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MODENA E REGGIO EMILIA



Phenotypic and genomic analysis of *Komagataeibacter* strains to elucidate the cellulose biosynthesis mechanism

University of Modena and Reggio Emilia, Research Doctorate in agri-food sciences, technologies and biotechnologies

Tutor: Dr. Maria Gullo

Candidate: Salvatore La China

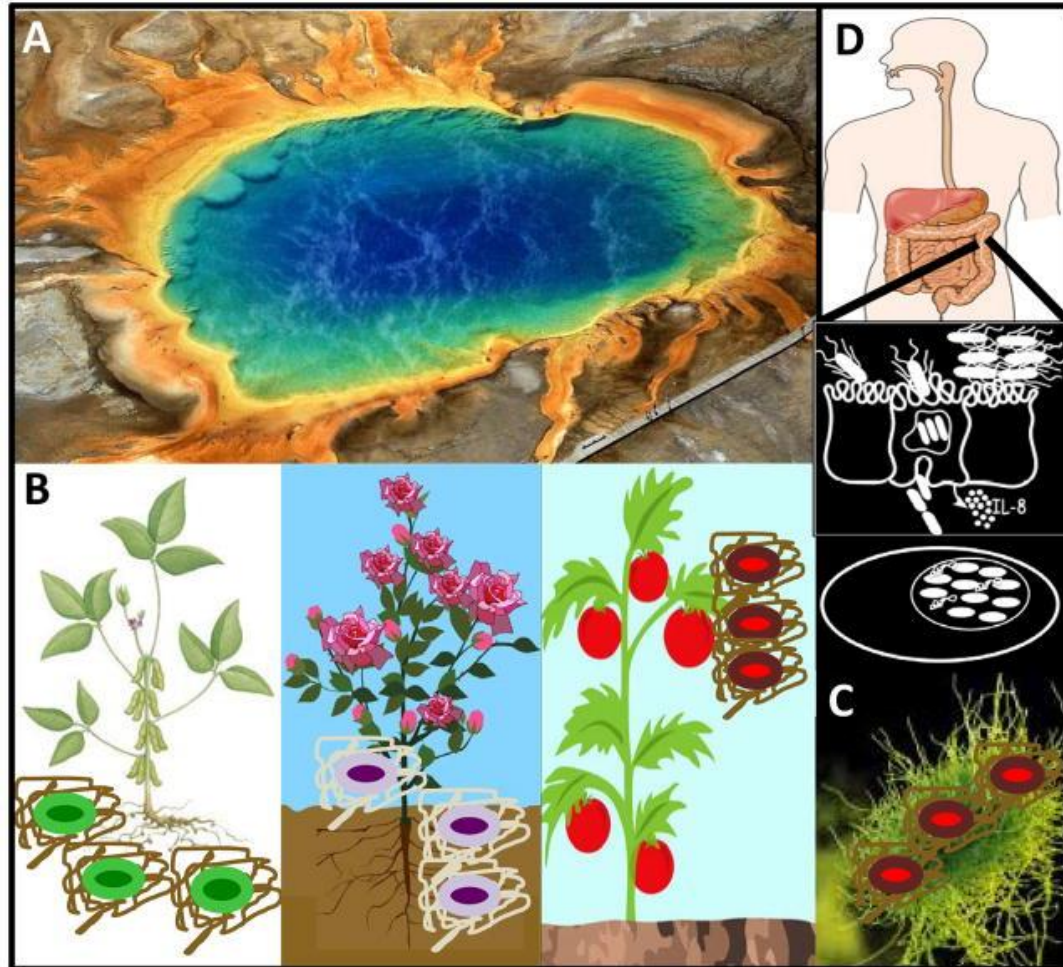
Coordinator: Prof. Alessandro Ulrici

