Chicory (***Cichorium intybus*** L) for space-oriented production of prebiotic rich plants under controlled conditions for astronaut wellbeing.

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Background

Humans wellbeing depends on a positive interaction with the surrounding and internal microbiota that can be promoted by **prebiotics.**

General working hypothesis:

We might limit the negative effects of space induced stress on astronauts with an adequate supply of prebiotics.

Specific aims were to:

A) select prebiotic rich plants suitable for bioregenerative life support system;
B) test the response of selected plants to growth under controlled conditions;
C) verify produce prebiotic effects on selected bacteria;
D) acquire data for system dimensioning;
E) provide prebiotics rich produce for tests *in vivo*.

CURRENTLY ACCEPTED DEFINITION OF PREBIOTICS
Consensus statement 2016

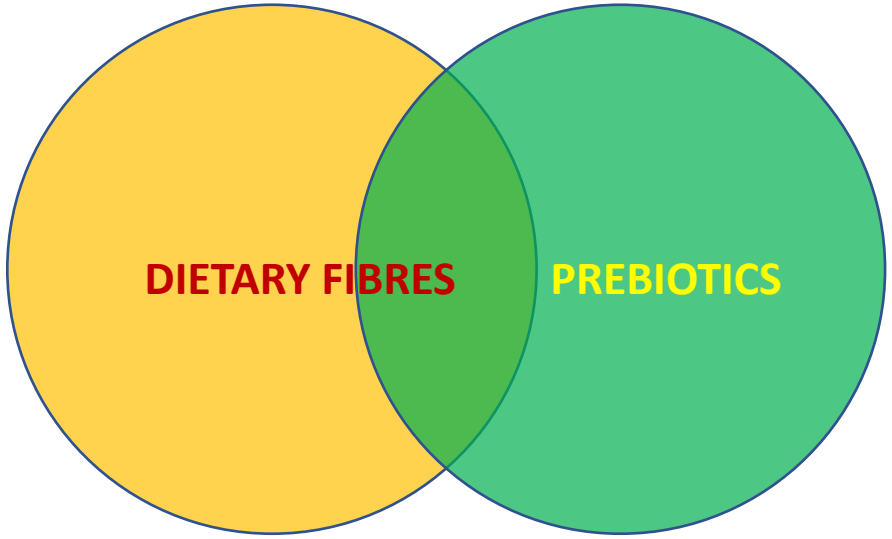
"….a substrate that is selectively utilized by host microorganisms conferring a health benefit"

AN OPEN DEFINITION

- Many microbe species
- Many host organisms
- Many target organs
- Many target functions
- Many candidate molecules
- …….

EFSA- NUTRITION CLAIM HIGH IN FIBRE:

- at least 6 g of fibre per 100 g
- at least 3 g of fibre per 100 kcal.



NO EFSA authorised CLAIMS referring to PREBIOTICS

OUR TARGET: GUT MICROBIOTA

EXPERIMENTS WITH PREBIOTICS ARE TARGETED TO BLSS

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Relationship between the Gut Microbiome and Energy/Nutrient Intake in a Confined Bioregenerative Life Support System

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….In addition, alterations in …. such as the synthesis of propionate, butyrate, and glutamate, in our study also indicated ***that the gut microbiome may play a role in mental disorders such as anxiety … and depression … in those confined in the BLSS****….*

TARGET:

