Electronic Supplementary Material (ESI) for Chemical Communications

Supporting Information for:

Phosphate starvation as an antimicrobial strategy: the controllable toxicity of lanthanum oxide nanoparticles

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Content:

A) X-ray diffraction analysis

Figure S1 shows the XRD spectra of the lanthanum oxide nanoparticles (La_2O_3) as received from the commercial supplier and of lanthanum oxide nanoparticles prepared by flame spray synthesis. In the commercial product different lanthanum species were identified: lanthanum oxide carbonate ($La_2O_2CO_3$) in orthorhombic crystal structure (Ref. code: 01-070-5539), lanthanum oxide carbonate ($La_2O_2CO_3$) in monoclinic crystal structure (Ref. code: 00-023-0435), and lanthanum hydroxide ($La_2(OH)_3$) (Ref. code: 01-075-1900). As described in the main text, these species are related and can be transformed. To investigate pure lanthanum oxide, we produced La_2O_3 nanoparticles by flame spray synthesis as described in detail by Loher et al.[1] Briefly, for the La_2O_3 precursor, 30.052 g lanthanum(III)-nitrat hydrat (99.9%, Aldrich-Fine Chemicals) was dissolved in 116.41 g tetrahydrofuran (>99.8%, Fluka-Chemie AG). The liquid precursor was pumped through a needle (5 mL min^{-1}), dispersed by oxygen (5 L min^{-1}) and ignited with a methane (1.2 L min^{-1})/oxygen (2.4 L min^{-1}) flame. The production rate was about 10 g h^{-1}. As-formed nanoparticles were collected on a fiber filter unit mounted above the flame by the aid of a vacuum pump. The peaks were correlated to lanthanum oxide (Ref. code: 01-083-1344). The flame sprayed particles were added to *E. coli* cultures. The observed effect was very similar to the effect demonstrated with the commercially obtained particles.

The flame sprayed particles were added to *E. coli* cultures and showed a similar effect than the commercial ones.

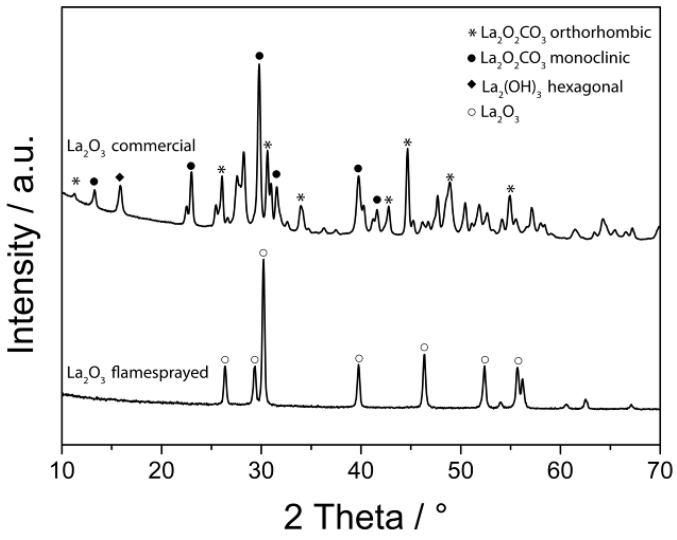

Fig. S1 X-ray diffraction spectra of commercial lanthanum oxide nanoparticles and flame spray synthesized lanthanum oxide nanoparticles.

B) Phosphate absorption capacity of lanthanum oxide nanoparticles

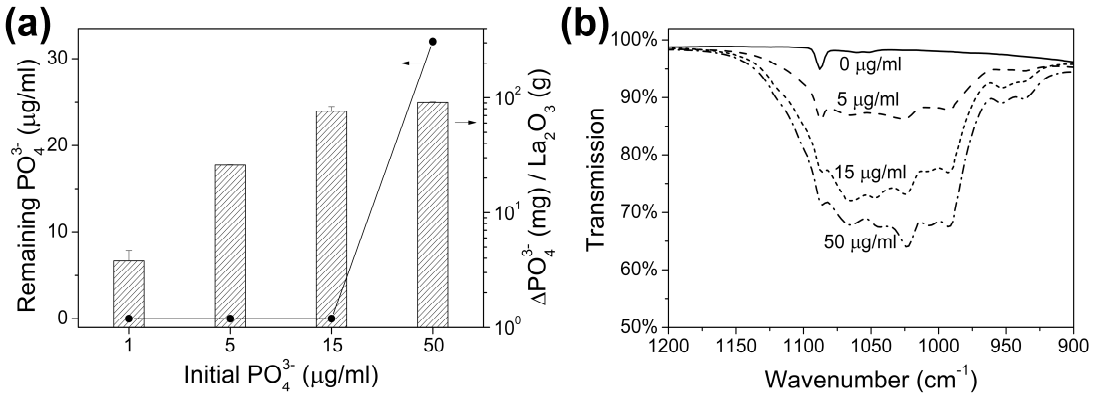

Fig. S2 Phosphate absorption capacity of lanthanum oxide nanoparticles. (a) To four solutions with initial phosphate levels of 1, 5, 15, resp. 50 µg/ml a defined amount of La_2O_3 was added. After 24 hours the phosphate in the solution was measured (left axis). A saturation value was reached between the 15 µg/ml and 50 µg/ml experiment. This corresponds to a value on the secondary y-axis of 80-100 mg phosphate per gram lanthanum oxide. (b) Infrared spectra revealed different transmission for particles that were exposed to different amounts of phosphate. The more phosphate was absorbed to the particles, the lower was the transmission in the P-O relevant vibration range around 1'000 cm^{-1}.

