Sociedad Argentina de Microbiología General

# XIX CONGRESO DE LA SOCIEDAD ARGENTINA DE MICROBIOLOGÍA GENERAL

22 al 25 de octubre del 2024
Centro cultural y Pabellón Argentina de la Universidad Nacional de Córdoba, Córdoba, ARGENTINA.

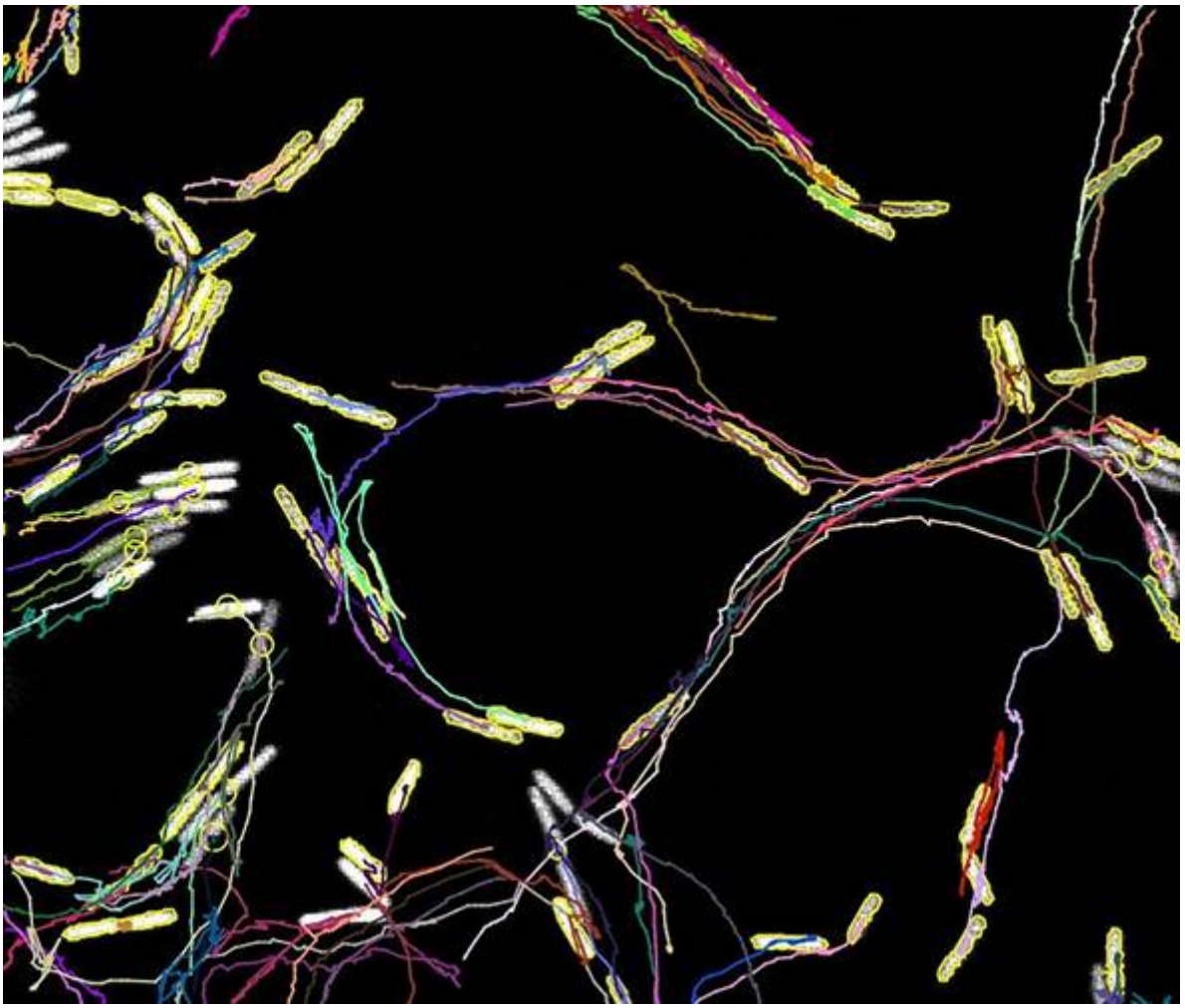

Foto: Se hace camino al andar. Celeste Dea. 1er puesto. Concurso fotográfico SAMIGE 20 años.

# MORPHOLOGICAL PARAMETERS OF *Pseudomonas extremaustralis* 2E-UNGS AGGREGATES UNDER DIFFERENT NUTRIENT CONCENTRATIONS

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*Pseudomonas extremaustralis* 2E-UNGS (NCBI GenBank NZ_CP091043.1) was isolated from the highly contaminated Reconquista River basin (Buenos Aires, Argentina) and can grow either in planktonic states, or suspended aggregates or even in biofilm states. This microorganism is of environmental interest as proved in the development of biotreatments and bioreactors applying immobilised cells for Cr(VI) biotransformation or for the Cu(II), Zn(II), Cd(II) biosorption. The aim of this work was to study the kinetics of *P. extremaustralis* 2E-UNGS aggregate formation and characterise their morphology over time. The final objective is to modulate the development of bacterial flocs to apply in further biotreatments. The aggregate formation kinetics was studied for 24 h using the commercial formulation of nutrient broth (NB) and its 1:2 dilution (NB-½). Cultures in duplicates were inoculated with 5 mL of overnight NB-cultures in 50 mL of fresh medium (NB or NB-½) and incubated at 32 ºC under agitation. Growth parameters were recorded as a function of time, monitoring pH and optical density at 600 nm (OD). To microscopically characterise the aggregate morphology, eight 1 mL-samples from 0 to 24 h were studied. The cell-free control samples were only taken at t3=5 h and t7=24 h to obtain the illumination pattern of the system. Stereomicroscopy was used to acquire eight non-overlapping images by placing 0.3 mL of each sample in confocal petri-dishes (by duplicates) and were analysed with the FIJI® software. The % self-aggregation was determined in the exponential, deceleration and stationary phases of growth. For this purpose, cells from 10 mL cultures were washed once and suspended in 150 mM NaCl, registering the OD decrease in 2.5 mL of each bacterial suspension along 4 h. Both media promoted the formation of small and compact cell aggregates of *P. extremaustralis* 2E-UNGS. The NB-½ doubled the number of flocs detected especially at 5 h when the deceleration phase began. The morphological parameters of the aggregates had similar behaviours during exponential phases of growth in both media. The circularity, the roundness and the solidity increased up to the first 5 h, while a decrease in the aspect ratio was evidenced. After 5 h, the morphological parameters remained constant for the NB medium. Whereas, from this deceleration growth phase and continuing in stationary phase with the NB-½, the morphological parameters changed to the

initial (t=0) values. Coincidently, around 50% self-aggregation was registered for NB-½ medium while 20% for NB medium was observed at the deceleration growth stage. In the stationary phase, the self-aggregation abruptly decreased with a low total number of aggregates (10%). These results contribute to the understanding of the cell aggregation mechanism to optimise the design of bioreactors for the treatment of metal effluents.

Palabras clave: Pseudomonas extremaustralis - Cellular aggregation - Morphology - Digital image analysis