Fructans

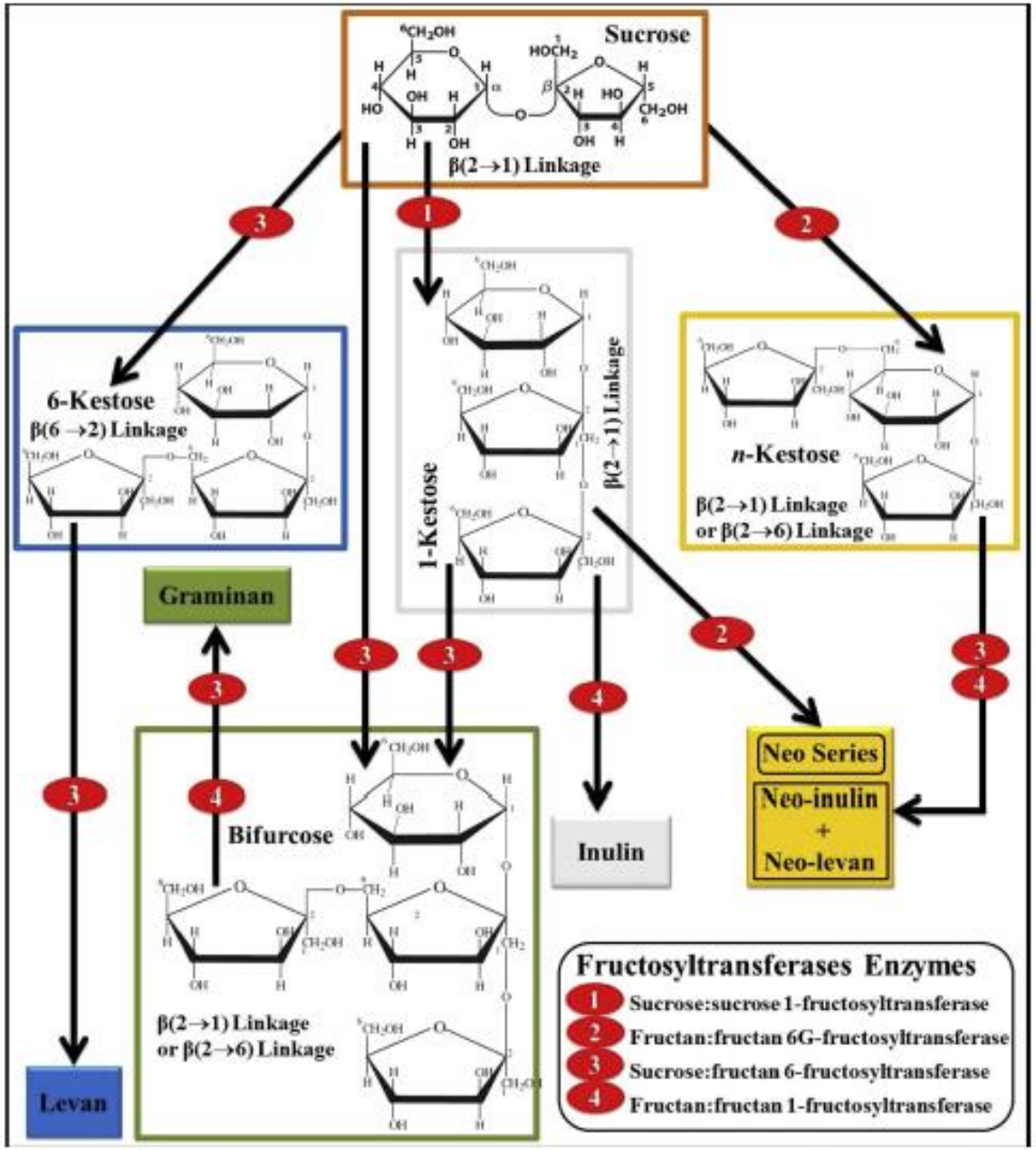

Fig. 2. Formation of five different forms of fructans from the basic fructan oligosaccharides in plants, as follows: 1) Inulin, 2) Levan, 3) graminan, 4) Neo-inulin, and 5) Neo-levan (Van Laere and Van den Ende, 2002; Cimini et al., 2015).

- Synthesised from sucrose
- Sucrose as a signal for the synthesis
- Variable degree of polymerisation (DP)
- Genetic control of presence and accumulation
- Environmental control of accumulation and DP
- **Recognised PREBIOTICS**

Preliminary results in previous projects

EDEN ISS

HORIZON 2020

European Commission

ID: 636501

FOS in different lettuce tissues obtained in the FEG facility during the mission in Antarctica. (% of the DW).

