APPLICATION NOTE

FLOW CYTOMETRY

MoFlo™ | Bacterial Proliferation and PHB Production

Population dynamics were followed during growth on phenol in response to adaptation processes of *Cupriavidus necato*, bacterial individuals morphologically nearly uniform, yet structurally and functionally diverse. The species belongs to the group of Betaproteobacteria and is known for its capability to degrade numerous toxic substances and therefore is used often as a model organism.[1]

Overflow metabolism is induced in cases of imbalanced substrate supplies and temporary changes in nutrient sources, which is thought to be part of a survival strategy. An example is the synthesis of poly-β-hydroxybutyrate (PHB).[2] Variations were analyzed in bacterial structural cellular parameters like chromosome contents (via 4´, 6-diamino-2´phenylindole -DAPI) and PHB (via Nile red).

Materials and Methods

C. necator JMP 134 was grown aerobically at 30 °C and at pH 7.0 in minimal medium in 0.5 l shake flasks for batch experiments and with 0.02 percent phenol as carbon and energy source. The harvested cells were centrifuged at 3,200 x g for six minutes, fixed with 10 percent NaN_3, and stored at 4 °C. For flow cytometric measurements[3] the preserved cells were centrifuged again, washed in NaCl-phosphate buffer (0.4 M Na_2HPO_4/NaH_2PO_4, 150 mM NaCl, pH 7.2) and resuspended at a concentration of 3 x 10^8 cells/mL.

DNA was stained with 4´, 6-diamino-2´phenylindole (DAPI) according to Meistrich et al.[4] The staining is AT-specific and stochiometric and thus provides quantitative information about the chromosome multiples within single bacterial cells.

To analyse the PHB content of the cells Nile red was used as follows: 40 µl of Nile red stock solution (1 mg/mL acetone) was added to 3 x 10^8 cells/mL DAPI stained cells, and incubated for eight minutes.

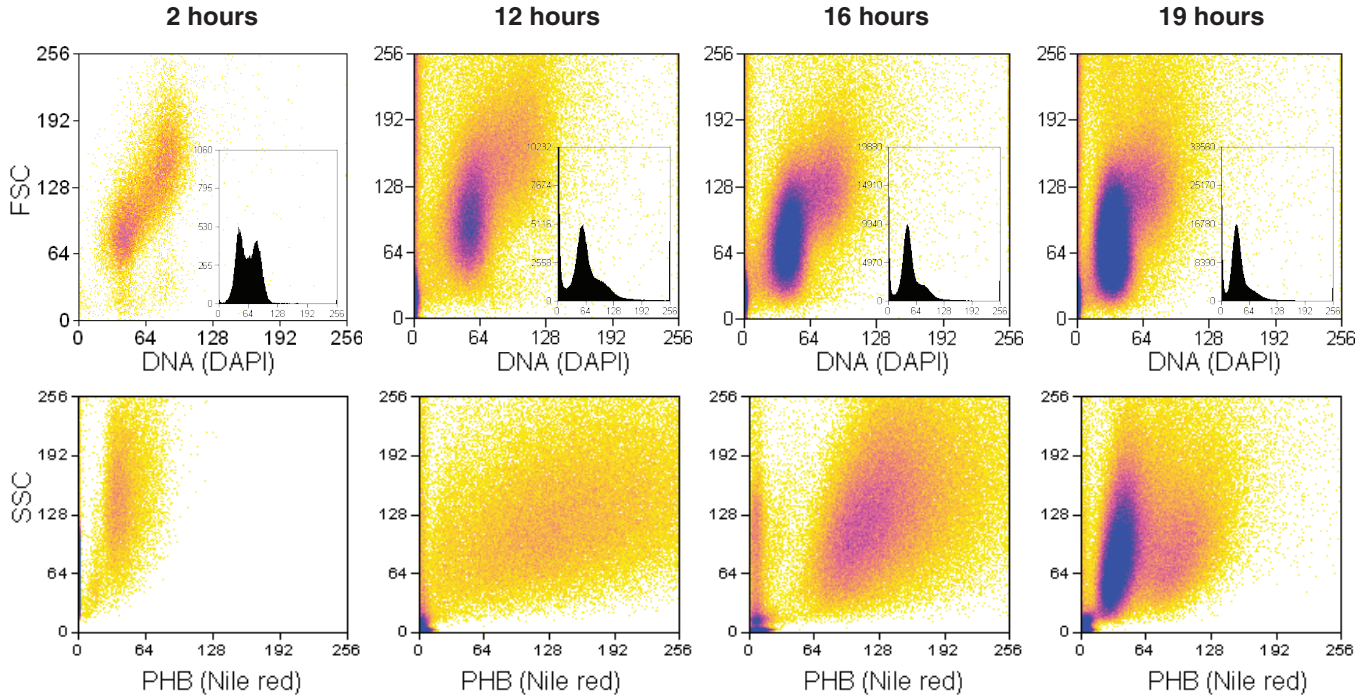
Flow cytometric measurements were performed using a MoFlo Cell Sorter equipped with two water-cooled argon-ion lasers (Innova 90C and Innova 70C). A wavelength of 488 nm (500 mW) was used to excite Nile red at the first observation point. DAPI was excited by multi-line-UV (333-365 nm, 110 mW) at the second observation point. Forward scatter signals (FSC; cell size related) were analyzed using the 488 nm line and a neutral density filter with an optical density of 2.3. The orthogonal side scatter signal (SSC; cell granularity related) was reflected first by a beam-splitter and then recorded after reflection by a 555 nm long-pass dichroic mirror using a D 488/10. Nile red and DAPI fluorescence was passed through a 580/30 and a 450/65 band-pass filter, respectively. Amplification was linear for all signals and measurement of the events was triggered by the SSC signal. Fluorescent beads (Polybead Microspheres: 0.483 µm diameter; flow check BB/Green compensation kit, Blue Alignment Grade) were used to align the MoFlo (CV-value about 2 percent). Data was acquired and analyzed using Summit software.

Results

Individual batch grown cells of ***C. necator*** were characterized with respect to their PHB content, FSC/SSC behavior and proliferation activity. After inoculation, the cells increased in size and an eukaryotic-like cell cycle behavior was observed (Figure 1: 2 hours). PHB production was not yet initiated. The low fluorescence intensity observed is due to unspecific staining of the lipid membranes of the cells. With the decline in nutrient or oxygen concentrations within the cultivation flask proliferation stopped. The surplus of carbon was stored as PHB in cellular granules (Figure 1: 12 hours). When the carbon and energy sources were depleted these storage materials were consumed during cell division and maintenance (Figure 1: 16 – 19 hours).

Figure 1

DNA content (vs. FSC, upper row) and PHB content (vs. SSC, lower row) of batch cultivated *C. necator* JMP134 cells grown on 0.02% phenol. Sampling times are indicated. The insets represent the chromosome contents.



Conclusion

The cell cycle distribution profiles of *C. necator,* represent an eukaryotic-like cell cycle behavior when grown on phenol under batch conditions. Since the species is usually able to induce uncoupled DNA synthesis on other substrates, these patterns provide information on phenol being a good but sub-optimal growth substrate. Also the strain is able to use PHB to survive disturbed growth conditions and to use it for maintaining turnover processes and cell-homeostasis.

References

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PRODUCT	CODE
MoFlo High-Performance Sorter	S2500

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The protocols in this application note might deviate from the normal recommended protocol/specification guidelines that are included with the Dako product or any other non-Dako product.

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