

Presentazione dei risultati degli studi interlaboratorio sui Biostimolanti

5 Dicembre 2023

Dr Sandro Spezia

TC 455 Standardization Request

- 2021: Accettazione da parte del CEN della Standardization request (SR) formulata dalla Commissione UE in vista dell'entrata in vigore del nuovo Regolamento Europeo sui fertilizzanti.
- In pratica, la SR chiede al CEN che vengano predisposte 68 nuove norme armonizzate, quale riferimento per i necessari controlli di qualità dei prodotti che rientrano all'interno del Regolamento,
- E' stata acquisita la convenorship del WG 3 "Pathogenic and non-pathogenic microorganisms" del TC 455 "Plant biostimulants" e WG 5
- E' stato quindi predisposto un piano operativo pluriennale delle attività assegnate ai due WG, che rientrano nel piano di competenza del TC 455

TS pubblicati

1. CEN/TS 17700-1:2022 (WI=00455039) Plant biostimulants - Claims - Part 1: General principles
2. CEN/TS 17700-2:2022 (WI=00455045) Plant biostimulants - Claims - Part 2: Nutrient use efficiency resulting from the use of a plant biostimulant
3. CEN/TS 17700-3:2022 (WI=00455054) Plant biostimulants - Claims - Part 3: Tolerance to abiotic stress resulting from the use of a plant biostimulant
4. CEN/TS 17700-4:2022 (WI=00455038) Plant biostimulants - Claims - Part 4: Determination of quality traits resulting from the use of a plant biostimulant
5. CEN/TS 17700-5:2022 (WI=00455044) Plant biostimulants - Claims - Part 5: Determination of availability of confined nutrients in the soil or rhizosphere
6. CEN/TS 17701-1:2022 (WI=00455058) Plant biostimulants - Determination of specific elements - Part 1: Digestion by aqua regia for subsequent determination of elements
7. CEN/TS 17701-2:2022 (WI=00455059) Plant biostimulants - Determination of specific elements - Part 2: Determination of total content of Cd, Pb, Ni, As, Cr, Cu and Zn
8. CEN/TS 17701-3:2022 (WI=00455012) Plant biostimulants - Determination of specific elements - Part 3: Determination of mercury
9. CEN/TS 17702-1:2022 (WI=00455035) Plant biostimulants - Sampling and sample preparation - Part 1: Sampling
10. CEN/TS 17702-2:2022 (WI=00455036) Plant biostimulants - Sampling and sample preparation - Part 2: Sample preparation
11. CEN/TS 17703:2022 (WI=00455011) Plant biostimulants - Determination of chromium(VI)
12. CEN/TS 17704:2022 (WI=00455025) Plant biostimulants - Determination of dry matter
13. CEN/TS 17705:2022 (WI=00455028) Plant biostimulants - Determination of phosphonates
14. CEN/TS 17706:2022 (WI=00455034) Plant biostimulants - Determination of inorganic arsenic
15. CEN/TS 17707:2022 (WI=00455037) Plant biostimulants - Determination of the yeast and mould content
16. CEN/TS 17708:2022 (WI=00455040) Plant biostimulants - Preparation of sample for microbial analysis
17. CEN/TS 17709:2022 (WI=00455041) Plant biostimulants - Determination of Azotobacter spp.

TS pubblicati

18. CEN/TS 17710:2022 (WI=00455042) Plant biostimulants - Detection of *Listeria monocytogenes*
19. CEN/TS 17711:2022 (WI=00455043) Plant biostimulants - Detection of *Vibrio* spp.
20. CEN/TS 17712:2022 (WI=00455046) Plant biostimulants - Detection of *Staphylococcus aureus*
21. CEN/TS 17713:2022 (WI=00455047) Plant biostimulants - Determination of *Azospirillum* spp.
22. CEN/TS 17714:2022 (WI=00455048) Plant biostimulants - Determination of microorganisms' concentration
23. CEN/TS 17715:2022 (WI=00455049) Plant biostimulants - Detection of *Shigella* spp.
24. CEN/TS 17716:2022 (WI=00455050) Plant biostimulants - Determination of *Escherichia coli*
25. CEN/TS 17717:2022 (WI=00455051) Plant biostimulants - Detection of *Salmonella* spp.
26. CEN/TS 17718:2022 (WI=00455052) Plant biostimulants - Determination of *Rhizobium* spp.
27. CEN/TS 17719:2022 (WI=00455053) Plant biostimulants - Determination of the anaerobic plate count
28. CEN/TS 17720:2022 (WI=00455055) Plant biostimulants - Determination of Enterococcaceae
29. CEN/TS 17721:2022 (WI=00455056) Plant biostimulants - Determination of the pH for liquid microbial plant biostimulants/pH in microbial products - Determination of pH
30. CEN/TS 17722:2022 (WI=00455057) Plant biostimulants - Determination of mycorrhizal fungi
31. CEN/TS 17723:2022 (WI=00455060) Plant biostimulants - Determination of chloride
32. CEN/TS 17724:2022 (WI=00455061) Plant biostimulants - Terminology
33. CEN/TS 17725:2022 (WI=00455062) Plant biostimulants - Determination of the quantity (indicated by mass or volume)