Lettuce variety and tissue	% of Fructo-Oligosaccharides	
	Mean	s.e.
Batavia Leaf	0,84	0,01
Batavia Root	1,97	0,27
Outredgeous Leaf	1,55	0,19
Outredgeous Root	1,23	0,63

INT. GEN. X TISSUE

Limited amounts on vegetables not specifically selected for prebiotic content

First step:
Selection of species that:

- Are small in size
- Fast growing
- Known to be edible (the full plant!)
- Contain a lot of prebiotics
- Safe produce
- Can grow nicely in fully controlled environment
- Respond to modulation of growth conditions

Chicory

Test the response of selected plants under controlled conditions (environmental and agronomic variables).

Effects of daily light distribution on growth and fructans accumulation

Photoperiod	Leaf FW (g plant^{-1})	Leaf DW (g plant^{-1})	Root FW (gplant^{-1})	Root DW (g plant^{-1})	S/R
	Mean	Mean	Mean	Mean	Mean
12 h	158 a	15,1 a	10,9 b	2,3 b	6,5 a
24 h	78 b	8,5 b	17,3 a	3,7 a	2,3 b

24 h photoperiod:

- reduced assimilation
- more allocation in roots
- more fructans in roots

Carbohydrate % on DM												
P. period	Fructans		Glucose		Fructose		Sucrose		Total Carbohydrates		DP av	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
12 h	52,8	0,70 b	0,20	0,02	0,24	0,07	4,0	0,16	57,2	0,64 b	9,62	0,40
24 h	60,5	1,12 a	0,20	0,04	0,41	0,22	3,6	0,14	64,8	1,33 a	10,2	0,42

Level of significance: *P = 0.05 are defined by different letters; absence of letters denotes a non-significant effect.

DLI= 21,6 mol quanta m^{-2} day^{-1}

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Effects of nitrate availability on root fructans content and productivity

Table 5. Effects of nitrogen supply on the carbohydrate content on **roots** of chicory (*Cichorium intybus* L.) cv Pan Zucchero and (*Cichorium endivia* L.) cv Romanesca. Data are reported as % of DM.

	Chicory roots		
	Fructans %	**Total NSC %**	**Fructans (g / m2)**
CV			
Pan Zucchero	38,94 ± 2,30	43,52 ± 2,39 **b**	4,02 ± 0,44
Romanesca	44,20 ± 2,92	50,16 ± 2,94 **a**	7,77 ± 2,04
TR			
C	38,49 ± 1,80	43,16 ± 1,69 **b**	3,26 ± 0,40
N	44,65 ± 3,16	50,52 ± 3,30 **a**	8,53 ± 1,86
CV x TR			
Pan Zucchero C	40,05 ± 2,37 **b**	43,58 ± 2,57	4,03 ± 0,41 **b**
Pan Zucchero N	37,83 ± 4,18 **b**	43,46 ± 4,36	4,01 ± 0,84 **b**
Romanesca C	36,92 ± 2,77 **b**	42,74 ± 2,49	2,49 ± 0,48 **c**
Romanesca N	51,47 ± 2,08 **a**	57,58 ± 2,26	13,06 ± 2,17 **a**

C, control; N, $CaNO_3$ 12 mM. Different letters indicate significant differences between the means (n=5) for each parameter, according to one-way ANOVA and Fischer's LSD test at p = 0.05.

Interaction genotype X N on both

- fructans content in root tissue
- fructans productivity on an area basis (but most of the effect is due to promotion of growth)

