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Synchronisation of *S. acidocaldarius* with Acetic Acid

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Abstract

This protocol describes how to synchronise a *S. acidocaldarius* cell culture. The first step is administering acetic acid to arrest the cells in a G2-like state. Once washed, the culture proceeds synchronously through division and DNA replication for about two cell cycles.

Related publication(s): Tarrason Risa et al, 2020 (this version) and Lundgren et al., 2004 (original version)

Background

S. acidocaldarius belongs to the TACK super phylum (Guy and Ettema, 2011). At the time of writing, these archaea are the closest archaeal relatives of eukaryotes that can be easily grown and studied in a lab. As such, there is considerable interest in understanding their cell biology. However, the field currently lacks the tools to tag and observe *S. acidocaldarius* proteins in vivo. Synchronisation is therefore key to cell cycle studies in this archaeon. Only one alternative to acetic acid synchronisation currently exists, which uses physical separation based on cell size (Duggin, McCallum, and Bell 2008). Comparing the two, acetic acid synchronisation is simpler and enables higher throughput. However, it is still not known how acetic acid arrests the cell cycle of *S. acidocaldarius*. Nevertheless, it has become the synchronisation method of choice due to the ease of conducting biochemical and microscopy analyses downstream.

Materials

Product name	Brand / manufacturer	Catalogue number / SKU	Notes
S. acidocaldarius	DSMZ, Leibniz Institute	DSM No.: 639	Kept at exponential growth for 3 days. OD600 should be between 0.1 and 0.3 when starting the experiment.
Brock medium	Home made		See Brock medium protocol
Absolute ethanol	EMPARTA / Merck	1070172511	NB fire hazard
100% acetic acid	Sigma-Aldrich / Merck	695092	NB Strong smell, use fume hood

Equipment

Equipment name	Brand / manufacturer	Catalogue number / SKU	Notes
Erlenmeyer flasks	Pyrex / Corning	SLW4980-500-6EA	For cells to grow. 20% culture and 80% air
Spectrophotometer cuvettes	Supelco / Merck	C5291-100EA	For OD600 readings
Falcon tubes 15mL	Falcon / Corning	14-959-49A	Flow cytometry & microscopy
Falcon tubes 50mL	Falcon / Corning	14-959-53A	Westerns & qPCR & Omics
Screw cap tubes	Merck	AXYSCT150CS	qPCR and westerns
Gilson pipettes and tips	Gilson		For liquid handling
Pipette controller and serological pipettes	Fisherbrand / Thermo Fisher Scientific		For liquid handling
Shaking incubator at 75°C	Thermotron / Infors HT		Must have a shaker capable of maintaining high temperatures

Protocol

1. Grow 750mL of *S. acidocaldarius* (In a 2L+ Erlenmeyer flask) to exponential growth phase (0.1-0.3)
    i. Collect control samples:
        a. Measure OD600
        b. Take a 3mL sample and “fix” with 7mL 100% Ethanol (on ice).
        c. Take 2 x 25mL samples, spin down at 4,500 rcf for 7 min —> remove liquid —> transfer to a 1mL tube screw cap using 1mL of Brock medium —> spin down at 16,000 rcf for 1 min —> remove liquid and store in a freezer (1 x 25mL for qPCR and 1 x 25mL for western)

2. Add Acetic acid to the exponentially growing culture to a final concentration of 3mM (~0.02mL of 100% acetic acid/100mL culture)

3. Let cells arrest in acetic acid for 4.5 hrs.

i. Record the effect every hour (keeping the culture at 75°C by using a waterbath during):
    a. Measure OD600 (should not change)
    b. Take a 3mL sample and "fix" with 7mL 100% Ethanol (on ice).
    c. Take 2 x 25mL samples, spin down at 4,500 rcf for 7 min —> remove liquid —> transfer to a 1mL tube screw cap using 1mL of Brock medium —> spin down at 16,000 rcf for 1 min —> remove liquid and store in a freezer (1 x 25mL for qPCR and 1 x 25mL for western)

4. Wash out the acetic acid
    i. Take the contents of the arrested cultures and spin down at 4,500 rcf for 5 min
    ii. Resuspend the pellet in fresh and warm Brock media (50mL)
    iii. Spin down at 4,500 rcf for 5 min and resuspend in 50mL fresh and warm Brock
    iv. Repeat this two times to remove any residual acetic acid

