LVII SAIB Meeting - XVI SAMIGE Meeting

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Living organisms have developed precise time-regulated clocks to adapt to the 24 h solar cycle of light and dark alternation. The cellular oscillator is composed of the molecular circadian clock of transcription and translation and of a cytosolic oscillator that may work together to temporally regulates the physiology and behavior in all vertebrates. These oscillators are present in organs, tissues and even in individual cells to control cellular metabolisms in a circadian manner. Among the metabolisms subject to circadian control, the synthesis and degradation of lipids seem to be one of the most highly modulated across time at the level of total content, enzyme expression and activities. Indeed, there is evidence that the chronic mismatch between our lifestyle caused by modern life (prolonged artificial lighting, high-calorie diets, night work, etc.) and the rhythm dictated by our internal clock is associated with an increased risk of various diseases, including metabolic syndrome, obesity, diabetes, cardiovascular disease, inflammatory disorders and even cancer. In particular, the liver is a crucial organ for physiology as a major metabolic integrator. It is a central hub for lipid and energy homeostasis, being involved in triglyceride (TG) and glycerophospholipid (GPL) metabolism. Different factors cause a metabolic disorder which promote an abnormal lipid accumulation in organelles named lipid droplets (LDs) -hepatic steatosis- which is the metabolic syndrome manifestation, and it can progress to a hepatocellular carcinoma (HCC), the most common primary liver malignancy worldwide. Here we investigated in HepG2 cells, a human HCC-derived cell line, metabolic rhythms and their link with the circadian clock in control (B-WT) and in cells disrupted for Bmal1 (Bmal1-knocked down cells, B-KD), one of the main components of the molecular clock. We observed marked temporal oscillations in mRNA and protein abundance of key GPL synthesizing enzymes (Chokα, Pemt, Pcyt2 and Lipin1) as well as in TG and LD content in normal HepG2 cells (B-WT). Strikingly, when the circadian clock was disrupted (B-KD model), lactate levels were highly increased while the lipid metabolism was severely altered with a significant decrease in PC/PE ratio, TGs and LD content and rhythmicity, with marked changes in expression of several enzymes as Chokα and Lipin1. These and other results obtained in our group suggest a very strong cross-talk between the molecular clock and the GPL metabolism, and highlight a different and complex level of regulation driven by the biological clock. Moreover, this precise and coordinated multi-task metabolic network likely responds to the cell requirements offering a novel time-related level of organization.

MICROBIOLOGY - BIODIVERSITY

MI-P001-38

TAXONOMIC CLASSIFICATION OF 62 GENOMOSPECIES BELONGING TO THE *Bacillus cereus* GROUP, USING A MACHINE LEARNING APPROACH

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The *Bacillus cereus* group is usually categorized into three clades, Clade 1 has pathogenic strains as *Bacillus anthracis*, Clade 2 is composed of *Bacillus cereus sensu stricto*, and *Bacillus thuringiensis*, the former is associated with food poisoning while the latter is used for agronomic purposes for pest control. Clade 3 is the most phylogenetically diverse clade; the strains that compound it have been isolated from very diverse sources. Classification between species within the *B. cereus group* has proven to be very challenging, having reported multiple cases of incorrect classifications or incoherences between taxonomic classification and genomic or phenotypic characteristics. Nevertheless, the correct assignment is of great importance because these assignments are used to predict the performance and safety of bacteria, thus affecting their use for industrial or agronomic purposes. We evaluated, employing the Machine Learning algorithm "Random Forest", gene markers used for the classification of these genomospecies. For this, we downloaded from GenBank, 2460 sequences belonging to the three clades. Of these, 2117 were previously classified by us, while 343 were recently uploaded to the databases; all of which were quality filtered, eliminating 267 sequences. Of the remaining 2191 sequences, 63 were not included in the analysis because they lacked housekeeping genes, suggesting that they are incomplete. The species-level taxonomic identity of the study strains was validated or reassigned using Average Nucleotide Identity (ANI) and multi-locus sequence analysis (MLSA). Thus, 47.13% of the sequences recently uploaded to the database were reassigned. In turn, 5 strains were classified as new genomospecies, named genomospecies 38, 39, 40, 40, 41, and 42. Subsequently, to generate the Random Forest-based classifier, the sequences of 22 gene markers for each of the strains in each clade were divided into a training group and a testing group. From the training group, predictive classification models were generated, which were shown to have accuracy values greater than 98% to assign Clade 1, 2, and 3 species, being the classifiers based on *gyrB*, *pyc*, or *lon* genes those with the highest accuracy. Finally, the testing group was used to see the error of the classifiers, being for Clades 1 and 2 less than 1% and for Clade 3, less than 4%. Therefore, these classifiers will allow mass assignments in metagenomic analysis, as well as assignments of new isolates of the *B. cereus* group with greater precision.

MI-P002-232

ANTAGONISM OF *Bacillus safensis* STRAIN AGAINST PHYTOPATHOGENIC BACTERIA *Xanthomonas citri* pv. *citri*.