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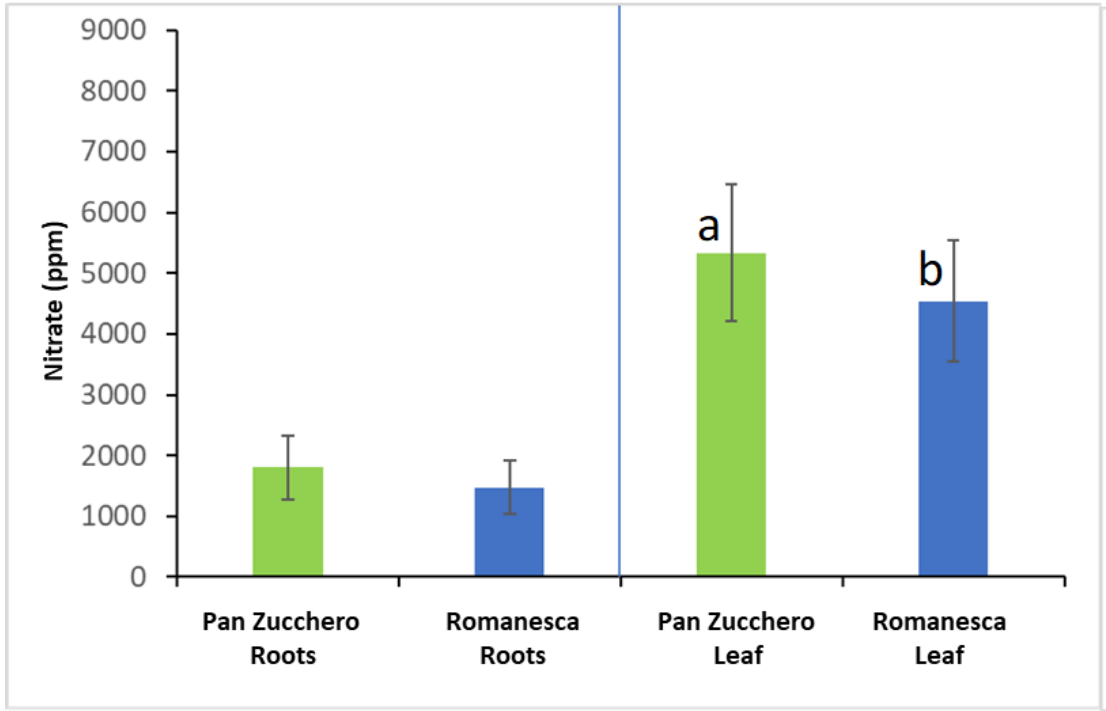
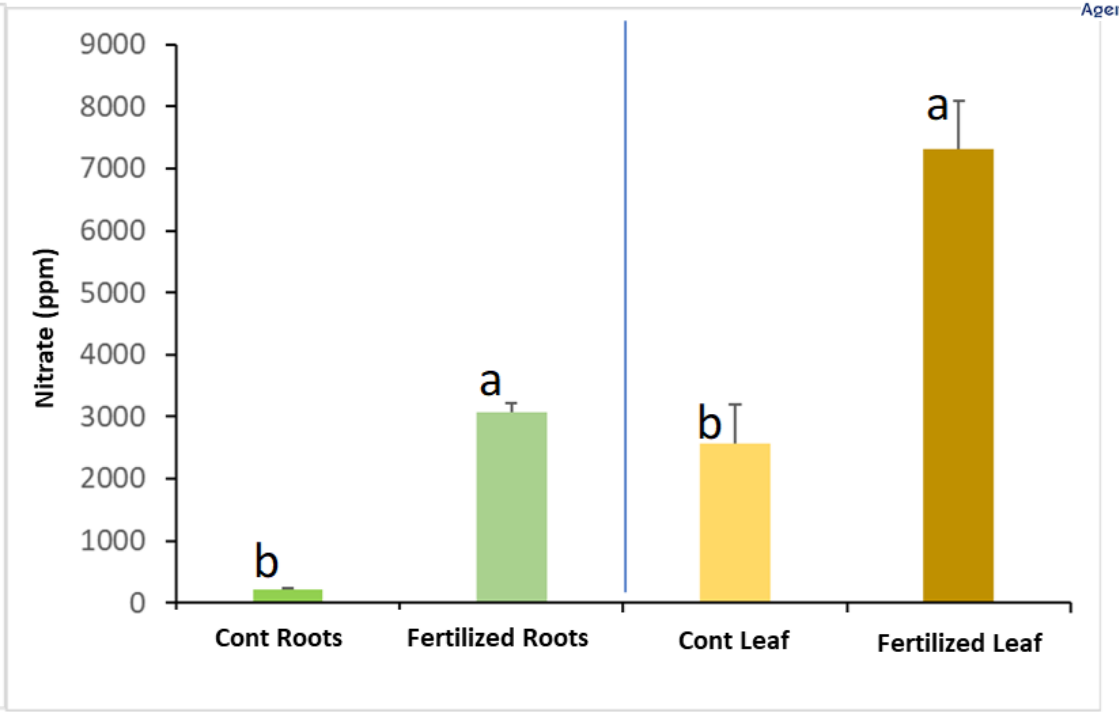
FOOD SAFETY: **N** FERTILISATION AND NITRATE ACCUMULATION





Effects of nitrogen supply on the nitrate content of leaves and roots of chicory (*Cichorium intybus* L.) cv Pan Zucchero and (*Cichorium endivia* L.) cv Romanesca. Data are reported as ppm (mg/kg) on a fresh weight basis. Different letters indicate significant differences between the means (n=5) for each parameter, according to one-way ANOVA and Fischer's LSD test at p = 0.05.