C) Phosphate limited growth of *E. coli*

In order to investigate the effect of phosphate limitation on the growth of microorganisms, cultures of *Escherichia coli (E. coli)* were grown in growth media with defined phosphate concentrations (See ESI, Fig. S3). Within 24 hours a colony of 4'500 CFU/ml (colony-forming units per milliliter) grew to 10^7 CFU/ml under excess of phosphate (100 µg/ml). Under the limitation of phosphate (≤ 5 µg/ml) the size of the colony at the same time point was more than 100 times smaller. This clearly shows that the colony growth was substantially hindered by limiting the amount of available phosphate, which is also in agreement with literature data.[2] It should be noted that even if no additional phosphate was added to the original colony (0 µg/ml) some growth could be observed, which can be attributed to minute amounts of phosphate available within the seed colony.

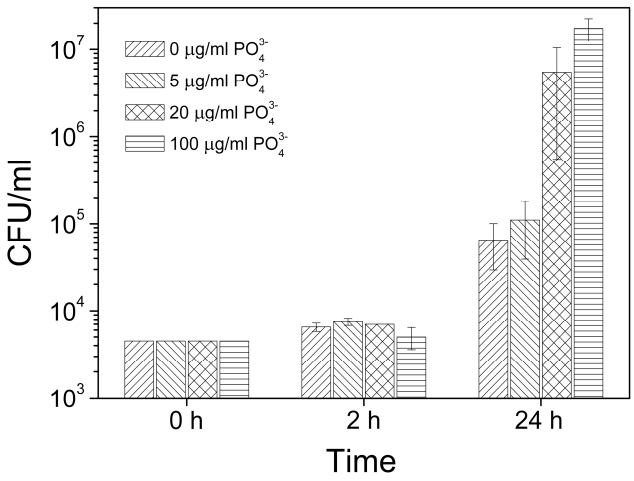

Fig. S3 Growth of *E. coli* in different media. *E. coli* were exposed to four growth media varying in phosphate concentration. Starting with 4'500 CFU/ml the populations evolved. After 24 hours the growth limitation due to lacking phosphate is well visible. While populations with low phosphate content grew to ~10^5 CFU/ml, populations with 100 µg/ml phosphate provided, grew to 10^7 CFU/ml. Slow growth in phosphate-free culture medium is attributed to nutrients remaining from the cultivation of the bacteria in full medium.

D) Experimental Section

Lanthanum oxide nanoparticles were purchased from Nanoamor (La_2O_3, 99.99%, 15-30 nm, Stock #: 2920RE; Houston, USA). X-ray diffraction patterns were recorded on an X'Pert PRO-MPD diffractometer (Cu-Kα radiation, X'Celerator linear detector system; PANalytical, Netherlands) with a step size of 0.033°. Scanning electron microscopy (SEM) images were recorded on a LEO Gemini 1530 (Zeiss, Oberkochen, Germany). For this purpose, the samples were sputtered with 5 nm of platinum. The specific surface area was determined on a Tristar Micromeritics (Norcross, GA, USA) using the Brunauer-Emmett-Teller (BET) method at 77 K. The BET analysis was measured after out gassing for 90 minutes at 250 °C.

Escherichia coli (strain C43) and *Staphylococcus carnosus* (strain TM300) were grown in Difco™ LB broth (Chemie Brunschwig) for 4 h at 37 °C and gently agitation to a concentration of about 10^8 CFU/ml. This suspension was diluted to the required concentration with physiological saline (0.9 wt% NaCl in water) or where medium free (in particular phosphate free) was needed, repeatedly (5 times) centrifuged for 60 minutes at RCF=1'300 (Mistral 3000E, 2'500 rpm), the supernatant removed, and the bacterial pellet resuspended in minimal media. Minimal media was produced from 5 g Glucose, 1 g NH_4Cl, 5.4 g NaCl, 0.1 g $MgSO_4$ and 0.02 g $CaCl_2$ hexahydrate filled up to 1 liter with ddH_2O (MilliQ, Millipore, resistivity 18 MΩ cm) and sterilized by autoclaving at 121 °C for 15 minutes. To quantify the CFU load in a sample, a dilution row (10^{-1}-10^{-8}) was plated in duplicate on dextrose agar plates (PDA, VWR BDH Prolabo). The plates were incubated at 37 °C for 24 hours before readout.