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Citrus canker caused by *Xanthomonas citri* subsp. *citri* (Xcc), is a bacterial disease which affects all the citrics. One alternative to manage it is the use of antagonist bacteria. The aim of this work was to investigate the antagonist activity of *Bacillus safensis* (S9) against Xcc. The activity was tested by diffusion assays. Xcc and S9 were grown overnight in Luria Bertani (LB) and potato dextrose (PD) medium, respectively, with continuous agitation at 28°C and then, were diluted to a concentration of 10^8 CFU/mL. Petri dishes were covered with 15 mL of LB-agar containing 100 µL of the Xcc dilution. Once the medium was solidified, 4 µL drops of S9 were inoculated 3 times in each Petri dish, and the experiment was made by triplicate. After 48 hours of incubation at 28°C, the inhibition zone was measured, and the average inhibition area was calculated as IA = average area of the inhibition zone - average area of the colony. A significant inhibition area of 5.18 cm^2 was obtained (one-sample t-test, $p<0.05$). At the same time, diffusion assays with the supernatant were made to prove its inhibitory ability. Petri dishes were prepared as described above. The supernatant was obtained by centrifugation of the S9 culture grown in PD medium, and then by bacteria filtration. Three filter paper discs embedded with the supernatant were placed per Petri dish, by triplicate. The inhibition zone was measured after 48 hours and calculated the IA. A significant inhibition area of 2.29 cm^2 was obtained (one-sample t-test, $p<0.05$). Besides, a study at genomic level comparing S9 with ten *Bacillus* strains was made. Different clusters of secondary metabolite synthesis pathways were detected, three common with *B. velezensis* strains (surfactin, basilicin and bacilibactin). These strains were tested as inhibitors of Xcc and they did not show inhibition (*Bacillus sp* and *B. megaterium*) or showed less inhibition (*B. velezensis*). The difference might be on the expression level of the clusters. These results suggest the potential use of S9 as a canker control agent and further studies will be necessary to identify the Xcc-inhibitor metabolite.

MICROBIOLOGY – BIOREMEDIATION and BIOCONTROL

MI-P003-4

BIOCONTROL OF GREEN MOULD IN ORANGES BY EPIPHYTIC BACTERIA AND BIOACTIVE COMPOUNDS

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Penicillium digitatum is a filamentous fungus that infects citrus fruits through injuries and wounds produced on the peel during harvest and post-harvest handling, causing rots known as green mould. The fruits are more susceptible to this infection in packing facilities and storage rooms, especially in those lacking appropriate hygiene, where high concentrations of spores prevail. The decays caused by *P. digitatum* result in significant production losses; therefore, strategies aimed to control this fungus are highly relevant. The application of synthetic fungicides is the mainly applied approach to control *P. digitatum.* However, the intensive usage of fungicides has led to the proliferation of *P. digitatum* strains with resistance to one or more fungicides. Besides, this practice poses a risk for the human health, decreases the population of fungal crop symbionts, produces soil and water pollution and is incompatible with the organic market. These concerns demand alternative approaches, which must be harmless to human and environmental health and fulfil the restrictions of different countries regarding to limit values of chemical residues on fruits. Biological control and natural bioactive compounds are promising alternatives to the control of post-harvest decays and may contribute to sustainable production of citrus. The objective of this work is to evaluate the potential of native bacterial strains isolated from the surface of oranges and the application of a natural bioactive compound to control *P. digitatum* growth. Eleven bacterial strains were isolated from oranges peel and identified by sequencing of 16S rRNA gene. The strains corresponded to *Micrococcus luteus*, *Staphylococcus xylosus*, *Bacillus mojavensis*, *Bacillus velezensis*, *Bacillus subtilis* and *Pseudomonas psychrotolerans*. Three of them showed effective antagonist performance *in vitro* against *P. digitaum* A21, a strain resistant to the fungicide pyrimethanil previously isolated by our group. Reductions of green mould growth by 80%-90% were obtained when culture filtrates were used by the poison agar method. Reproducible results were also obtained upon *in vivo* conditions and preventive treatments. The capability of 6 pentyl-alpha-pyrone (6PP) to inhibit the growth of *P. digitatum* was also assayed. This harmless compound has shown fungicide activity against different crop pathogens and is produced by the saprophytic fungus *Trichoderma atroviride*, which was isolated by our group. Significant differences were observed with respect to the control in curative treatments. These results suggested that epiphytic bacteria and 6PP are optimal tools for the control of green mould spreading in post-harvest citrus fruits. The combination of these tools with supplementary strategies such temperature regulation, UV irradiation and GRAS substances could lead to sustainable management of green mould decays, preserving post-harvest quality of oranges and dispensing with synthetic fungicides.

MI-P004-11

EVALUATION OF BACTERIAL ISOLATES FROM STRAWBERRY PLANTS (*Fragaria x ananassa* Duch.) AS BIOLOGICAL CONTROL AGENTS OF *BOTRYTIS CINEREA.*

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