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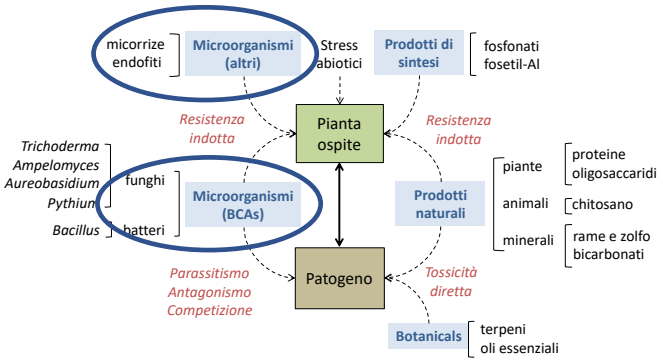
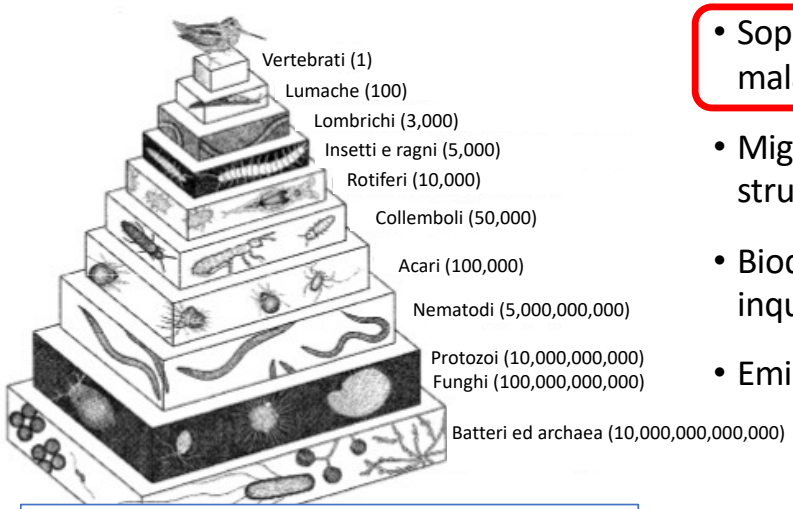
DISTAS

UNIVERSITÀ CATTOLICA del Sacro Cuore

I biopesticidi: dai prodotti naturali agli agenti di biocontrollo

La biodiversità microbica del suolo alla base del biocontrollo

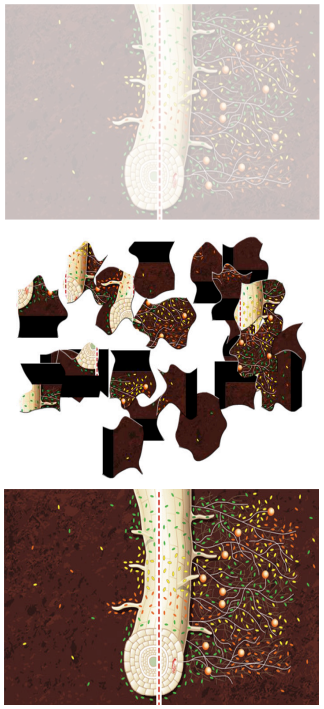




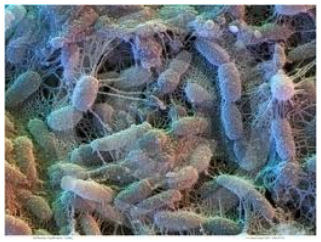
La biodiversità di 1 metro cubo di suolo

Cosa fanno?

- Decomposizione della sostanza organica
- Ciclo dei nutrienti
- Fissazione dell'N_2
- Soppressione delle malattie delle piante
- Miglioramento della struttura del suolo
- Biodegradazione degli inquinanti
- Emissioni di gas serra



Chi sono? Quanti sono ?