Cycle XXXIII

Workshop 4th December 2020

Cellulose producing bacteria and where found them

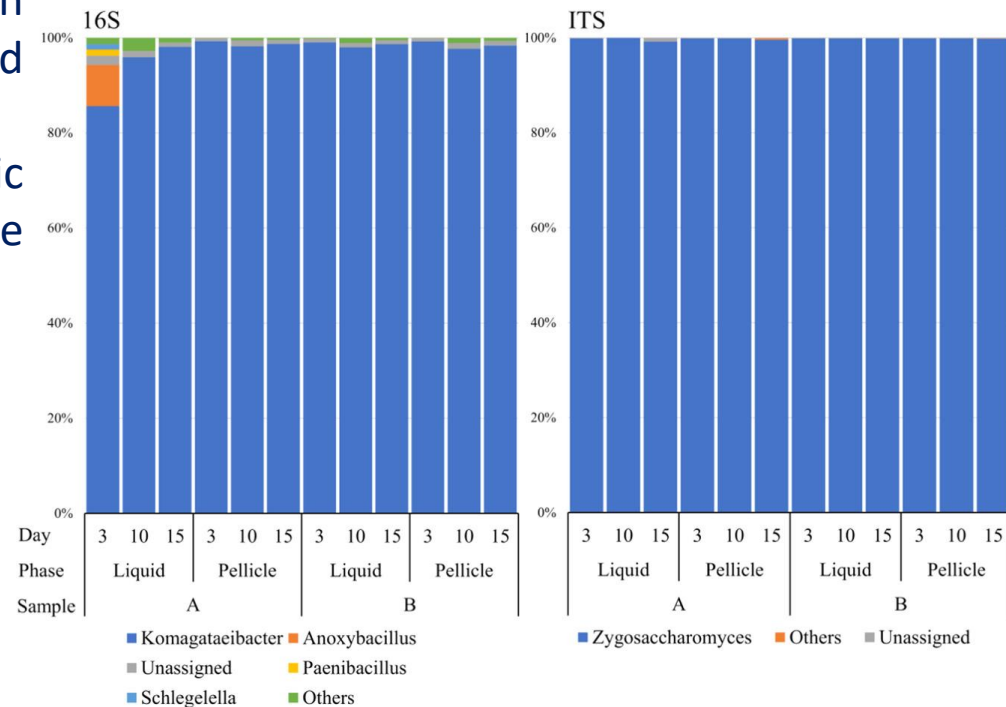
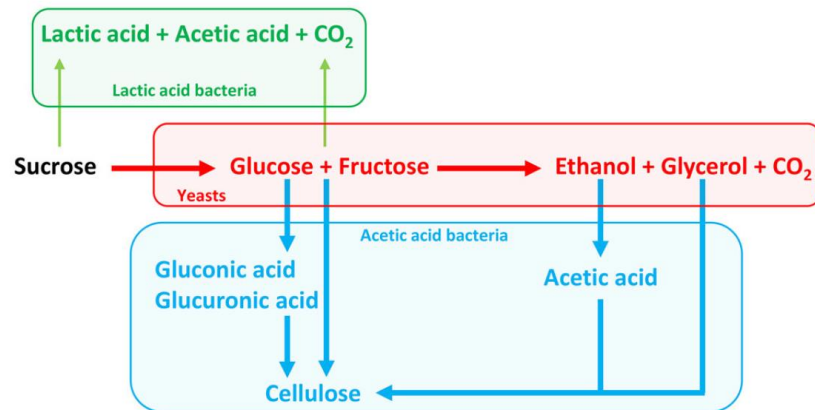
- *Cyanobacteria*: *Nostoc* and *Anabaena*;
- *Enterobacteriaceae*: *Escherichia* and *Salmonella*;
- *Pseudomonadaceae*: *Pseudomonas*
- *Acetobacteraceae*: *Komagataeibacter*



Kombucha tea as reservoir of cellulose producing bacteria

Kombucha tea:

