Glutathione attenuates uranyl toxicity in *Lactococcus lactis*

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We investigated the role of intracellular glutathione (GSH), which in a large number of taxa plays a role in the protection against the toxicity of heavy metals. Anaerobically grown *Lactococcus lactis* containing an inducible GSH synthesis pathway was used as a model organism allowing the study of GSH-dependent uranyl detoxification without interference from additional reactive oxygen species. Microcalorimetric measurements of the metabolic heat showed that intracellular GSH attenuates the toxicity of uranium at a concentration in the range of 10–150 µM. Isothermal titration calorimetry revealed the endothermic binding of U(VI) to the carboxyl group(s) of GSH. The data indicate that the primary detoxifying mechanism is the intracellular sequestration of carboxyl-coordinated U(VI) into an insoluble complex with GSH.[1]

EXPERIMENTAL. The strain NZ9000-pNZ3203 of *L. lactis* subsp. *cremoris* (NIZO food research B.V., The Netherlands) carries a plasmid with the *gshA* and *gshB* genes under the control of the *nisA* promoter, rendering the expression of the two genes, which are required for GSH synthesis, inducible by nisin. Bacteria were grown at pH 7.2 in M17 medium. Rate constants for exponential growth phases were calculated using the relation: $\int P(t)\,dt = H(t)$, with P(t): heat flow at time t (µW), and H(t): integrated heat (Joule). For exponential growth, the slope of a linear stretch in a heat flow *vs.* integrated heat plot is $dP/dH = k$ which is the growth rate k of the culture.

RESULTS. Plots of the heat flow *vs.* integrated heat recorded from *L. lactis* cultures in the presence of varying concentrations of uranyl are shown in Fig. 1. Linear stretches represent exponential growth phases. Non-induced cells do not produce GSH and the rate of their second exponential growth phase (proportional to the slope of the fitted lines) declines pronouncedly with increasing uranyl concentration (left panel). In the presence of nisin, the cells produce intracellular GSH and their second exponential growth becomes more resistant to uranyl (right panel). The quantitative analysis in Fig. 2 shows that uranyl does not affect initial growth rate k_1 but specifically interferes with the metabolism at later exponential growth described by k_2. GSH clearly attenuates the toxicity of uranyl during that phase, allowing larger exponential growth still up to 100 µM uranyl nitrate. The molecular mechanism of GSH-dependent modulation of uranyl toxicity is most likely due to the formation of an insoluble GSH uranyl complex as revealed by isothermal titration calorimetry of uranyl nitrate in aqueous GSH solution. Figure 3 shows the transition from complex formation (endotherm) to precipitation (exotherm) The determined enthalpic and entropic terms agree with the binding of uranyl the carboxylate function of GSH.[2]

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[1] Obeid, M. *et al.* (2016) *Appl. Environ. Microbiol.* **82**, 3563–357.
[2] Bismondo, A. *et al.* (1992) *Thermochim. Acta* **196**, 131–136.

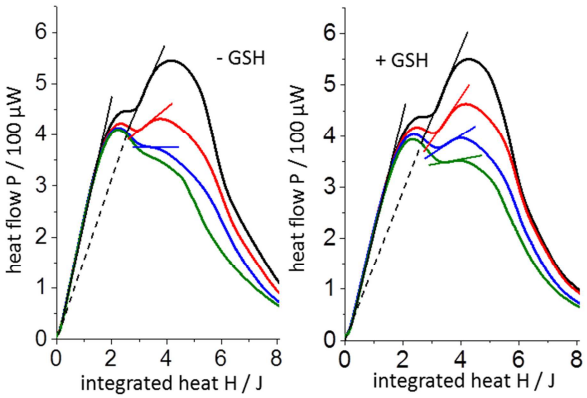

Fig. 1: Metabolic thermal power *vs.* integrated metabolic heat. Colored lines indicate exponential growth at 0, 50, 100, 150 µM uranyl. Left: growth without GSH production, right: growth with GSH production. Slopes scale with growth rates k_1 and k_2 (H > 3 J).

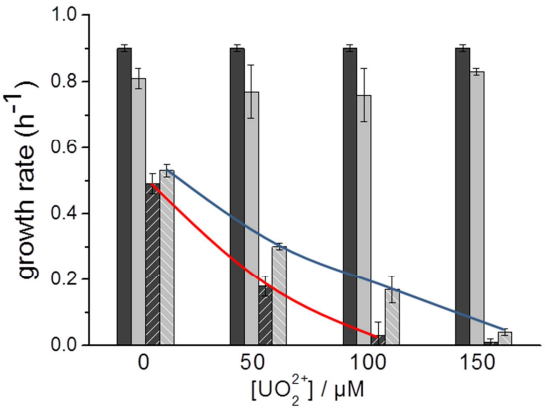

Fig. 2: Growth rates k_1 (no texture) and k_2 (hatched) of early and late exponential growth, respectively, as function of uranyl nitrate concentration. Rate k_1 is independent of GSH production (light grey), whereas k_2 (dark grey) decreases with uranyl concentration (red trace). The decrease is less pronounced in the presence of GSH (blue trace).

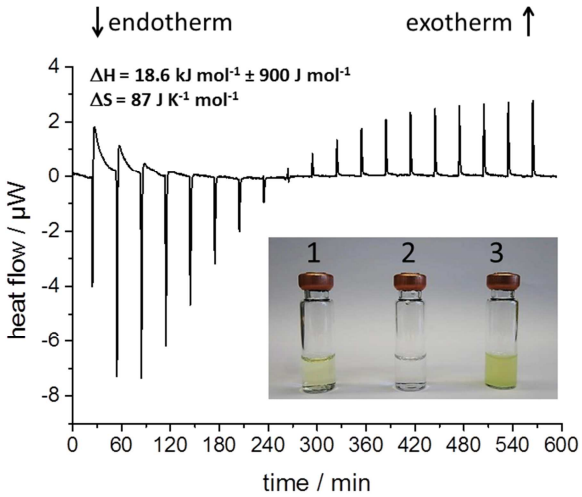

Fig. 3: Isothermal titration calorimetry. Ten-microliter aliquots of a 200 mM solution of GSH were injected into 5 mM uranyl nitrate at pH 4.5. The picture shows the uranyl (1) and GSH (2) solution before mixing. The turbidity upon mixing reveals an insoluble complex (3).