FOOD SAFETY:

- **NITRATE ACCUMULATES MORE IN LEAVES THAN IN ROOTS**
- **HIGH N AVAILABILITY STRONGLY INCREASES NITRATE ACCUMULATION IN ROOTS.**

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Effects of length of the growth period and light intensity on chicory performances.

		Leaves		Roots			
Days after sowing	Light intensity	Leaf DW (g plant^{-1})	Total NSC % (DW)	Root FW (g plant^{-1})	Root DW (g plant^{-1})	Fructans % (DW)	S/R
76	LL	6,7 ± 0,9	5,0± 0,7	27 ± 5,5	5,5 ± 0,9	59,2± 2,6	1,3 ± 0,04
	HL	9,6 ± 0,9	7,4± 1,0	37 ± 5,4	7,7 ± 1,1	56,4± 3,0	1,2 ± 0,11
91	LL	16,7 ± 0,8	5,3 ± 0,6	88 ± 6,5 b	20,6 ± 1,5	59,1 ± 2,6	1,0 ± 0,19
	HL	17,5 ± 1,6	4,6 ± 0,4	112 ± 13,0 a	28,8 ± 3,6	59,6 ± 3,5	0,7 ± 0,06

LL= low light intensity= 300 PPFD; HL= high light intensity of 600 PPFD. NCS= non-structural carbohydrates.
Within columns, means followed by different letters differ significantly at the level of P = 0,05: absence of letters denotes a non-significant effect.

Early and high fructans accumulation under fully controlled conditions
HL might determine an increase in fructans productivity

Proietti et al. in preparation

agronomy MDPI

Gabriele Paglialunga [1,2], Simona Proietti [1], Mariateresa Cardarelli [2], S
Stefano Moscatello [1], Giuseppe Colla [2,*] and Alberto Battistelli [1,*]

Agenzia Spaziale Italiana

Chicory taproot production: effects of biostimulants under partial or full controlled environmental conditions

In press

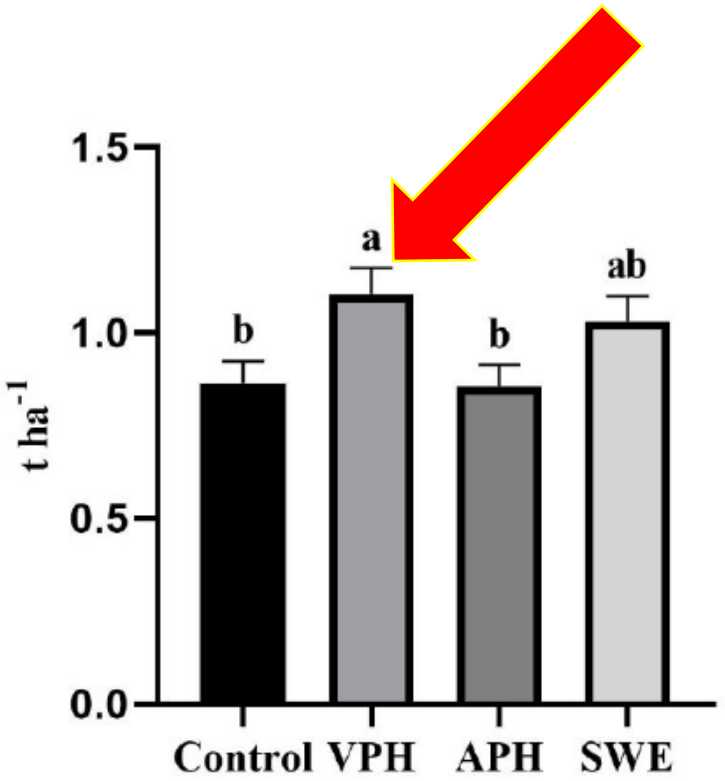


Figure 1. Effect of biostimulants on inulin yield per hectare in greenhouse chicory. Bars indicate the standard errors of the mean values. Different letters indicate significant differences according to Tukey's multiple-range test (P = 0.05). VPH= Vegetal Protein Hydrolysates; APH=Animal Protein Hydrolysates; SWE=Seaweed Extracts.