La biomassa microbica in 1 ha di suolo pesa circa come una vacca

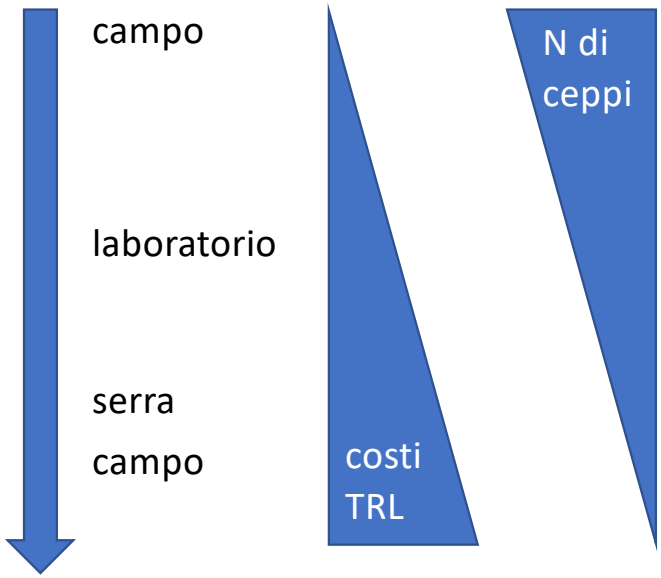
Dotazione microbiologica di un g di suolo di buona qualità:

- Oltre 1 miliardo di cellule
- Centinaia di migliaia di specie microbiche

Come scegliere i migliori agenti di biocontrollo: il bioprospecting

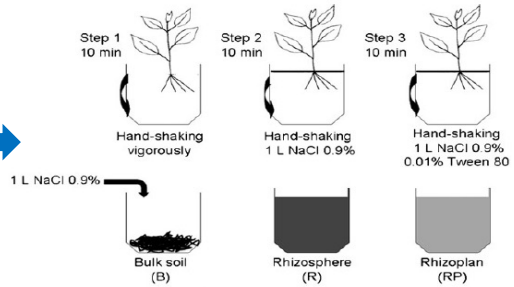
Bioprospecting -> *Sistematica ricerca di prodotti utili derivati da risorse biologiche incluse piante, animali e microorganismi che possono essere sfruttati per la commercializzazione ed il beneficio della società*

1. Isolamento di ceppi microbici dal suolo e dalla rizosfera
2. Dereplicazione ed identificazione tassonomica
3. Valutazione fenomica *in vitro* di proprietà di biocontrollo
4. Ranking quantitativo delle proprietà di biocontrollo
5. Analisi genomiche per confermare le proprietà, valutare sicurezza ed unicità (IP)
6. Rese in biomassa e scale-up industriale
7. Test di serra e di campo
8. Registrazione e commercializzazione

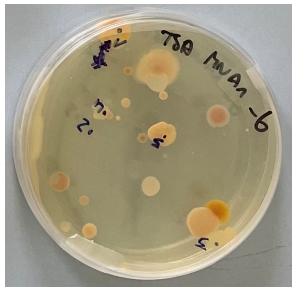


Bioprospecting: dal laboratorio al campo

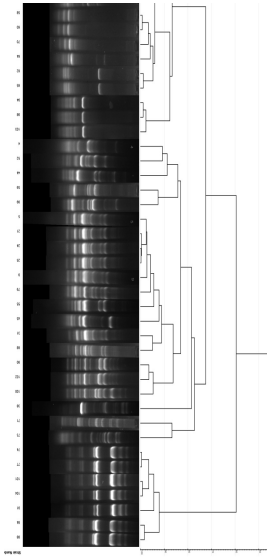
rizosfera



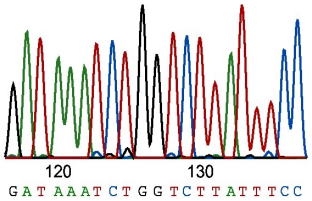
Tecniche di isolamento



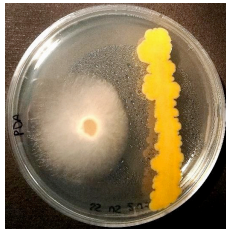
Culturomica



Dereplicazione molecolare

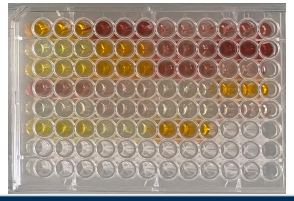


Identificazione molecolare



screening

biocontrollo



Eliminazione patogeni

Ranking

Test in planta

Genomica

Registrazione

Ranking quantitativo delle proprietà di biocontrollo (e biostimolazione)

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Table 4. Ranking of the rhizobacteria based on their in vitro PGP (plant growth promoting) and antifungal assay.