Progetti

Project	Project Leader
Position 1 - Plant Biostimulants - Determination of Shigella spp (TS and hEN)	Emanuela Biagioni Angeli
Position 2 - Plant Biostimulants - Determination of Staphylococcus aureus (TS and hEN)	Sara García Figuera
Position 3 - Plant Biostimulants - Determination of Azospirillum spp (TS and hEN)	Karine Picard
Position 4 - Plant Biostimulants - Determination of Listeria monocytogenes (TS and hEN)	Maurizio Ruzzi
Position 5 - Plant Biostimulants - Determination of Salmonella spp (TS and hEN)	Marie-Laure Guillotin
Position 6 - Plant Biostimulants - Determination of Rhizobium spp (TS and hEN)	Manuele Ricci
Position 7 - Plant Biostimulants - Anaerobic plate count (TS and hEN)	Maurizio Ruzzi
Position 8 - Plant Biostimulants - Determination of mycorrhizal fungi (TS and hEN)	Christian Hecker
Position 9 - Plant Biostimulants - Determination of the pH for liquid microbial plant biostimulants/pH in microbial products (TS and hEN)	Simonetta Gallerini
Position 10 - Plant Biostimulants - Determination of Enterococcaceae (TS and hEN)	Sara García Figuera
Position 11 - Plant Biostimulants - Determination of Vibrio spp (TS and hEN)	Simonetta Gallerini
Position 12 - Plant Biostimulants - Determination of Escherichia coli (TS and hEN)	Martina Buccella
Position 13 - Plant Biostimulants - Determination of Azotobacter spp. fungi (TS and hEN)	Manuele Ricci
Position 14 - Plant Biostimulants - Determination of the yeast and mould content (TS and hEN)	Martina Buccella
Position 15 - Plant Biostimulants - Determination of microorganisms concentration (TS and hEN)	Karine Picard
Position 16 - Plant Biostimulants - Preparation of sample for microbial analysis (TS and hEN)	Emanuela Biagioni Angeli

Biostimolanti – Prove interlaboratorio realizzate

	Working Group di riferimento	oggetto	Periodo di esecuzione
1° Gruppo	WG3	Determination of mycorrhizal fungi	Maggio-Giugno 2022
	WG5	Quantity (mass and Volume)	
2° Gruppo (Le prove del gruppo tutte su "substrato" Azotobacter)	WG3	Determination of Azotobacter spp.	Giugno – Luglio 2022
	WG3	Detection of Vibrio spp	
	WG3	Determination of Escherichia coli	
	WG3	Determination of the Yeast and Mould content	
3° Gruppo (Le prove del gruppo tutte su "substrato" Rhizobium)	WG3	Determination of Rhizobium spp.	Agosto – Settembre 2022
	WG3	Detection of Listeria	
	WG3	Detection of Salmonella spp.	
	WG3	Determination of Enterococcaceae	
	WG3	Determination of pH in microbial products	
4° Gruppo (Le prove del gruppo tutte su "substrato" Azospirillum)	WG3	Determination of Azospirillum spp.	Settembre – Ottobre 2022
	WG3	Detection of Shigella spp .	
	WG3	Detection of Staphylococcus aureus	
	WG3	Determination of the anaerobic plate count	
	WG5	Determination of the chloride	
Partnership con ILSA Lab	WG4	Determination of Chromium VI	Ottobre – Novembre 2022

Interlaboratory Studies

Specific commercial products

*CEN/TC 455/WG 5
“Labelling and denominations”*

*CEN/TC 455/WG 3
“Pathogenic and non-pathogenic microorganisms”*

❖ Quantity (density)

3 LIQUID PRODUCTS

- Liquid
- Thick liquid
- Very viscous

❖ Quantity (mass)

6 SOLID PRODUCTS

(laboratory will have to determine the weight of each one, then will have to send the packages to another laboratory; every 4 laboratories, the packages will be returned to the organizer for an intermediate check).

