elisa Brasili^a, Alessio Valletta^a, Iris Sammarco^c, Eleonora Carnevale^d, Gabriella Pasqua^a and Stefania Loreti^b

^aDepartment of Environmental Biology, Sapienza University of Rome, Rome, Italy; ^bCouncil for Agricultural Research and Economics, Research Centre for Plant Protection and Certification, Rome, Italy; ^cInstitute of Botany, The Czech Academy of Sciences (CAS), Pŕuhonice, Czech Republic; ^dDepartment of Public Health and Infectious Diseases, Sapienza University of Rome, Rome, Italy

ABSTRACT

Pseudomonas syringae pv. *actinidiae* (Psa), the causal agent of bacterial canker of kiwifruit, is considered the main pathogen of yellow-, green- and red-fleshed kiwifruit. All major kiwifruit producing countries in the world have been affected by this bacterial pathogen, leading to substantial economic losses. The control of bacterial canker of kiwifruit is based only on preventive methods or on the use of copper compounds that can cause phytotoxicity problems. In this study, the *in vitro* antibacterial activity of seven different plant extracts against eight Psa strains has been evaluated. The inhibition of 100% of the Psa growth was observed, after 24 h, for the extracts of *Polygonum cuspidatum* roots (POL-roots), *Hypericum perforatum* roots elicited with chitosan oligosaccharides (HYP-COS roots) and non-fermented grape pomace (ITA-pomace). The strongest antibacterial activity was exhibited by POL-roots, with a geometric mean of minimum inhibitory concentration of 100% of growth (GMMIC₁₀₀) of 105.11 µg/mL after 24 h, and with a GMMIC₁₀₀ value of 148.65 µg/mL after 48 h. Moreover, POL-roots extract showed the best bactericidal activity with a GMMBC of 210.22 µg/mL. No phytotoxic activity was observed up to 15 days in the leaves of *Actinidia chinensis* "Belen" treated with plant extracts at 500 µg/mL.

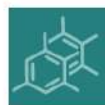
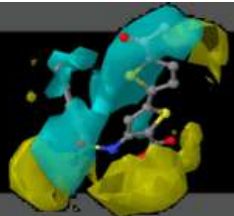
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KEYWORDS





Pseudomonas syringae pv. *actinidiae*; *Actinidia chinensis* "Belen"; plant extracts; antibacterial activity; non-fermented grape pomace extract; *Polygonum cuspidatum* root extract





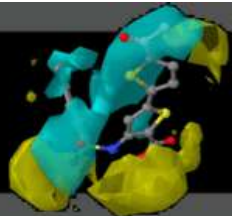
Article

A Synergic Potential of Antimicrobial Peptides against *Pseudomonas syringae* pv. *actinidiae*

Nuno Mariz-Ponte ^{1,2,3,*} , Laura Regalado ^{1,2}, Emil Gimranov ^{1,2}, Natália Tassi ⁴, Luísa Moura ⁵, Paula Gomes ⁴ , Fernando Tavares ^{1,3} , Conceição Santos ^{1,2} and Cátia Teixeira ⁴ 

Abstract: *Pseudomonas syringae* pv. *actinidiae* (Psa) is the pathogenic agent responsible for the bacterial canker of kiwifruit (BCK) leading to major losses in kiwifruit productions. No effective treatments and measures have yet been found to control this disease. Despite antimicrobial peptides (AMPs) having been successfully used for the control of several pathogenic bacteria, few studies have focused on the use of AMPs against Psa. In this study, the potential of six AMPs (BP100, RW-BP100, CA-M, 3.1, D4E1, and Dhvar-5) to control Psa was investigated. The minimal inhibitory and bactericidal concentrations (MIC and MBC) were determined and membrane damaging capacity was evaluated by flow cytometry analysis. Among the tested AMPs, the higher inhibitory and bactericidal capacity was observed for BP100 and CA-M with MIC of 3.4 and 3.4–6.2 μ M, respectively and MBC 3.4–10 μ M for both. Flow cytometry assays suggested a faster membrane permeation for peptide 3.1, in comparison with the other AMPs studied. Peptide mixtures were also tested, disclosing the high efficiency of BP100:3.1 at low concentration to reduce Psa viability. These results highlight the potential interest of AMP mixtures against Psa, and 3.1 as an antimicrobial molecule that can improve other treatments in synergic action.