Other agronomic tools might help fructans production optimisation

In Situ Resources BioUtilization per il Supporto alla Vita
ReBUS

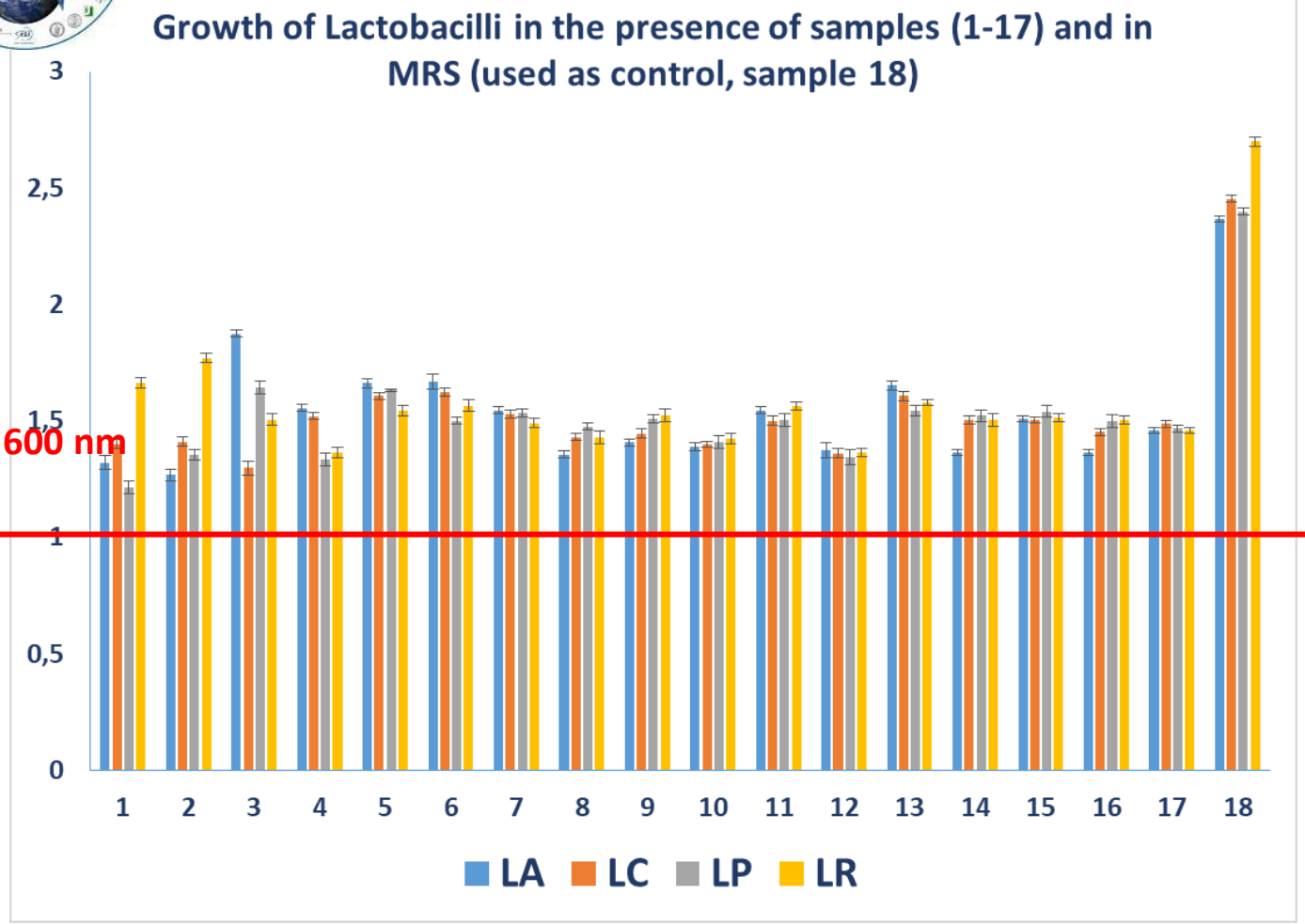
Space oriented system dimensioning for fructans production