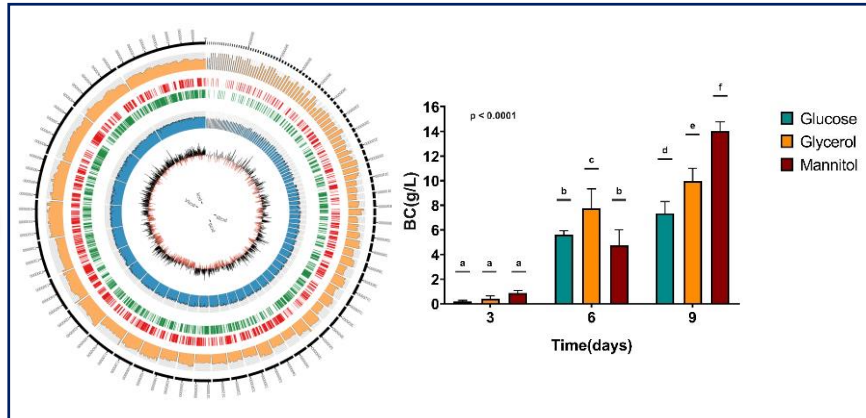
- Yeasts: help bacteria in the fermentation process producing glucose, fructose and ethanol
- Bacteria oxidize glucose into gluconic acid and polymerize it for cellulose production



Main project activities and outputs

Time line

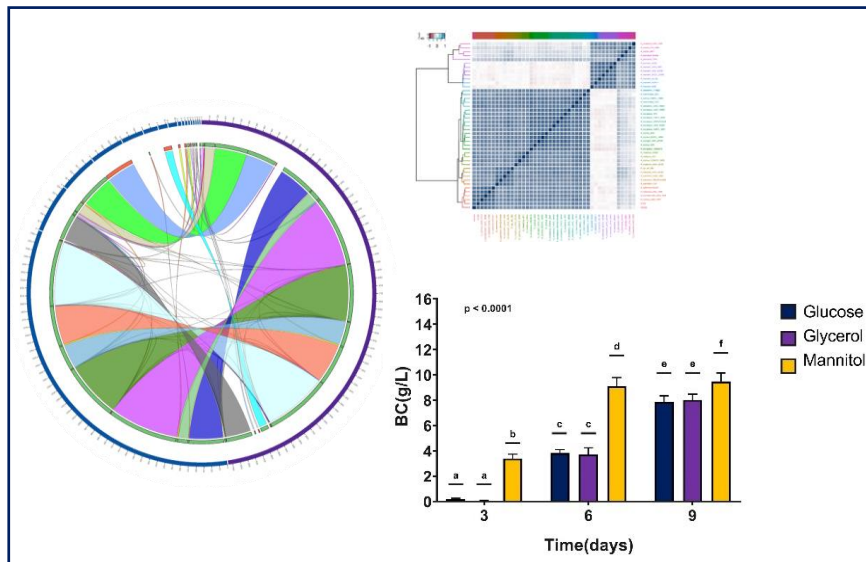
1st year



- K2G30 genome was sequenced and analyzed
- Phenotypic data were obtained

- Gullo M, La China S, Falcone PM, Giudici P (2018) *Appl Microbiol Biotechnol* **102**, 6885–6898. <https://doi.org/10.1007/s00253-018-9164-5>
- La China, S., Zanichelli, G., De Vero, L, Gullo M (2018) *Biotechnol Lett* **40**, 1289–1302 <https://doi.org/10.1007/s10529-018-2591-7>
- Gullo M, La China S, Petroni G, Di Gregorio S and Giudici P (2019) *Front. Microbiol.* **10**:58. doi: 10.3389/fmicb.2019.00058

2nd year



- K1G4 genome was sequenced and analyzed
- Phenotypic data were obtained
- Comparative genomics

- La China S, Bezzecchi A, Moya F, Petroni G, Di Gregorio S, Gullo M (2020) *Biotechnol Lett* **42**, 807–818 <https://doi.org/10.1007/s10529-020-02811-6>

