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EXPERIMENTAL ARTICLES

Bacterial Degradation of EDTA

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Abstract—Degradation of EDTA (ethylenediaminetetraacetic acid) or metal–EDTA complexes by cell suspensions of the bacterial strain DSM 9103 was studied. The activity of EDTA degradation was the highest in the phase of active cell growth and decreased considerably in the stationary phase, after substrate depletion in the medium. Exponential-phase cells were incubated in HEPES buffer (pH 7.0) with 1 mM of uncomplexed EDTA or EDTA complexes with Mg^{2+}, Ca^{2+}, Mn^{2+}, Pb^{2+}, Co^{2+}, Cd^{2+}, Zn^{2+}, Cu^{2+}, or Fe^{3+}. The metal–EDTA complexes (Me–EDTA) studied could be divided into three groups according to their degradability. EDTA complexes with stability constants K below 10^{16} ($\log K < 16$), such as Mg–EDTA, Ca–EDTA, and Mn–EDTA, as well as uncomplexed EDTA, were degraded by the cell suspensions at a constant rate to completion within 5–10 h of incubation. Me–EDTA complexes with $\log K$ above 16 (Zn–EDTA, Co–EDTA, Pb–EDTA, and Cu–EDTA) were not completely degraded during a 24-h incubation, which was possibly due to the toxic effect of the metal ions released. No degradation of Cd–EDTA or Fe(III)–EDTA by cell suspensions of strain DSM 9103 was observed under the conditions studied.

Key words: EDTA, metal–EDTA complexes, degradation, bacteria, metal toxicity.

Ethylenediaminetetraacetic acid (EDTA), owing to its ability to form stable water-soluble complexes with most metal ions, is employed for the decontamination of nuclear power plants and in photography, the textile and paper industries, galvanic processes, and production of detergents and soap powders [1]. At present, annual EDTA production has reached 100 000 tons [2]. A steady environmental accumulation of EDTA has been revealed; little or no elimination of EDTA occurs during wastewater treatment, and the only known sink of EDTA is UV degradation of Fe(III)–EDTA at the water surface [3, 4]. Contamination of subsoil waters with EDTA deteriorates the quality of drinking water because of the conversion of heavy and toxic metals to a soluble state.

Microbial degradation of aminopolycarboxylic acids, including EDTA, was reviewed by Bucheli-Witschel and Egli [4]. As a result of 40 years of study, three pure cultures of EDTA-degrading bacteria have been isolated: an isolate assigned to the genus *Agrobacterium* degraded the Fe(III)–EDTA complex [5]; strain BNC-1, a gram-negative bacterium, was able to degrade Mg–EDTA, Ca–EDTA, Mn–EDTA, and Zn–EDTA [6, 7]; the third isolate, DSM 9103, a gram-negative bacterium, was assigned to *Proteobacteria* [8]. From the latter strain, the enzyme complex EDTA monooxygenase, catalyzing primary degradation of EDTA, was isolated and purified [4, 9]. This enzyme complex was found to exhibit high specificity towards certain metal–EDTA complexes, which could be ranged in the following order with respect to their degradability: Mg–EDTA > Zn–EDTA > Mn–EDTA > Co–EDTA > Cu–EDTA, whereas EDTA complexes with Ca^{2+}, Ni^{2+}, and Fe^{3+}, as well as uncomplexed EDTA, were not utilized as substrates by EDTA monooxygenase [9]. Earlier, we established the conditions optimal for the degradation of Mg–EDTA by suspensions of exponential-phase cells of strain DSM 9103 (the concentrations of biomass and EDTA and the procedure of cell washing) [10].

The aim of this work was further study of the degradation of various metal–EDTA complexes and uncomplexed EDTA by nongrowing cells of the bacterial strain DSM 9103.