Note that step 4 should take around 30 minutes to complete at most

5. Re-suspend cells in 50mL fresh and warm Brock media and add them to a 2L Erlenmeyer flask with 650mL fresh and warm Brock media (the culture should have a final OD600 of about 0.08-0.15).
    i. Every 20 min from time 0 (for at least a total of 200 min for a full cycle):
        a. Measure OD600 (should not change)
        b. Take a 3mL sample and "fix" with 7mL 100% Ethanol (on ice).
        c. Take 2 x 25mL samples, spin down at 4,500 rcf for 7 min —> remove liquid —> transfer to a 1mL tube screw cap using 1mL of Brock medium —> spin down at 16,000 rcf for 1 min —> remove liquid and store in a freezer (1 x 25mL for qPCR and 1 x 25mL for western)

6. Store the EtOH fixed samples in the fridge

Note that you need about 1mL of EtOH fixed cells for flow cytometry and about 100uL for immunofluorescence

7. Following this protocol, synchronised cells can next be studied by flow cytometry, immunofluorescence microscopy, qPCR, and western blotting, etc.

Data analysis

Typical OD600 readings and flow cytometry profiles from a synchronisation experiment are exemplified in figure 1 and figure 2 below (Tarrason Risa, 2021).

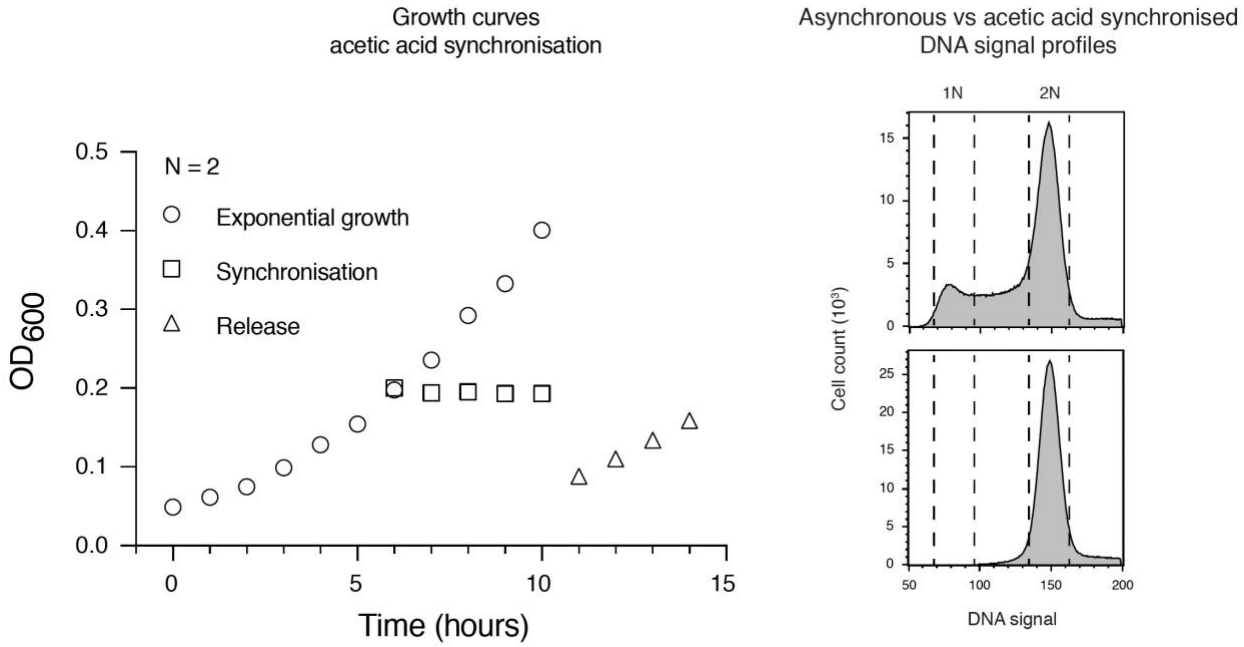

Figure 1: The effect of acetic acid on the growth and cell cycle of S. acidocaldarius. (Left) Growth curve of S. acidocaldarius as detected by a spectrophotometer. Cell growth was arrested upon addition of acetic acid. Washing out the acetic acid allowed the cells to resume growth, albeit more slowly. (Right) Flow cytometry histograms showing the DNA signal profiles of S. acidocaldarius before and 4.5 hours after treatment with acetic acid. After 4.5 hours with acetic acid treatment, no cells were observed with less than 2N DNA signal, indicating completed synchronisation. Representative plots shown, n = 10^6. Reprinted with permission (Tarrason Risa, 2021).