K1G4 and K2G30 roots



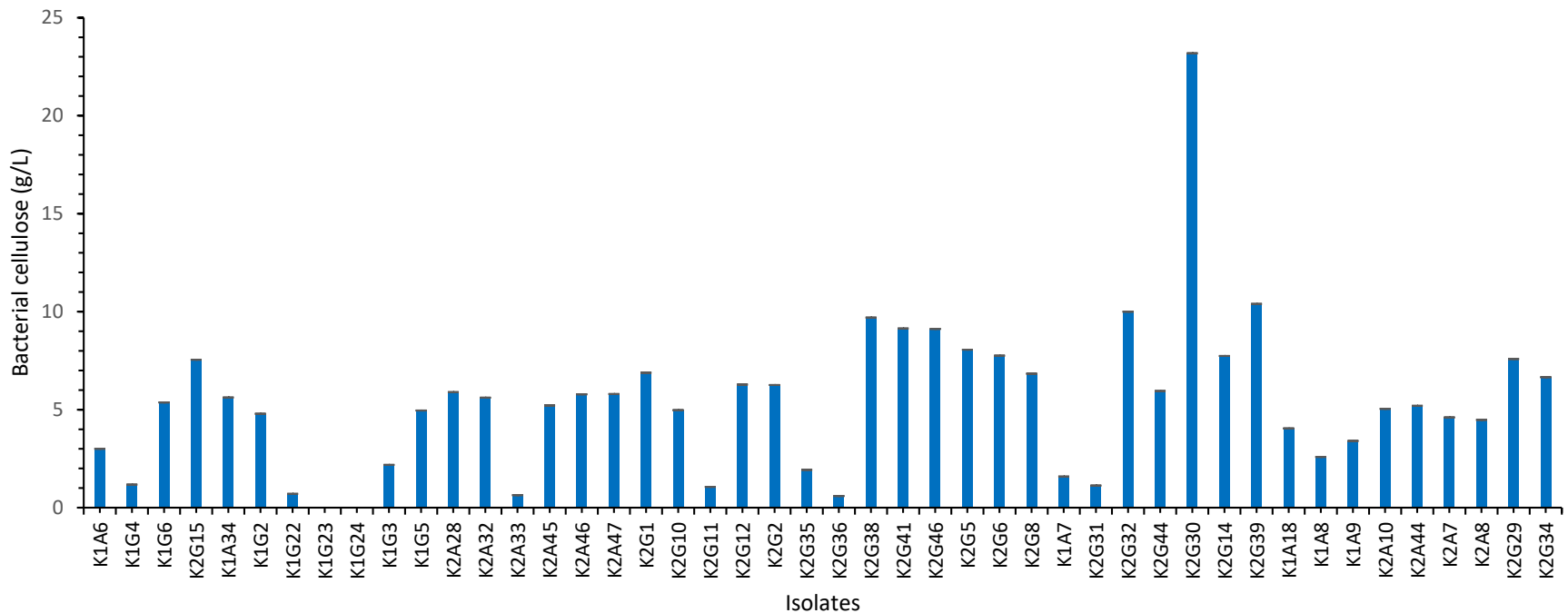
- K1G4 and K2G30 came from a dataset of 184 isolates
- The strains were selected based on phenotypic traits, as colony morphology, Gram stain, ethanol oxidation and acetic acid production
- Collected isolates were stored in the Unimore microbial culture collection

K. xylinus K2G30 = UMCC 2756

K. xylinus K1G4 = UMCC 2947

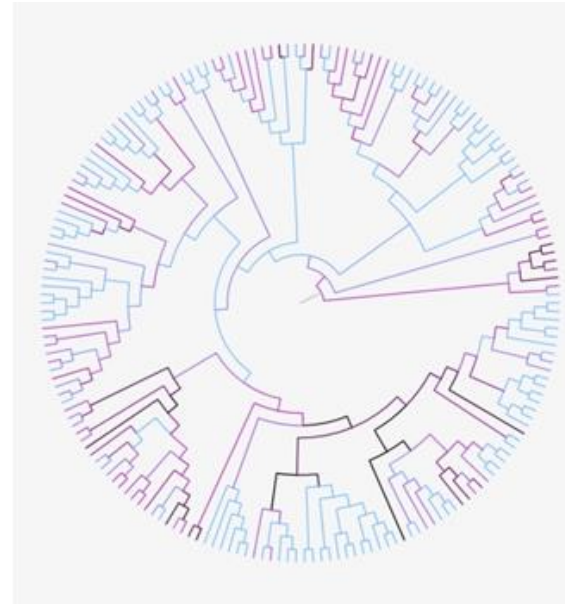
Cellulose production

- Candidate strain were selected based on bacterial cellulose production
- The cellulose production yields was highly variable among all isolates
- The most abundant yield was obtained by the strain K2G30

