The „BactoCat Microfluidic Laboratory Disc“ a droplet-based microfluidic screening tool for soil bacteria with valuable metabolic activities

G.Alexander Groß[1]*,Jialan Cao[1], Katja Bergmann[1], Marc Kielpinski[2], Erica Kothe[3], Martin Roth[4], Thomas Henkel[2], J.Michael Köhler[1]*

[1]*Institute for Chemistry and Biotechnology, Ilmenau University of Technology, Germany Dept. of Physical Chemistry and Microreaction Technologies,*
[2]*Institute of Photonic Technology (IPHT) Jena, Germany, Dept. Microfluidics,*
[3]*Friedrich Schiller University Jena, Germany, Institute of Microbiology, Dept. of Microbial Communication,*
[4]*Leibniz Institute for Natural Product Research and Infection Biology e.V. Hans-Knöll-Institute (HKI) Jena, Germany.*

Introduction

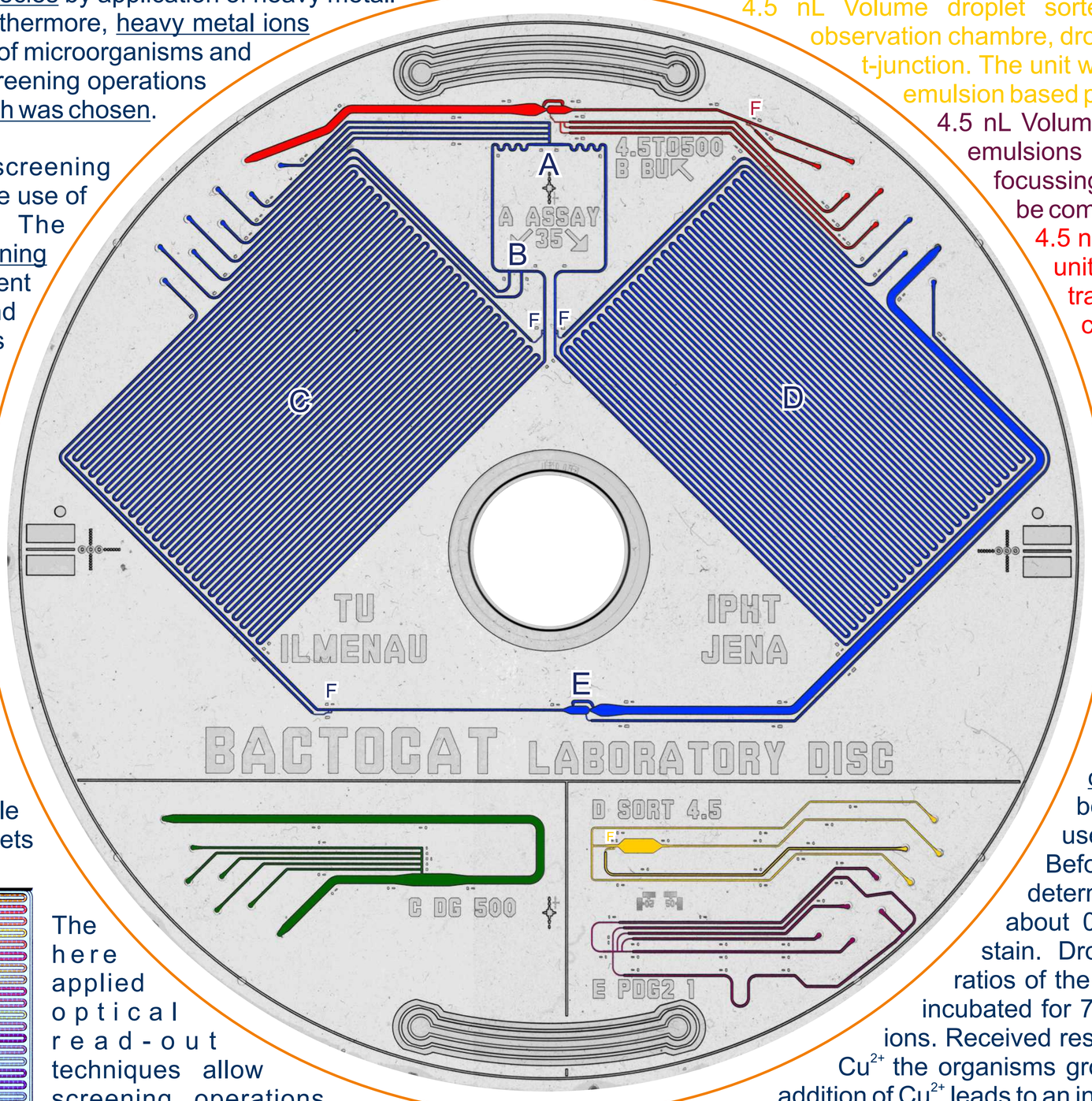
Soil is one of the most complex microbial ecosystems on the planet. Evolutionary processes evolved complex microorganism communities which are adapted to the local environmental conditions. Most of the microorganisms are „unculturables“ because they depend on the existence of other microorganisms, special nutrition conditions or stimulation by secondary metabolites in a complex way. Hence, soil microbial communities are an promising screening resource for new valuable enzymes, antibiotics or biocatalytic activities. Especially the behavior of soil microbial communities on heavy metall ions is a nearly unexplored research field.
The BactoCat consortium focus on the selection of reduced microbial communities or unknown heavy metal tolerant species by application of heavy metall ion stress on soil communities. Furthermore, heavy metal ions can induce secondary metabolism of microorganisms and communities. For the necessary screening operations a droplet based microfluidic approach was chosen.