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Molecules 2018, 23, 482; doi:10.3390/molecules23020482



Article

Antimicrobial and Antibiofilm Activity and Machine Learning Classification Analysis of Essential Oils from Different Mediterranean Plants against *Pseudomonas aeruginosa*

Marco Artini ^{1,†}, Alexandros Patsilnakos ^{2,3,4,†}, Rosanna Papa ¹, Mijat Božović ^{2,5}, Manuela Sabatino ^{2,3}, Stefania Garzoli ², Gianluca Vrenna ¹, Marco Tilotta ¹, Federico Pepi ², Rino Ragno ^{2,3,4,*} and Laura Selan ¹

Molecules 2020, 25, 2452; doi:10.3390/molecules25102452



Article

Experimental Data Based Machine Learning Classification Models with Predictive Ability to Select in Vitro Active Antiviral and Non-Toxic Essential Oils

Manuela Sabatino ^{1,†}, Marco Fabiani ^{2,†}, Mijat Božović ³, Stefania Garzoli ⁴, Lorenzo Antonini ¹, Maria Elena Marcocci ², Anna Teresa Palamara ^{2,5,*}, Giovanna De Chiara ^{6,†} and Rino Ragno ^{1,4,†}



Molecules 2019, 24, 890; doi:10.3390/molecules24050890



Article

Machine Learning Analyses on Data including Essential Oil Chemical Composition and In Vitro Experimental Antibiofilm Activities against *Staphylococcus* Species

Alexandros Patsilnakos ^{1,2,†}, Marco Artini ^{3,†}, Rosanna Papa ³, Manuela Sabatino ², Mijat Božović ⁴, Stefania Garzoli ⁵, Gianluca Vrenna ³, Raissa Buzzi ⁶, Stefano Manfredini ^{6,*}, Laura Selan ^{3,*} and Rino Ragno ^{1,2,*}

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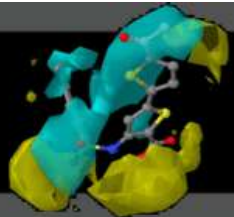


Article

Essential Oils Biofilm Modulation Activity, Chemical and Machine Learning Analysis—Application on *Staphylococcus aureus* Isolates from Cystic Fibrosis Patients

Rosanna Papa ^{1,†}, Stefania Garzoli ^{2,†}, Gianluca Vrenna ¹, Manuela Sabatino ^{2,3}, Filippo Sapienza ^{2,3}, Michela Relucenti ⁴, Orlando Donfrancesco ⁴, Ersilia Vita Fiscarelli ⁵, Marco Artini ¹, Laura Selan ^{1,*} and Rino Ragno ^{2,3,*}





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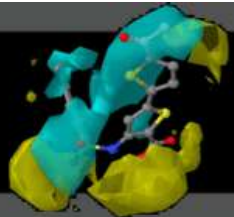
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Una miscela di oli essenziali quale potenziale rimedio per combattere il cancro del kiwi

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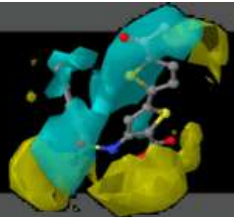


Conclusioni

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- Isolati dall'essudato o dal tronco condividevano lo stesso ceppo di Psa
- La produzione di EPS è correlata alla resistenza batterica e protezione contro diverse condizioni ambientali.
- EO hanno attività contro Psa
- I risultati chiaramente dimostrano che una miscela di tre EO (EOMS, REO e TTO), è in grado di uccidere il Psa a una concentrazione circa sedici volte inferiore ai corrispondenti valori MIC del singolo EO utilizzato da solo, dopo 1 h di esposizione.
- L'OE nei confronti dei batteri patogeni del kiwi suggerisce la possibilità di utilizzare le sostanze anche in questo raccolto.





Ringraziamenti

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