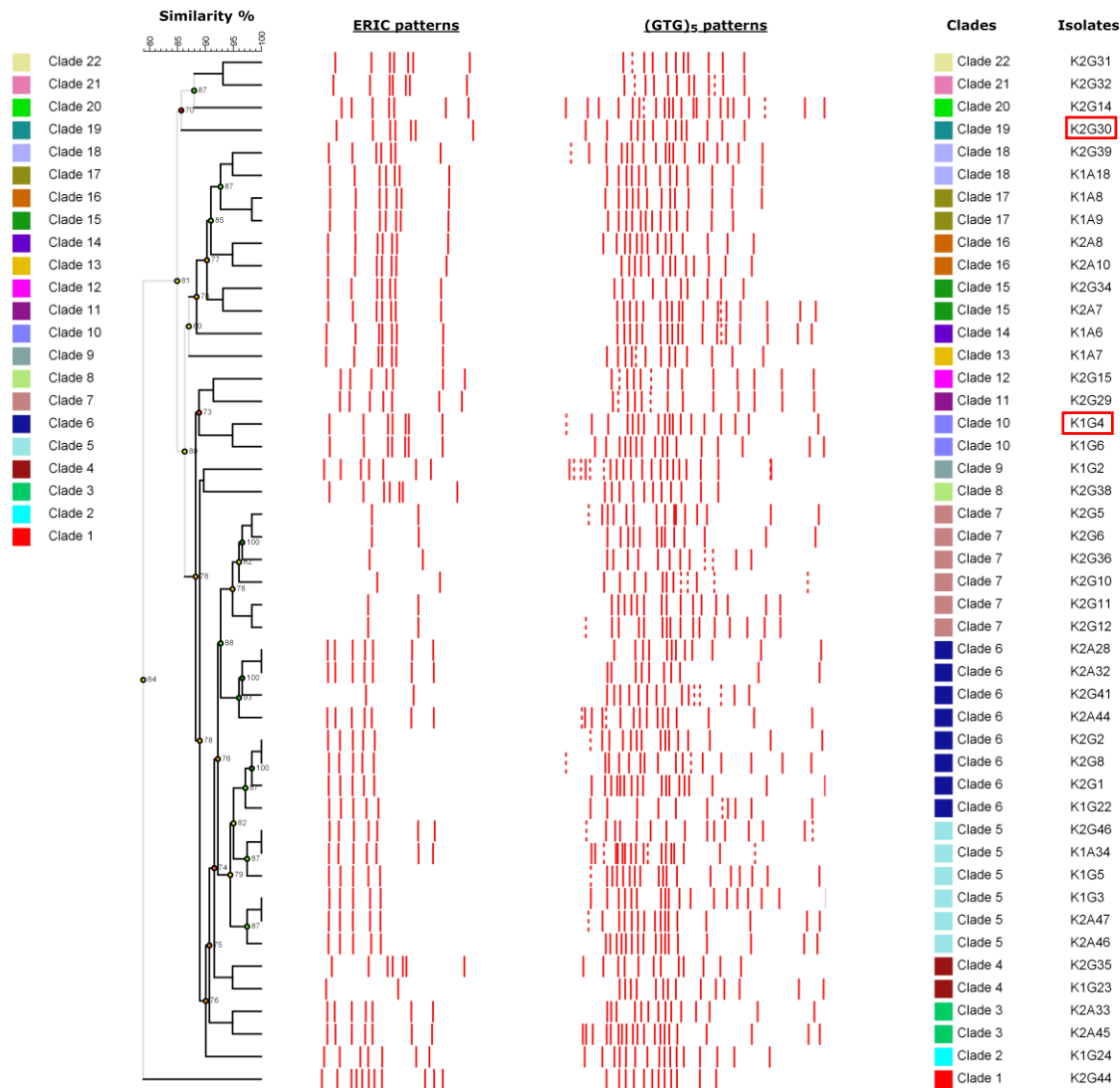


Genotyping study

- The isolates were genotyped in order to detect the number of strains
- The genotyping was performed using the (GTG)₅ REP-PCR, analyzing the interspersed GTG repetitive regions
- In comparison, also the ERIC rep-PCR was applied to improve the accuracy of typing approach