A droplet-based microfluidic screening strategy was developed which make use of an unique “LabDisc” device. The „LabDisc“ enables complex screening procedures comprising three different droplet volumes: 4.5 nL, 35 nL and 500 nL. Optical readout techniques were used to analyze the bacterial growth on the one hand and the selection of colorimetric active colonies on the other hand in dependence on heavy metal or nanoparticle concentrations.

Operation Units

35 nL Volumen fluidic network for bacterial screening procedures comprises following operation units: A: Droplet generator utilizing 4 different fluids (3D-Experiments) B: Observation and droplet selection unit C: 1.Storage Array D: 2.Storage array E: Blow-up unit for volume expansion from 35 nL to 500 nL F: Droplet distance control unit for dense droplet packing or increase of the droplet distance.
500 nL Droplet Generator: Generation of 500 nL droplets for pipe based segmented flow experiments. Droplets can be composed of 5 different fluids (4D-Experiments).
4.5 nL Volume droplet sorter: The sorting unit comprise a observation chambre, drop distance control unit and a sorting t-junction. The unit was designed to select droplets from emulsion based processes.
4.5 nL Volume droplet generator: Generation of emulsions of 4.5 nL droplets by a flow-focussing junction technique. Droplets can be composed of three different fluids.
4.5 nL to 500 nL Volume droplet blow-up unit: Experimental blow-up unit for transfer of emulsion based droplets to channel confined droplet processing

The LabDisk was designed to unify three different droplet volume levels within one device to enable complex screening procedures e.g. serial transfer experiments.



Read-Out

Two image based readout techniques were used:
1.) Digital microscope imaging of the complete droplet storage arrays C and D (see Figure 1 - 3).
2.) Local video analysis of single channels containing flowing droplets (see Figure 4).

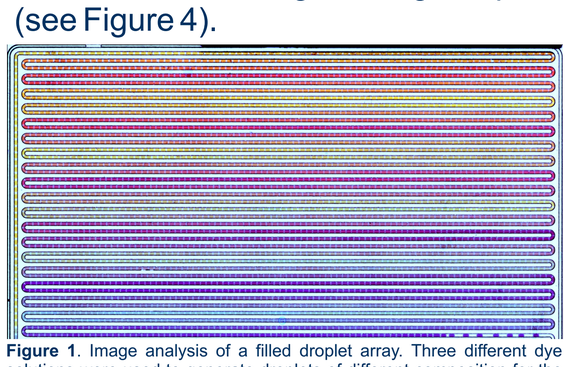


Figure 1. Image analysis of a filled droplet array. Three different dye solutions were used to generate droplets of different composition for the validation of the fluidic system.