2 BLEND PRODUCTS:

- Liquid blend (liquid biostimulant + liquid fertilizer)
- Solid blend (solid biostimulant + solid fertilizer) (1 solid blend for genotype identification= solid biostimulant + solid fertilizer)

3 BIOSTIMULANT PRODUCTS:

- Liquid
- Solid 1 Pellet containing one or more beneficial (azospirillum, azotobacter etc.)
- Solid 2 Growing substrate (based on peat, organic substances with N, P and carbonates, and containing one or more beneficial - azospirillum, azotobacter etc.)

Number of samples

Working group	Analysis	Project leader	N. Laboratories	N. Samples
WG3	Detection of <i>Shigella</i> spp.	Biagioni Angeli	8	7 (tot. 56)
	Detection of <i>Staphylococcus aureus</i>	Garcia Prospero & Partners	11	7 (tot. 77)
	Determination of <i>Azospirillum</i> spp.	Picard	8	10 (tot. 80)
	Detection of <i>Listeria monocytogenes</i>	Ruzzi Prospero & Partners	11	7 (tot. 77)
	Detection of <i>Salmonella</i> spp .	Guillotini	11	7 (tot. 77)
	Determination of <i>Rhizobium</i> spp.	Ricci	8	10 (tot. 80)
	Anaerobic plate count	Ruzzi Prospero & Partners	9	7 (tot. 63)
	Determination of mycorrhizal fungi	Hecker	11	10 (tot. 110)
	Determination of pH	Gallerini	12	7 (tot. 84)
	Determination of <i>Enterococcaceae</i>	Garcia Prospero & Partners	9	7 (tot. 63)
	Detection of <i>Vibrio</i> spp.	Gallerini	8	7 (tot. 56)
	Determination of <i>Escherichia coli</i>	Buccella	12	7 (tot. 84)
	Determination of <i>Azotobacter</i> spp.	Ricci	8	10 (tot. 80)
	Determination of the yeast and mould content	Buccella	12	7 (tot. 84)
WG5	Quantity (mass)	Chenon Prospero & Partners	9	15 (tot. 135)
	Quantity (density)		9	3 (tot. 27)
	Determination of the Chloride	Gallerini	11	5 (tot. 55)

ILS structure

Four groups of analysis

1st Group

WG3
Determination of mycorrhizal fungi
WG5
Quantity (density)
Quantity (mass)

2nd Group

WG3
Determination of <i>Azotobacter</i> spp.
Detection of <i>Vibrio</i> spp
Determination of <i>Escherichia coli</i>
Determination of the Yeast and Mould content

WG3

Determination of <i>Rhizobium</i> spp.
Detection of <i>Listeria</i>
Detection of <i>Salmonella</i> spp.
Determination of <i>Enterococcaceae</i>
Determination of pH in microbial products

3rd Group

WG3

Determination of <i>Azospirillum</i> spp.
Detection of <i>Shigella</i> spp .
Detection of <i>Staphylococcus aureus</i>
Determination of the anaerobic plate count
WG5
Determination of the chloride

4th Group

Shigella

Sample	Matrix	l	n	o %	Real value	N° of correct results	N° of false positive/negative	Correct results %
01_ML_01+02	Liquid blend	11	33	0	Absent	31	2	93,9%
01_MS_01+02	Solid blend	11	33	0	Absent	33	0	100%
01_BL_01	Bio.liquid	11	33	0	Present	33	0	100%
01_PR_01	Solid 1	11	33	0	Present	30	3	90,9%
01_PS_01	Solid 2	11	33	0	Absent	30	3	90,9%
l n o	number of laboratories after outlier rejection number of individual test results after outlier rejection percentage of outliers							

Staphylococcus aureus

Sample	Matrix	l	n	o %	Real value	N° of correct results	N° of false positive/negative	Correct results %
02_ML_01+02	Liquid blend	13	39	0	Absent	38	1	97,4%
02_MS_01+02	Solid blend	13	39	0	Absent	37	2	94,9%
02_BL_01	Bio.liquid	13	39	0	Present	37	2	94,9%
02_PR_01	Solid 1	13	39	0	Present	38	1	97,4%
02_PS_01	Solid 2	13	39	0	Absent	37	2	94,9%
l n o	number of laboratories after outlier rejection number of individual test results after outlier rejection percentage of outliers							

