

REAL-TIME OD MONITORING FOR GROWTH-RATE CHARACTERISATION OF EVOLVED METHYLOTROPHIC *E. COLI*

Automated OD₆₀₀ monitoring from the RTS-8 Plus supported growth-rate characterization in an *E. coli* methanol-evolution study (¹Nieh et al., *Nat Commun.* 2024).

BACKGROUND

Methanol – derivable from CO₂ or methane – is a promising renewable feedstock for sustainable bioproduction. However, wild-type *E. coli* cannot utilise it, and existing synthetic methylotrophs grow far slower than native ones. Nieh *et al*¹ set out to evolve an *E. coli* strain capable of fast growth on methanol as its sole carbon source using adaptive laboratory evolution and a dynamic-copy-number bacterial artificial chromosome (ddp-BAC).

THE CHALLENGE

- Methanol metabolism produces formaldehyde, which causes DNA–protein crosslinking (DPC) – toxic damage that kills cells and creates a bottleneck for evolving faster growth.
- The evolution campaign required screening many strains across methanol concentrations (0.4–1.2 M) over hundreds of passages, with growth rate as the primary readout.
- After adaptive evolution and candidate selection, objective growth-rate characterisation was needed to compare improved strains under defined methanol-medium conditions.

ROLE OF THE RTS-8 PLUS IN THE WORKFLOW

- For growth-rate measurement, the authors cultivated strains in 20 mL of methanol minimal medium in single-use 50 mL cultivation tubes using the RTS-8 Plus at 37 °C and 2700 rpm (¹ Methods).
- The instrument recorded OD₆₀₀ automatically in real time, providing continuous growth curves without opening the vessel.
- Reverse-Spin® mixing supported automated cultivation and OD monitoring in the tube format; eight parallel channels allowed biological replicates or condition comparisons in a single run.
- The authors also used a benchtop spectrophotometer for diluted-sample OD₆₀₀ verification and a 2 L bench-scale bioreactor for high-cell-density fed-batch cultivation.

RESULTS

- A doubling time of approximately 3.5 h was recorded for the evolved strain SM7 – comparable to or faster than native methylotrophs *M. extorquens* AM1 (~4 h) and *B. methanolicus* (~5 h).
- The stabilised strain SM8 (SM7 with WT mutS restored) reached 20 g/L dry cell weight within 77 h in a 2 L bioreactor under fed-batch conditions – a separate cultivation step from the RTS-8 Plus screening.
- The RTS-8 Plus contributed continuous, non-invasive OD₆₀₀ data used by the authors for growth-rate characterisation during the strain characterization of synthetic methylotrophic *E. coli*.

¹ Nieh, L.-Y., Chen, F. Y.-H., Jung, H.-W., Su, K.-Y., Tsuei, C.-Y., Lin, C.-T., Lee, Y.-Q. & Liao, J. C. Evolutionary engineering of methylotrophic *E. coli* enables fast growth on methanol. *Nature Communications* **15**, 8840 (2024). <https://doi.org/10.1038/s41467-024-53206-4>

AT A GLANCE

APPLICATION

Growth-rate characterisation of synthetic methylotrophic *E. coli* · methanol medium · 37 °C

CHALLENGE

Formaldehyde toxicity (DPC) blocks fast methanol growth; continuous growth-rate data needed

APPROACH

Adaptive evolution + ddp-BAC

INSTRUMENT ROLE

Automated OD₆₀₀ in single-use 50 mL cultivation tubes
20 mL · up to 2700 rpm

OUTCOME

Doubling time ~3.5 h recorded for evolved strain (SM7/SM8)

RTS-8 Plus

THE INSTRUMENT



Automated, non-invasive OD₆₀₀ – continuous growth curves in single-use cultivation tubes

Built-in O₂ (0–100%) & pH (4.0–8.5) monitoring *via* non-invasive sensor spots (not used in this study)

8 parallel channels – biological repeats or media, strain & feeding screens in one run

OD range up to 100 OD₆₀₀ equivalent · Reverse-Spin® mixing for strong aeration