MATERIALS AND METHODS

The bacterial strain DSM 9103 [8] used in this study was maintained on agar medium [11] containing 1 g/l of EDTA as the source of carbon, energy, and nitrogen and the following mineral components (mg/l): $MgSO_4 \cdot 7H_2O$, 1000; KH_2PO_4, 260; $CaCl_2 \cdot 2H_2O$, 400; $Na_2HPO_4 \cdot 12H_2O$, 830; $FeCl_2 \cdot 4H_2O$, 1.5; H_3BO_3, 0.06; $MnCl_2 \cdot 4H_2O$, 0.1; $CoCl_2 \cdot 6H_2O$, 0.12; $ZnCl_2$, 0.07; $NiCl_2 \cdot 6H_2O$, 0.025; $CuCl_2 \cdot 2H_2O$, 0.015; $Na_2MoO_4 \cdot 2H_2O$, 0.025. As a source of vitamins, 0.1 g/l of yeast

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extract (Difco, USA) was used. The initial pH of the medium was 7.0.

The bacterium was cultivated in 750-ml flasks containing 100 ml of the aforementioned medium on a shaker (150–200 rpm) at 28°C.

Cells were harvested by centrifugation at 20°C and washed with a solution containing 2 mM EDTA and 5 mM $NaNO_3$ (pH 7.0) [10]. An aliquot of washed biomass was suspended in 50 ml of buffer (pH 7.0) containing 20 mM HEPES and 20 mM $NaNO_3$. HEPES buffer was chosen because of its low complexing capacity for metal ions [12]. The cell suspension was shaken at 28°C for 30 min; then 50 ml of uncomplexed EDTA or of a Me–EDTA complex in an appropriate concentration was added and the mixture was incubated at 28°C on a shaker (150–200 rpm) for 24 h. The amount of biomass in the assay mixture was 0.4–1.1 g/l [10]. Me–EDTA complexes (0.8–1.0 mM) were prepared no later than 24 h prior to use. Samples were withdrawn from the assay mixture at a preset time, centrifuged, and analyzed for EDTA. With the exception of $ZnSO_4$, all metal ions were applied in the form of chloride salts, and EDTA was applied as a tetrasodium salt.

Biomass density was determined with a Specol 221 spectrophotometer (Germany) at 546 nm after acidifying the cell suspension with 5% HNO_3 to pH 2.0 to dissolve precipitates formed during bacterial growth. The amount of dry biomass was calculated from the optical density of the cell suspension using a calibration curve.

To determine the number of colony-forming units (CFU), serial dilutions of the culture liquid in physiological saline (0.9% NaCl) were plated onto petri dishes with nutrient agar, and the colonies grown were enumerated.

The EDTA concentration was analyzed as described in [13] on an HPLC chromatograph (LKB, Sweden) equipped with a Nucleosil 100 column (Machery und Nagel, Germany) at 285 nm.

The efficiency of EDTA degradation, designated as Deg(t), was calculated as the amount of EDTA (mmol) degraded in a time t per the amount of biomass involved in the process (g) by the formula $\mathrm{Deg}(t) = [C(0) - C(t)]/X$, where $C(0)$ and $C(t)$ are EDTA concentrations (mM), initial and at the moment t, respectively; and X is biomass concentration (g/l), which is constant during the whole process of EDTA degradation by washed cells. This value is convenient since its derivative with respect to time (the slope ratio of the tangent line to curve Deg(t)) is equal to the specific rate of EDTA degradation:

$$q_S = \frac{d\mathrm{Deg}(t)}{dt} = -\frac{1}{X}\frac{dC}{dt}.$$

The values of Deg(t) for rectilinear dependences of q_S were calculated by the formula $q_S = \mathrm{Deg}(t)/t$.

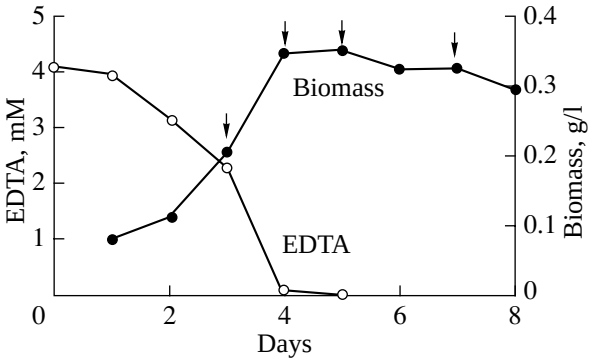

Fig. 1. Growth of bacterial strain DSM 9103 in EDTA-containing medium. Arrows indicate moments of sampling.