Listeria monocytogenes

Sample	Matrix	l	n	o %	Real value	N° of correct results	N° of false positive/negative	Correct results %
04_ML_01+02	Liquid blend	13	39	0	Present	39	0	100%
04_MS_01+02	Solid blend	13	39	0	Absent	36	3	92,3%
04_BL_01	Bio.liquid	13	39	0	Absent	36	3	92,3%
04_PR_01	Solid 1	13	39	0	Present	39	0	100%
04_PS_01	Solid 2	13	39	0	Present	39	0	100%
l n o	number of laboratories after outlier rejection number of individual test results after outlier rejection percentage of outliers							

Escherichia Coli

Sample	Matrix	l	n	o %	\bar{x} CFU/g	\bar{x} Log ₁₀	s _R Log ₁₀	s _r Log ₁₀
12_ML_01+02	Liquid blend	12	60	14	3,17*10 ²	2,501	0,389	0,241
12_MS_01+02	Solid blend	11	55	21	3,31*10 ²	2,521	0,537	0,197
12_BL_01	Bio.liquid	12	60	14	3,63*10 ²	2,560	0,500	0,191
12_PR_01	Solid 1	11	55	21	6,89*10 ²	2,838	0,478	0,1900
12_PS_01	Solid 2	10	50	29	2,80*10 ⁴	4,447	0,330	0,218
l n o \bar{x} s _R s _r	number of laboratories after outlier rejection number of individual test results after outlier rejection percentage of outliers overall mean of results (without outliers) s _R reproducibility standard deviation s _r repeatability standard deviation							

Enterococcaceae

Sample	Matrix	l	n	o %	\bar{x} CFU/g	\bar{x} Log ₁₀	s _R Log ₁₀	s _r Log ₁₀
10_ML_01+02	Liquid blend	9	41	18	1,05*10 ⁵	5,130	0,496	0,168
10_MS_01+02	Solid blend	9	42	18	3,22*10 ⁵	5,508	0,456	0,175
10_BL_01	Bio.liquid	8	36	27	4,21*10 ⁵	5,624	0,337	0,134
10_PR_01	Solid 1	8	39	27	3,12*10 ⁵	5,494	0,444	0,150
10_PS_01	Solid 2	8	36	27	4,02*10 ⁵	5,604	0,285	0,178
l	number of laboratories after outlier rejection							
n	number of individual test results after outlier rejection							
o	percentage of outliers							
\bar{x}	overall mean of results (without outliers)							
s _R	s _R reproducibility standard deviation							
s _r	s _r repeatability standard deviation							

Azospirillum spp.

Sample	Matrix	LAB	l	n	o %	\bar{x} CFU/g	\bar{x} Log ₁₀	s _R Log ₁₀	S _r Log ₁₀
01_ML_01+02	Liquid blend	9	6	17	5,5	3,75*10 ⁶	6,574	0,509	0,182
01_MS_01+02	Solid blend	9	6	18	0	8,12*10 ³	3,910	0,498	0,158
01_BL_01	Bio.liquid	9	6	18	0	1,61*10 ⁵	5,206	0,439	0,263
01_PR_01	Solid 1	9	5	15	16,7	2,13*10 ⁴	4,329	0,544	0,073
01_PS_01	Solid 2	9	5	15	16,7	8,07*10 ⁴	4,907	0,225	0,049
LAB	Total number of participant laboratories								
l	number of laboratories after outlier rejection								
n	number of individual test results after outlier rejection								
o	percentage of outliers (blunders excluded)								
\bar{x}	overall mean of results (without outliers)								
s _R	s _R reproducibility standard deviation								
S _r	s _r repeatability standard deviation								

Rhizobium spp.

Sample	Matrix	LAB	Blunders	l	n	o %	\bar{x} CFU/g	\bar{x} Log ₁₀	s _R Log ₁₀	s _r Log ₁₀
01_ML_01+02	Liquid blend	9	2	6	18	14	7,54*10 ⁹	9,878	0,532	0,152
01_MS_01+02	Solid blend	9	2	7	21	0	8,49*10 ⁸	8,929	0,539	0,168
01_BL_01	Bio.liquid	9	2	7	21	0	4,88*10 ⁹	9,689	0,504	0,115
01_PR_01	Solid 1	9	2	6	18	0	6,00*10 ⁸	8,778	0,756	0,097
01_PS_01	Solid 2	9	2	7	21	0	4,76*10 ⁸	8,678	0,333	0,116
LAB	Total number of participant laboratories									
l	number of laboratories after outlier rejection									
n	number of individual test results after outlier rejection									
o %	percentage of outliers (blunders excluded)									
\bar{x}	overall mean of results (without outliers)									
s _R	s _R reproducibility standard deviation									
s _r	s _r repeatability standard deviation									