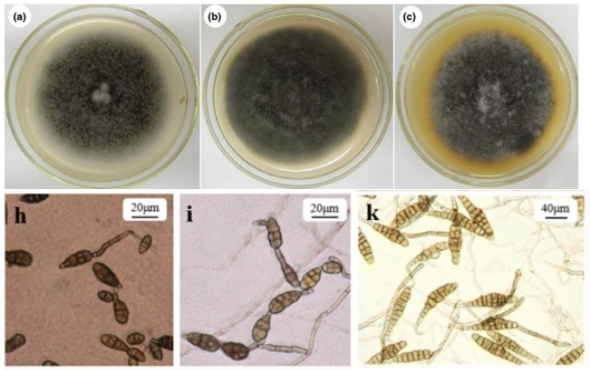
Code	Identity	*N* Fixation	*P* Solubilization	IAA Production		Antifungal Activity vs. *S. sclerotiorum*	Siderophore	Rank
				w/Try	w/o Try			
UC4094	*Enterobacter tabaci*	1	0.5	0.46	0.42	0.51	0.51	3.41
UC4098	*Stenotrophomonas rhizophila*	1	0.5	0.16	1,00	0.57	0.00	3.22
UC4109	*Enterobacter tabaci*	1	0.5	1.00	0.11	0.38	0.17	3.16
UC4127	*Klebsiella oxytoca*	1	0.5	0.25	0.22	1.00	0.09	3.06
UC4089	*Stenotrophomonas pictorum*	1	0.5	0.25	0.22	1.00	0.09	2.99
UC4105	*Stenotrophomonas pictorum*	1	0.25	0.17	0.03	0.75	0.78	2.98
UC4103	*[Pseudomonas] hibiscicola*	1	0.25	0.04	0.04	0.94	0.68	2.94
UC4123	*Klebsiella oxytoca*	1	0.5	0.25	0.21	0.93	0.02	2.92
UC4099	*Enterobacter tabaci*	1	0.5	0.36	0.09	0.45	0.51	2.90
UC4117	*Pseudomonas taiwanensis*	1	0.75	0.07	0.09	0.59	0.39	2.88
UC4113	*[Pseudomonas] hibiscicola*	1	0.25	0.04	0.03	0.85	0.68	2.86
UC4096	*Stenotrophomonas pavanii*	1	0.25	0.04	0.03	0.76	0.77	2.85
UC4090	*Aeromonas caviae*	1	0.5	0.18	0.12	0.74	0.30	2.84
UC4106	*Enterobacter ludwigii*	1	0.5	0.36	0.26	0.60	0.08	2.80
UC4093	*Stenotrophomonas pictorum*	1	0.25	0.02	0.01	0.60	0.91	2.79
UC4082	*Pseudomonas pseudoalcaligenes*	1	0.25	0.01	0.03	0.90	0.60	2.79
UC4084	*Kosakonia radicincitans*	1	0.5	0.04	0.02	0.65	0.58	2.78
UC4091	*Pseudomonas pseudoalcaligenes*	1	0	0.22	0.05	0.92	0.57	2.76
UC4101	*Klebsiella grimontii*	1	0.5	0.25	0.25	0.61	0.01	2.61
UC4118	*Klebsiella oxytoca*	1	0.5	0.26	0.22	0.43	0.11	2.52
UC4088	*Pseudomonas indoloxydans*	1	0	0.06	0.06	0.86	0.53	2.50
UC4087	*Pseudomonas indoloxydans*	1	0	0.05	0.06	0.82	0.55	2.48
UC4092	*Kosakonia radicincitans*	1	0.5	0.13	0.13	0.66	0.06	2.48
UC4104	*Stenotrophomonas rhizophila*	1	0.25	0.04	0.02	0.56	0.58	2.46
UC4110	*Kosakonia oryzendophytica*	1	0.5	0.06	0.04	0.60	0.18	2.39
UC4126	*Pseudomonas japonica*	1	0.5	0.18	0.13	0.11	0.42	2.33
UC4122	*Pseudomonas taiwanensis*	1	0.75	0.00	0.03	0.20	0.22	2.20
UC4125	*Delftia tsuruhatensis*	1	0	0.01	0.01	0.75	0.28	2.05
UC4102	*Chryseobacterium ureilyticum*	0	0.25	0.04	0.03	0.74	0.98	2.04
UC4120	*Chryseobacterium rhizosphaerae*	0	0	0.18	0.02	0.82	0.97	1.99
UC4086	*Klebsiella oxytoca*	0	0.5	0.54	0.31	0.42	0.19	1.96
UC4112	*Pseudomonas taiwanensis*	1	0.5	0.01	0.03	0.17	0.23	1.95
UC4081	*Chryseobacterium oranimense*	0	0.25	0.04	0.03	0.66	0.91	1.89
UC4083	*Stenotrophomonas acidamiphila*	0	0	0.05	0.04	0.77	0.75	1.61
UC4107	*Sphingobacterium canadense*	0	0	0.06	0.02	0.46	1.00	1.54
UC4108	*Chryseobacterium rhizosphaerae*	0	0	0.04	0.03	0.65	0.81	1.53
UC4080	*Sphingobacterium detergens*	0	0	0.04	0.01	0.45	0.79	1.29
UC4121	*Sphingobacterium siyangense*	0	0	0.00	0.00	0.40	0.87	1.27