The here applied optical read-out techniques allow screening operations focusing on: 1.) bacterial growth (e.g. MIC or ED_{50} determination), 2.) colony morphology analysis. (e.g. aggregation as response on toxic noxes) and 3.) colorimetric analyses.(e.g. screening for dye forming micro-organnisms)

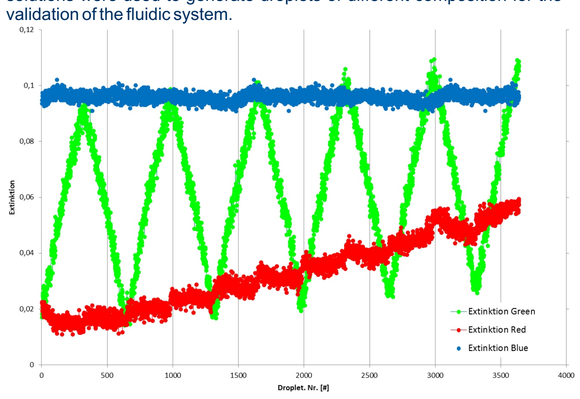


Figure 2.: Droplet compostion analysis. Colorimetric image analysis (RGB-Values) reveals the droplet composition accuracy corresponding to the fluidic protocol. Colorimetric information about dye forming microorganismen as well as the bacteria growth proportional grey-scale data can be received by this technique.

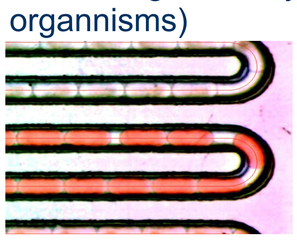


Figure 3. Image of a storage array section with bacteria. Droplet analysis was done along the red line to reveal bacteria growth and RGB-values.

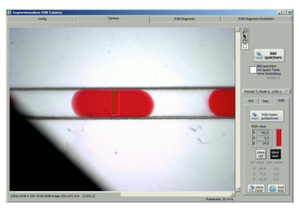


Figure 4. Video analysis of moving droplets. The revealed data can be used to trigger the droplet selection.

Results

Here, we present results from a screening run exploring the induction of the secondary metabolism by Cu-ions on co-cultures. A soil born yeast and a soil born streptomyces bacterium were used in co-culture with different ratios. Before the MIC Cu^{2+} tolerance was determined to: yeast >2 mM Cu^{2+} and about 0.5 mM Cu^{2+} for the streptomyces stain. Droplets with the different inoculum ratios of the two organism were generated and incubated for 7 day with and without 0.3 mM Cu^{2+} ions. Received results are shown in Figure 5. Without Cu^{2+} the organisms grow as expected as co-culture. The addition of Cu^{2+} leads to an inhibition of the yeast growth because the streptomyces stain dominates the inoculum (see figure 5). The received results indicate that the streptomyces produce a Cu^{2+} induced secondary metabolite which inhibits the yeast growth in the co-culture.

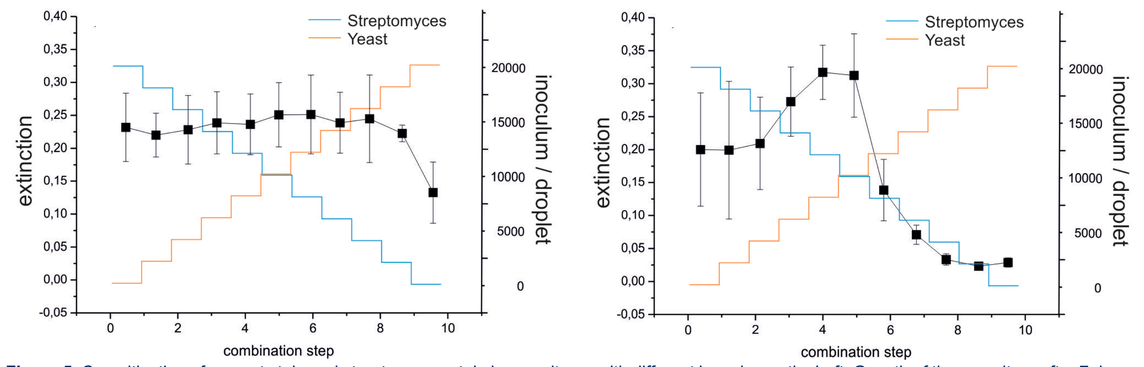


Figure 5: Co-cultivation of a yeast stain and streptomyces stain in co-cultures with different inoculum ratio. Left: Growth of the co-culture after 7 days incubation without Cu^{2+}. Right: Growth of the co-culture with 0.3 mM Cu^{2+} after 7 days. In the combination steps 3 - 5 the sub-lethal stimulation can be seen. Beyond step 5 the streptomyces inhibits the yeast growth by a Cu-induced secondary metabolite.

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CONTACT

*Dr. G. Alexander Groß
Phone: +49 3677 69-3716
E-Mail: alexander.gross@tu-ilmenau.de