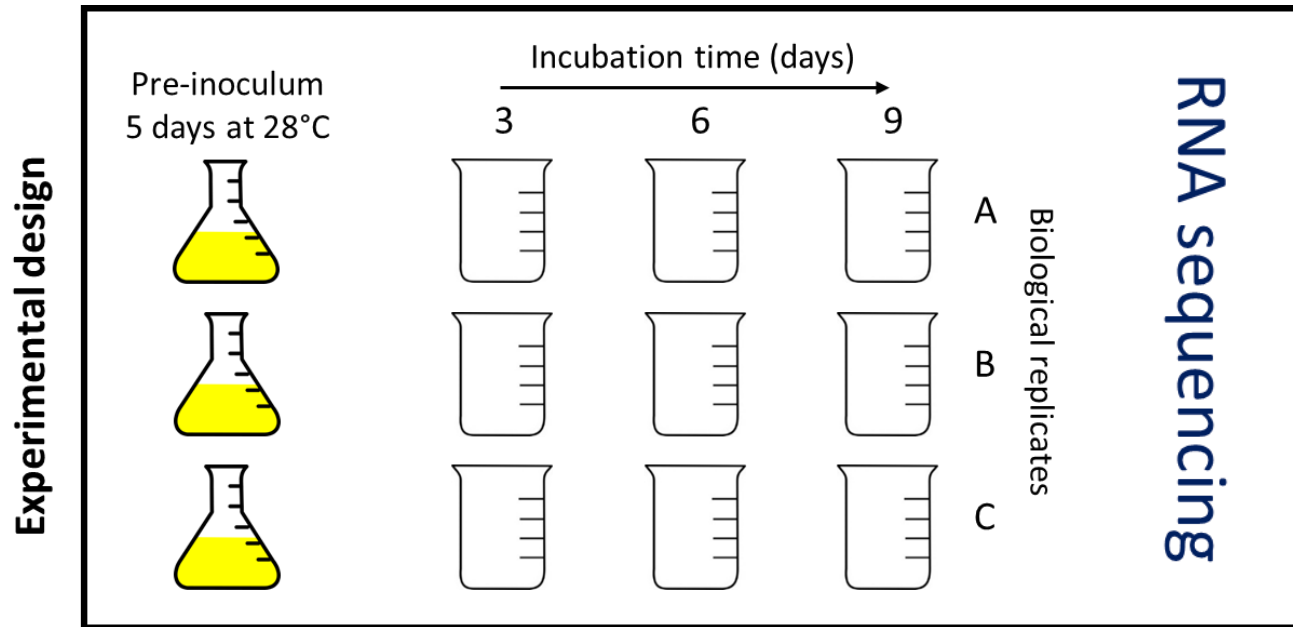
(GTG)₅ and ERIC rep-PCR fingerprint



- The minimum similarity percentage, based on the cluster cut-off analysis, was 88%
- The discriminatory index was 0.94
- A total of 22 clades were formed mixing the profiles from (GTG)₅ and ERIC PCR using a threshold of 94% of similarity
- Genotyping was able to discriminate at strain level

3 months Kaust project

- Temporal metabolic pathways reconstruction
 - How the metabolism shifts using different carbon source?
 - Which are the main metabolic differences among the strains?