w/Try and w/o Try stands for with or without DL-Tryptophan.

Attività di biocontrollo di *Alternaria* spp. ed alternariosi in pomodoro

96 isolati batterici
VS.
3 specie di *Alternaria*

(a) *A. tenuissima* (b) *A. alternata* (c) *A. solani*



Dual plate assay

ranking effetti positivi

REDUCTION OF FUNGI BIOMASS OR MICOTOXINS PRODUCTION	RANK
0	0
1-25%	0,5
25.1-50%	1,0
50.1-75%	1,5
75.1-100%	2

Ranking effetti negativi

INCREASE FUNGI BIOMASS OR MICOTOXINS PRODUCTION	RANK
0-25%	-1.0
25.1-50%	-2.0
>50%	-3.0

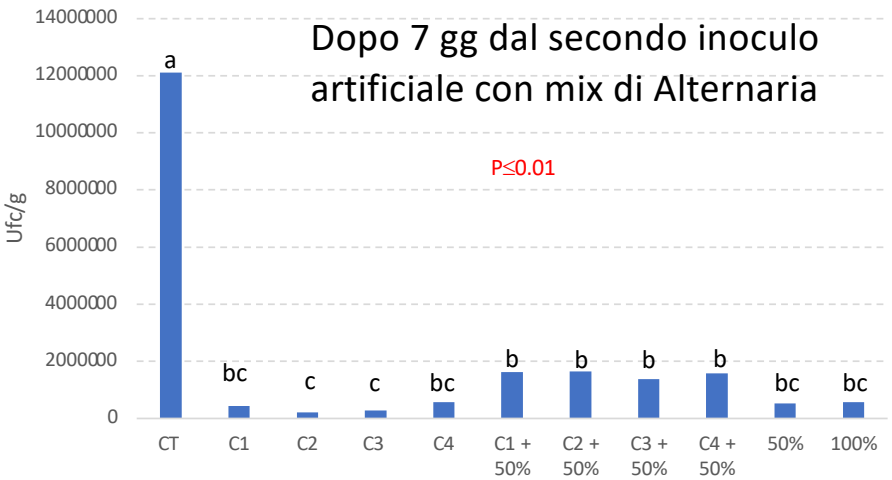
LC-MS/MS

Micotossine di *Alternaria spp.*:

- **Tenuazonic acid (TeA)**
- Alternariol (AOH)
- Alternariol-methyl-ether (AME)
- Tentoxin (TEN)

Misura della variazione in biomassa in confronto al controllo (*Alternaria spp*. da sola)

Test in vaso attività di biocontrollo di *Alternaria* spp.