	Fructans ($g \cdot m^{-2}$)	Estimated dietary fibre (fructans + 30% due to the cell wall, $g \cdot m^{-2}$)	DLI (moles quanta $\cdot$ m^{-2} for the full growing cycle)	Light to fibre conversion efficiency ($g \cdot$ moles quanta$^{-1)}$	Fibre productivity ($g \cdot m^{-2}$ day^{-1})	Growing area to provide the fibre need for one crew member (m^{-2})
High light	256,9	341,7	1944	0,132	3,80	6,59
Low light	198,8	264,4	972	0,205	2,94	8,51

Efsa considers a dietary fibre intakes of 25 g day^{-1} to be adequate for normal laxation in adults.

https://efsa.onlinelibrary.wiley.com/doi/epdf/10.2903/j.efsa.2010.1462

Prebiotic effect on selected bacterial species.



1. Chiavari foglie biostimolante controllo 9/12/2020
2. Chiavari foglie biostimolante proteico 9/12/2020
3. Chiavari foglie biostimolante algale 9/12/2020
4. Chiavari radici biostimolante controllo 9/12/2020
5. Chiavari radici biostimolante proteico 9/12/2020
6. Chiavari radici biostimolante algale 9/12/2020
7. Chiavari campione unificato biostimolante controllo 9/12/2020
8. Chiavari campione unificato biostimolante proteico 9/12/2020
9. Chiavari campione unificato biostimolante algale 9/12/2020
10. Chiavari foglie 17/9/2020
11. Chiavari radice 17/9/2020
12. Pan di Zucchero foglie 28/7/2020
13. Pan di zucchero radice 28/7/2020
14. Pan di zucchero foglie 27/4/2020
15. Pan di zucchero radici 27/4/2020
16. Romana foglie 27/4/2020
17. Romana radici 27/4/2020
18. MRS

Samples allowed a good growth of lactic acid bacteria (*L. acidophilus*, *L. casei*, *L. plantarum*, *L. rhamnosus*), as indicated by the OD 600 nm values, higher than 1. Samples grown in MRS (containing glucose instead of prebiotic substances) exhibited superior values of growth, but this is normal, as bacteria in this case had the availability of glucose immediately for their growth.

Providing produce rich in prebiotics for tests *in vivo*.

Agenzia Spaziale Italiana

Greenhouse production of ***Cichorium intybus*** **L.** tap roots, drying, grounding and shipping to a mice food producer company.





	Glucose	Fructose	Sucrose	Fructans
	%SS	%SS	%SS	%SS
Average	0,51	1,17	2,61	46,12
s.e.	0,06	0,07	0,28	0,63

Almost 8 g of fibre in 100 g of fresh produce

EFSA: High in fibre: ……at least 6 g of fibre per 100 g or at least 3 g of fibre per 100 kcal'

I STRONGLY SUGGEST TO FOLLOW NEXT PRESENTATION BY F. ZORATTO!!!

ReBUS – In Situ Resources BioUtilization per il Supporto alla Vita

Specific aims

A) Select prebiotic rich plants suitable for bioregenerative life support system.

B) Test the response of selected plants to growth under controlled conditions (environmental and agronomic variables).

C) Verify produce prebiotic effects on selected bacteria.

D) System dimensioning

E) Provide produce for tests *in vivo*.

Perspectives

- Prebiotic producing vegetables can be included in BLSS
- Species and genotypes selection is still an open question although promising candidate are available.
- Physiology (and molecular biology) of prebiotics accumulation is a largely open question.
- Growth variables in BLSS can help optimising prebiotics accumulation and productivity.
- Down stream applications are possible.
- The role of prebiotic rich vegetables on human psychophysical well-being is a relevant research topic for both the Earth and Space


Our terraforming target


Thank you for your attention!