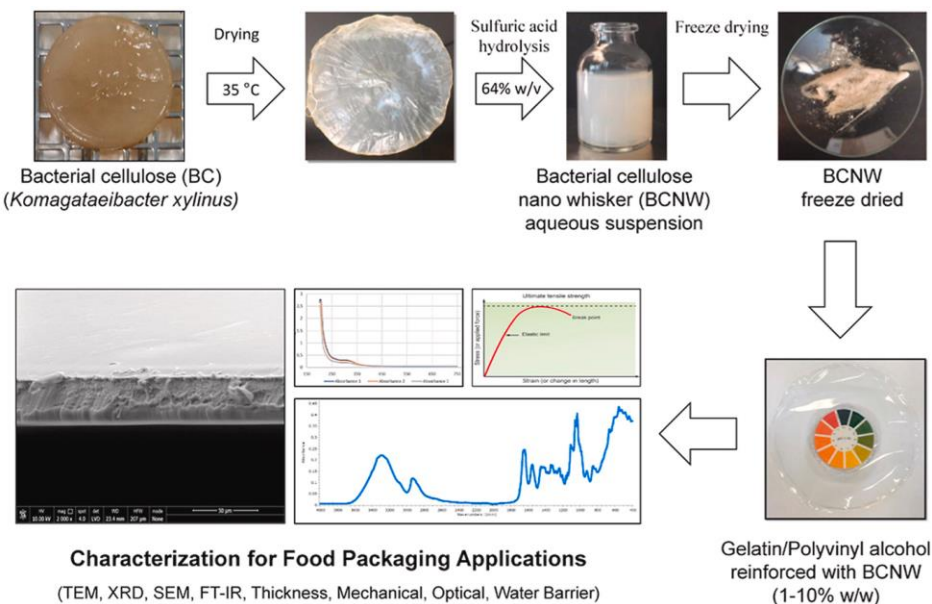
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للعلوم والتقنية
King Abdullah University of
Science and Technology

Ongoing

K2G30 as an industrial microorganism - promising applications from functionalized composites

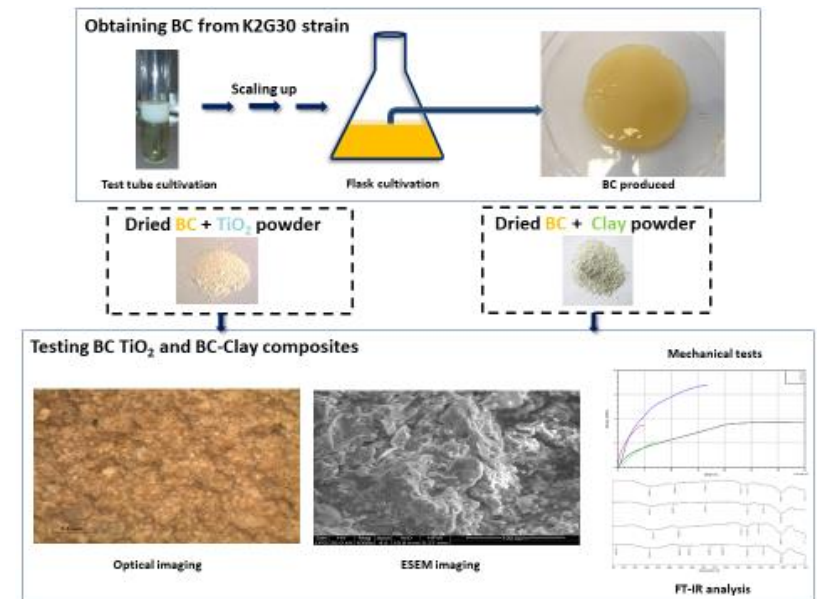
Characterization of bio-nanocomposite films based on gelatin/polyvinyl alcohol blend reinforced with bacterial cellulose nanowhiskers for food packaging applications

Hossein Haghighi ^{a,*},¹, Maria Gullo ^{a,1}, Salvatore La China ^a, Frank Pfeifer ^b, Heinz Wilhelm Siesler ^b, Fabio Licciardello ^{a,c}, Andrea Pulvirenti ^{a,c}



Mechanical and structural properties of environmental green composites based on functionalized bacterial cellulose

S. Barbi, C. Taurino, S. La China, K. Anguluri, M. Gullo, M. Montorsi



Conclusions

- Isolates from Kombucha tea were analyzed from phylogenetic point of view, discriminating at strain level
- Two strains from the 46 isolates were deeper tested using different approaches (at genome level and phenotypically)
- The tested strain will be used in a future study to understand all the metabolic changes that occur during time (cultivation period) and when the different carbon sources are supplied
- Three promising composites were formulated based on the cellulose produced by K2G30

Acknowledgements



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Il boschetto maremma toscana s.r.l