RESULTS AND DISCUSSION

In the first series of experiments, the effect of the growth phase on the EDTA-degrading activity of the cells was studied. Figure 1 shows typical curves of cell growth and EDTA consumption. Arrows indicate the moments of sampling for the determination of rates of Mg–EDTA degradation by washed cells. It should be noted that, in EDTA-free medium (control), biomass increased only slightly (from 0.04 to 0.06 g/l) over 7 days. Figure 2 presents data on the efficiency of Mg–EDTA degradation (mmol of EDTA degraded per g of biomass) by cells taken from the phase of active growth (curve *1*) or from the stationary phase and the phase of cell death (curves *2–4*). Actively growing cells (curve *1*) exhibited the highest specific degradation rate (0.257 ± 0.012 mmol EDTA/(g h)). Cells taken from the early stationary phase (curve *2*) exhibited initially a specific degradation rate fourfold less than that of actively growing cells, but within the first 5 h of incubation, their activity was restored to the level typical of the exponential-phase cells. Cells taken 1 day after EDTA depletion in the medium (curve *3*) showed markedly lower activity, and their specific EDTA degradation rate could not be restored to the maximum value characteristic of the exponential-phase cells. Three-day starvation resulted in an almost total loss of EDTA degrading activity by the cells (curve *4*). The results obtained suggest that EDTA monooxygenase is an inducible enzyme and rapidly loses its activity after substrate exhaustion. This inference is supported by the observation that cells grown on nutrient agar were unable to grow after their transfer to EDTA-containing medium. Determination of the dynamics of the CFU number in the course of batch cultivation of this strain in EDTA-containing medium showed that the number of viable cells decreased by 35–43% and 60–63% after 1 and 3 days of starvation, respectively, as compared to the number of viable cells in the end of the exponential phase. Taking these results into account, only cells from the exponential growth phase were used in further experiments.

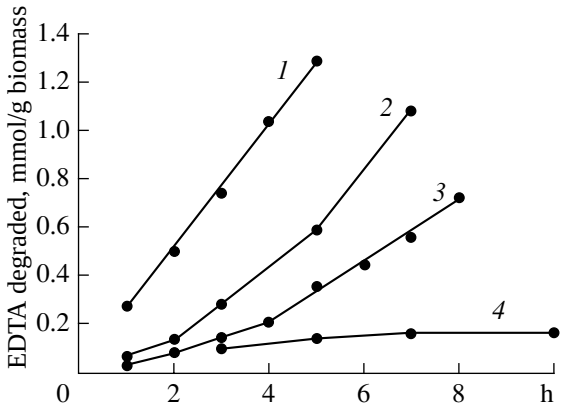

Fig. 2. Degradation of Mg–EDTA by cell suspensions of strain DSM 9103 taken from (*1*) exponential phase, (*2*) stationary phase, or (*3*, *4*) phase of cell death.

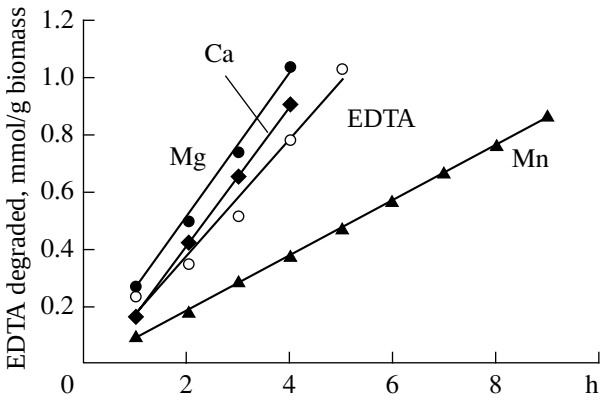

Fig. 3. Degradation of uncomplexed EDTA or EDTA complexes with Mg^{2+}, Ca^{2+}, or Mn^{2+} by cell suspensions of strain DSM 9103.