pH

Sample	Matrix	l	n	o %	\bar{x} upH	s_R upH	C_{VR} %	s_r upH	C_{Vr} %
09_ML_01+02	Liquid blend	13	65	7,7	7,855	0,122	1,5%	0,014	0,2%
09_BL_01	Biostimulant in liquid formulation containing Bradyrhizobium japonicum	14	70	0	4,168	0,347	8,3%	0,019	0,4%
09_BL_02	Fluid protein concentrate completely of vegetal origin with a high content of amino acids, low MW peptones, vitamins, macro and microelements.	14	70	0	4,107	0,134	3,3%	0,018	0,4%
09_BL_03	Natural Adjuvant carboxymethylcellulose (CMC) based solution and derived from glucides constituting plant cell wall.	14	70	0	7,267	0,217	2,9%	0,022	0,3%

l number of laboratories after outlier rejection
n number of individual test results after outlier rejection
o % percentage of outliers
 \bar{x} overall mean of results (without outliers)
 s_R s_R reproducibility standard deviation
 $C_{V,R}$ $C_{V,R}$ coefficient of variation of reproducibility
 s_r s_r repeatability standard deviation
 $C_{V,r}$ $C_{V,r}$ coefficient of variation of repeatability

Cloruri

Sample	Matrix	l	n	o %	\bar{x} g/kg	s_R g/kg	C_{VR} %	s_r g/kg	C_{Vr} %
17_ML_01+02	Liquid blend	7	35	36	24,80	0,81	3,2%	0,46	1,8%
17_MS_01+02	Solid blend	8	40	27	14,05	1,46	10,4%	0,79	5,6%
17_BL_01	Bio.liquid	6	30	0	0,14	0,97	70,8%	0,011	8,3%
17_PR_01	Solid 1	7	35	36	29,09	1,39	4,8%	1,28	4,4%
17_PS_01	Solid 2	8	40	27	2,55	0,29	11,4%	0,13	5,1%
l n o \bar{x} s_R $C_{V,R}$ s_r $C_{V,r}$	number of laboratories after outlier rejection number of individual test results after outlier rejection percentage of outliers overall mean of results (without outliers) s_R reproducibility standard deviation $C_{V,R}$ coefficient of variation of reproducibility s_r repeatability standard deviation $C_{V,r}$ coefficient of variation of repeatability								

Cr VI

Sample	Matrix	l	n	o %	X	\bar{x} mg/kg	η	s_R mg/kg	C_{VR} %	s_r mg/kg	C_{Vr} %
LB-001	Liquid blend	12	60	0	46,30	46,98	98,6%	0,91	1,96%	0,55	1,19%
SB-002	Solid blend	11	55	8,3%	24,86	25,76	96,5%	1,48	5,97%	1,48	5,97%
LPB-003	Liquid biostimulant	11	55	8,3%	8,18	8,25	99,1%	0,19	2,27%	0,14	1,71%
SPB-004	Solid 1	12	60	0	19,62	19,08	102,8%	1,34	6,83%	1,34	6,83%
SPB-005	Solid 2	10	50	16,6%	30,48	31,54	96,6%	0,76	2,48%	0,70	2,28%

l	number of laboratories after outlier rejection
n	number of individual test results after outlier rejection
o	percentage of outliers
X	Theoretical Value
\bar{x}	overall mean of results (without outliers)
η	recovery
s_R	s_R reproducibility standard deviation
$C_{V,R}$	$C_{V,R}$ coefficient of variation of reproducibility
s_r	s_r repeatability standard deviation
$C_{V,r}$	$C_{V,r}$ coefficient of variation of repeatability

Conclusioni

- La determinazione di patogeni e microorganismi nei biostimolanti è complessa, a causa della matrice particolarmente carica di lieviti e muffe
- Nelle prove di presenza/assenza quasi sempre presenti percentuali di falsi positivi e in misura molto minore falsi negativi, anche se generalmente, (con qualche eccezione) con una percentuale $< 5\%$.
- Le prove di «enumeration» hanno fornito spesso scarti tipo di riproducibilità intorno (se non superiori) ai $0,5 \log_{10}$, e solo per una tipologia di matrice intorno ai $0,3 \log_{10}$
- Come ci si attendeva, le prove inorganiche hanno presentato molti meno problemi con in genere ottimi risultati
- Sicuramente la poca esperienza dei laboratori con questo tipo di matrice ha giocato un ruolo importante in questi risultati.
- Da qui nasce la proposta di iniziare a proporre dei circuiti UNICHIM che propongano a rotazione i parametri microbiologici su campioni di biostimolanti commerciali (più probabilmente dal 2025, ma si tenterà anche di proporre qualcosa nella seconda metà del 2024)