Penicillium roqueforti (Thom, anamorph deposited as *Penicillium roquefortii*, ATCC 42294, LGC Standards) was grown in 75 cm^2 T-flasks at 22 °C in 50 ml potato dextrose broth (PD, Fluka) for two weeks before usage. To quantify the amount of fungi, the complete sample was high-shear mixed (IKA, Ultra-Turrax T10, 30'000 rpm) in 20 ml deionized water (dH_2O) for 1 minute at maximum speed. A dilution row (10^0 to 10^{-5} in NaCl 0.9%) was plated on PDA. The plates were incubated at ambient conditions for 2-3 days before readout.

Chlorella vulgaris (SAG 211-11b, Sammlung von Algenkulturen, University of Goettingen) was grown in a algae growth medium very similar as proposed by OECD.[3] This medium contained $NaHCO_3$ 50.00 mg/l, NH_4Cl 15.00 mg/l, $MgCl_2$ ·6(H_2O) 12.00 mg/l, $CaCl_2$·2(H_2O) 18.00, $MgSO_4$·7(H_2O) 19.65 mg/l, K_2HPO_4·3(H_2O) 2.097 mg/l, $FeCl_3$·6(H_2O) 0.08 mg/l, Na_2EDTA·2(H_2O) 0.10 mg/l, H_3BO_3 0.185 mg/l, $MnCl_2$ 0.264 mg/l, $ZnCl_2$ 0.003 mg/l, $CoCl_2$·6(H_2O) 0.0015 mg/l, Na_2MoO_4·2(H_2O) 0.007 mg/l, $CuCl_2$ 0.0079 µg/ml. Stock solutions were used. After 7 days of growth in a climate chamber (Binder KBF-ICH 240, Tullingen, Germany) at 23 °C, 80% RH with constant illumination according to ICH guideline Q1A (R2)[4] 5 ml of the grown algae were added to 45 ml of fresh medium for every experiment. To samples with no additional phosphate 0 mg, 0.1 mg, resp. 1.0 mg La_2O_3 was added. To samples '100 µg/ml PO_4^{3-}' 0 mg, 33 mg, resp. 658 mg La_2O_3 was added. After 5 days in the climate chamber as above, algae cells were counted under a transmitted-light microscope by the aid of a hemocytometer.

Phosphate dependent growth of *E. coli*.: To 30 ml of minimal medium containing $10^{3.65}$ CFU/ml 0.00 ml, 0.0075 ml, 0.03 ml, respectively 0.15 ml of a phosphate solution (12.19 g disodium hydrogen phosphate dodecahydrate (Na_2HPO_4·12H_2O, AppliChem) and 1.14 g potassium dihydrogen phosphate (KH_2PO_4, AppliChem) dissolved in 200 ml ddH_2O) was added to obtain the growth media with 0 µg/ml, 5 µg/ml, 20 µg/ml, respectively 100 µg/ml phosphate. The specimens were incubated at 37 °C under slight agitation and samples were taken after 2 and 24 hours. This experiment was conducted in triplicate.

Binding capacity: To 1 liter ddH_2O 50 µl, 250 µl, 750 µl, respectively 2.5 ml of a phosphate solution (12.19 g Na_2HPO_4·12H_2O and 1.14 g KH_2PO_4 dissolved in 200 ml ddH_2O) were added to obtain solutions with 1 µg/ml, 5 µg/ml, 15 µg/ml, respectively 50 µg/ml phosphate. To each of these samples 200 mg La_2O_3 were added and the suspension subsequently sonicated (UP400S, 24 kHz, Hielscher GmbH, 50% amplitude, 100% pulse) for 5 minutes. After 24 hours, 50 ml of each sample was centrifuged for 60 minutes at RCF=1'300 (Mistral 3000E, 2'500 rpm). The phosphate concentrations in the supernatants were measured by the ascorbic acid method provided by the American Public Health Association[5] that is based on Murphy & Riley.[6] The sedimented particles were washed three times by repeated centrifugation and resuspension in ddH_2O. After drying at ambient condition, IR transmission (DRIFT-FTIR) was recorded on a Bruker Vertex 70. Presaturated La_2O_3 particles were obtained by immersing 1 g La_2O_3 in a solution of 5.13 g Na_2HPO_4·12H_2O in 1 liter ddH_2O for 24 hours and subsequent repeated centrifugation and resuspension in ddH_2O.

E) Recyclability

The lanthanum oxide nanoparticles could be reused after they have been saturated with phosphate. Since the formed lanthanum phosphate is a very stable chemical compound, the chemistry required to do this is very harsh (98% H_2SO_4, 150 °C, several hours followed by precipitation with oxalate and conversion to lanthanum oxide). As this is common chemistry in the preparation of lanthanum oxide from monazite ores in the first place, recycling of use particles seems feasible.[7]

References

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