Degradation of Uncomplexed EDTA or Me–EDTA Complexes by Nongrowing Cells of Strain DSM 9103

The complexes of EDTA with Mg^{2+}, Ca^{2+}, Mn^{2+}, Pb^{2+}, Co^{2+}, Cd^{2+}, Zn^{2+}, Cu^{2+}, and Fe^{3+} were used as substrates; the stability constants of these complexes ($\log K$) are 10.6, 10.7, 15.6, 18.0, 18.1, 18.2, 18.3, 20.5, and 25.0, respectively [9]. The Me–EDTA complexes studied can be divided into three groups with respect to their degradability by cell suspensions of strain DSM 9103. The first group comprises Mg–EDTA, Ca–EDTA, and Mn–EDTA complexes with comparatively low stability constants ($\log K$ below 16) and uncomplexed EDTA, which were degraded by washed cells completely at rates that were constant for each substrate within 5–10 h of incubation. The data on the efficiency of degradation of these Me–EDTA complexes (mmol EDTA degraded/g biomass) by cell suspensions are given in Fig. 3. Specific degradation rates for Mg–EDTA, Ca–EDTA, uncomplexed EDTA, and Mn–EDTA were 0.255, 0.245, 0.146, and 0.096 mmol EDTA/(g h), respectively. Thus, there is an inverse correlation between the stability of Me–EDTA complexes and the rates of their degradation by bacterial cells.

It is noteworthy that uncomplexed EDTA and the Ca–EDTA complex were rapidly degraded by the cells, although they are not substrates for EDTA monooxygenase, an enzyme complex isolated from this bacterial strain [9]. Explanation of this phenomenon invites further investigations.

The second group of Me–EDTA complexes comprised Zn–EDTA, Co–EDTA, Pb–EDTA, and Cu–EDTA, which were degraded only partly during the incubation with cell suspensions of strain DSM 9103. As seen from Fig. 4, the degradation rates of these complexes by bacterial cells changed in the course of incubation. The initial specific degradation rates for Zn–EDTA, Pb–EDTA, Co–EDTA, and Cu–EDTA were 0.076, 0.141, 0.017, and 0.033 mmol EDTA/(g h), respectively. It should be mentioned that all the Me–

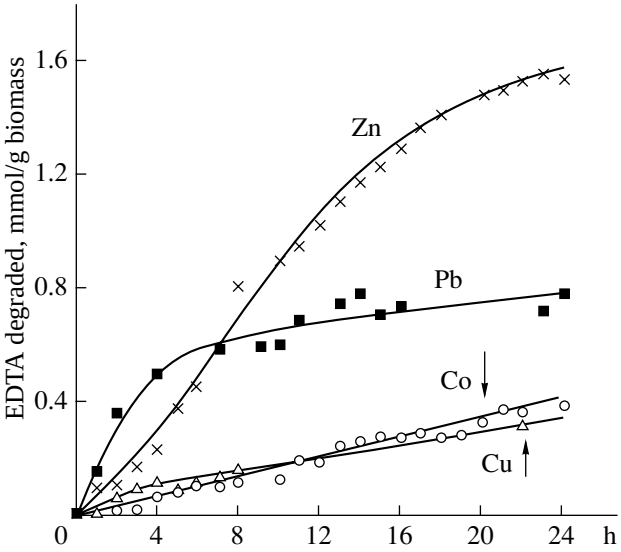

Fig. 4. Degradation of EDTA complexes with Co^{2+}, Cu^{2+}, Pb^{2+}, or Zn^{2+} by cell suspensions of strain DSM 9103.

EDTA complexes of this group were characterized by high stability ($\log K$ above 16); unlike the case with less stable complexes, no clear-cut relationship was observed between the stability constants and the degradation rates of these complexes. In particular, the degradation rate of Co–EDTA was considerably lower than that of Zn–EDTA, although their stability constants were very close. It is reasonable to assume that the sharp decline observed in the rate of Pb–EDTA degradation within first hours of incubation with bacterial cells is due to the toxicity of lead ions released through the degradation of this complex.

The third group of Me–EDTA complexes consisted of Cd–EDTA and Fe(III)–EDTA, whose degradation by the cells of strain DSM 9103 was not revealed under the conditions employed. The resistance of Fe(III)–EDTA

to bacterial degradation seems to be due to its extremely high stability and inertness. The degradation of Cd–EDTA was not revealed, possibly because of the high toxicity of cadmium ions, which inhibited the metabolism of bacterial cells even when released from the complex in trace amounts.

To conclude, most of the Me–EDTA complexes studied were degraded by washed cells of strain DSM 9103. The reasons for incomplete degradation of stable Me–EDTA complexes (log$K$ above 16) by bacterial cells remain to be investigated.

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