TESI	CODICE
Testimone non trattato	CT
Consorzi	C1
	C2
	C3
	C4
Consorzi + 50% fungicida	C1+50%
	C2+50%
	C3+50%
	C4+50%
50% fungicida	50%
100% fungicida	100%

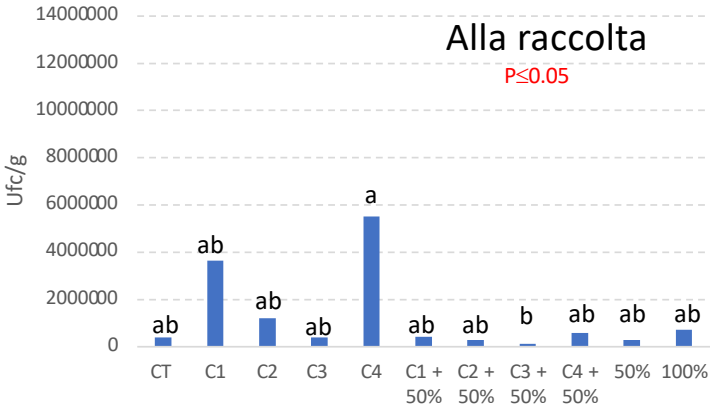


TABLE 3 | Biosynthetic gene clusters as predicted by antiSMASH in the nine *Pseudomonas* genomes are shown here.

	Region	Type	From	To	Most similar known cluster
R32	Region 1	NRPS-like	108,600	137,959	Mangotoxin
	Region 2	arylpolyene	301,125	344,738	APE Vf
	Region 3	NRPS	1,763,102	1,815,679	Pyoverdine
	Region 4	NRPS	3,581,365	3,648,859	Pyoverdine
R47	Region 1	NRPS-like	114,171	144,819	Mangotoxin
	Region 2	arylpolyene	499,588	543,208	APE Vf
	Region 3	other	4,075,994	4,117,076	Pyrrolnitrin
	Region 4	NRPS, resorcinol	4,846,097	4,925,365	Pyoverdine
	Region 5	NRPS	4,969,815	5,022,843	Pyoverdine
R76	Region 1	NRPS-like	159,850	202,848	Mangotoxin
	Region 2	arylpolyene	525,506	569,081	APE Vf
	Region 3	NRPS	2,841,936	2,945,551	Viscosin
	Region 4	NRPS	3,563,564	3,604,450	Pyochelin
	Region 5	NRPS	4,364,529	4,407,587	Poaeamide
	Region 6	NRPS	4,766,660	4,819,556	Pyoverdine
R84	Region 2	NRPS-like	131,789	161,456	Mangotoxin
	Region 3	arylpolyene	499,287	542,891	APE Vf
	Region 4	NRPS, terpene	2,169,525	2,234,015	Pyoverdine
	Region 5	NRPS	2,763,610	2,840,351	Anikasin
	Region 6	NRPS	4,533,076	4,586,074	Pyoverdine
S04	Region 1	arylpolyene	423,375	467,009	APE Vf
	Region 2	NRPS	2,828,784	2,901,359	Pyoverdine
	Region 3	terpene	4,329,654	4,350,538	Pyoverdine
S19	Region 1	arylpolyene	423,375	467,009	APE Vf
	Region 2	NRPS	2,828,790	2,901,365	Pyoverdine
	Region 3	terpene	4,329,659	4,350,543	Pyoverdine
S34	Region 1	NRPS-like	96,203	125,383	Mangotoxin
	Region 2	arylpolyene	431,851	475,456	APE Vf
	Region 3	NRPS	4,344,492	4,408,473	Pyoverdine
	Region 4	NRPS	4,455,540	4,508,538	Pyoverdine
S35	Region 1	NRPS-like	109,236	150,641	Mangotoxin
	Region 2	arylpolyene	474,763	518,338	APE Vf
	Region 3	terpene, NRPS	2,731,917	2,858,142	Viscosin
	Region 4	NRPS	4,185,638	4,230,966	WLIP
	Region 5	NRPS	4,576,688	4,629,596	Pyoverdine
S49	Region 1	NRPS-like	120,934	150,633	Mangotoxin
	Region 2	arylpolyene	473,010	516,614	APE Vf
	Region 3	NRPS	2,191,853	2,267,429	Pyoverdine
	Region 4	NRPS	3,906,071	3,982,443	Anikasin
	Region 5	NRPS	4,658,162	4,711,160	Pyoverdine

For each region, the biosynthetic cluster type, the genomic boundaries (in bp) and the most similar known biosynthetic gene cluster are shown.