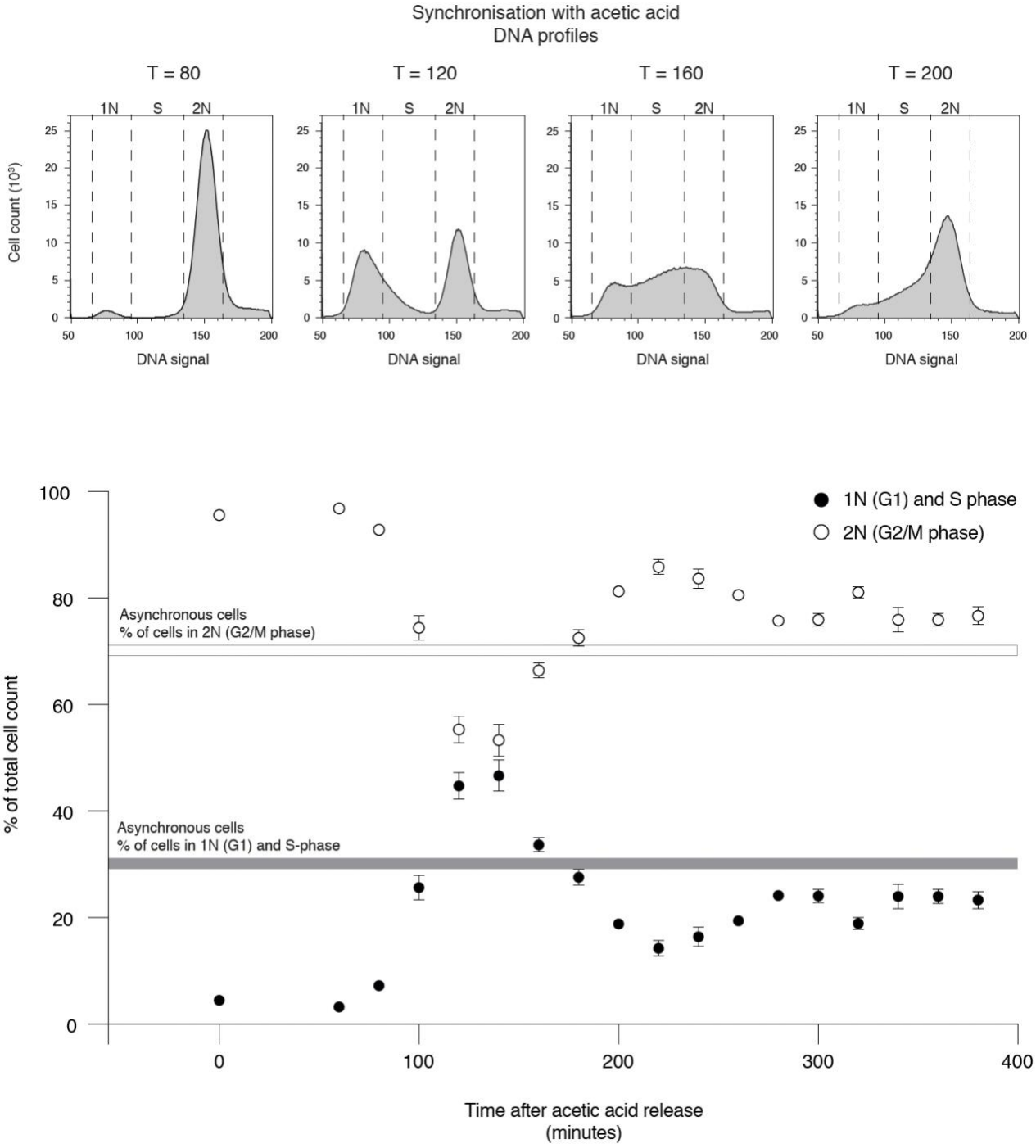

Figure 2: Synchronisation of S. acidocaldarius with acetic acid. (Top) Flow cytometry histograms showing the DNA signal profiles of a synchronised time course experiment with 40-minute intervals from 80 to 200 minutes after release. In this period the cells went from a 2N state (G2 and M-phase), through a 1N state (G1 phase), a between state (S-phase), and return to a majority 2N state (G2). Representative plots shown, n = 106. (Bottom) Quantification of three biological replicates showing the % of cells in a 1N or in between phase (G1 and S-phase) vs a 2N state. The synchrony of cells was nearly lost after one round of division, after which the DNA signal profile was similar to that of an asynchronous culture. N = 3, n = 10^6. Reprinted with permission Reprinted with permission (Tarrason Risa, 2021).

Additional Notes

Thermal management is critical to the success of the synchronisation. Sample collection and handling should be done at temperatures close to 75°C. If possible, preheating of all equipment including centrifuges is strongly recommended.

Competing interests

The authors declare that they have no conflict of interest.

Acknowledgments

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