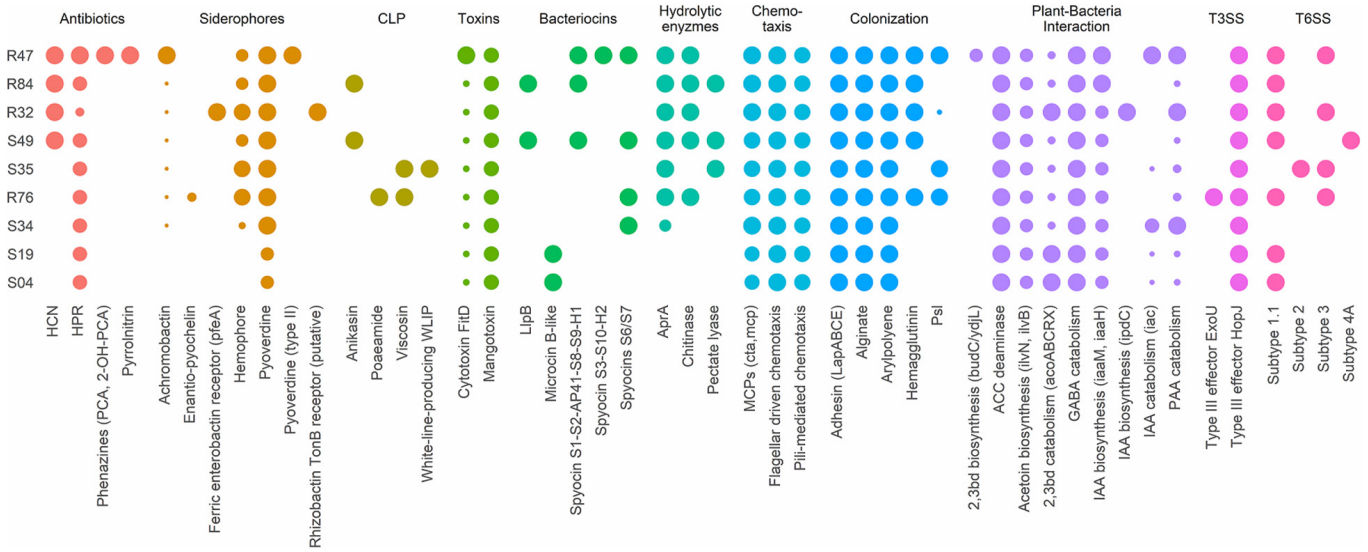


FIGURE 3 | Presence/absence table of known antibiotics, siderophores, cyclic lipopeptides (CLP), toxins, bacteriocins, and extracellular hydrolases with reported antibacterial, antifungal or anti-oomycetal properties (2,3bd, 2,3-butanediol; PAA, phenylacetic acid; IAA, indole-3-acetic acid, GABA, γ-aminobutyric acid; ACC, 1-aminocyclopropane-1-carboxylic acid). All genomes were also screened for genes implicated in chemotaxis, plant colonization, plant-bacteria interactions, type III secretion systems (T3SS) and type VI secretion systems (T6SS). Full sized dots indicate the presence of homologs for all necessary genes or complete gene clusters. Small dots indicate incomplete clusters and dot size is proportional to the number of homologous genes found. All genes and reference sequences, including the genes encoding for traits for which no homologous genes were found, can be found in **Supplementary Table S4**. Strains are grouped according to their phenotypical activity *in vitro* (R47, R84, R32, and S49) and on plant tissue (R32, S49, S35). The least inhibiting strains are at the bottom of the table (S34, S19, S04).

Conclusioni

- Il suolo, la rizosfera e la fillosfera sono una preziosissima riserva di agenti di biocontrollo
- Metodi ben consolidati per il bioprospecting e la quantificazione di attività di biocontrollo
- Ranking quantitativo fondamentale per selezionare i migliori ceppi
- Ricerca fondamwntale per comprendere e sfruttarre meglio alcuni processi come la resistenza sistemica indotta
- Rese in biomassa e scale-up industriale prerequisiti per l'economicità delle soluzioni
- Genomica fondamentale per valutare sicurezza e attività dei ceppi
- Ricerca necessaria per calibrare ed ottimizzare modi di produzione ed impiego, dosi di applicazione e co-applicazione con fertilizzanti e fitofarmaci
- Gli avanzamenti scientifici possono supportare la regolamentazione

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