

curiously spongy, represents the earliest record of perichondral, and therefore endoskeletal, bone in a primitive chondrichthyan. With few exceptions¹¹, the absence of endoskeletal bone is seen as a defining feature of extant chondrichthyans⁸, relative to its primitive presence in other jawed and fossil jawless vertebrates. This unexpected juxtaposition of bone and GCC may represent a further, persistent, primitive aspect of early chondrichthyan skeletal tissues.

Alternatively, the near-continuity of this bone layer with the fin spine could indicate an unusual distribution of dermal skeleton derived from the trunk or cranial neural crest¹². Finally, the disputed relationship of the brush to the fins^{1,5} is resolved by regarding it as a specialized fin-baseplate extension. Its endoskeletal location, histology and absence of fin radials support this idea.

M. I. Coates*, S. E. K. Sequeira*,

I. J. Sansom†, M. M. Smith‡

*Biology Department,

University College London,

London WC1E 6BT, UK

e-mail: m.coates@ucl.ac.uk

†School of Earth Sciences,

University of Birmingham,

Birmingham B15 2TT, UK

‡Department of Craniofacial Development,

School of Dentistry,

UMDS Guy's Hospital Campus,

London SE1 9RT, UK

- Zangerl, R. *J. Vert. Paleontol.* **4**, 372–378 (1984).
- Maisey, J. G. *Zool. J. Linn. Soc.* **66**, 161–183 (1979).
- Smith, M. M. & Hall, B. K. *Biol. Rev.* **65**, 277–373 (1990).
- Patterson, C. *Phil. Trans. R. Soc. Lond. B* **249**, 101–205 (1965).
- Lund, R. *Ann. Carnegie Mus.* **45**, 161–178 (1974).
- Williams, M. E. *Paleontographica* **190**, 83–158 (1985).
- Wood, S. P. *Nature* **297**, 574–577 (1982).
- Janvier, P. *Early Vertebrates* (Oxford Univ. Press, 1996).
- Orvig, T. *Arkiv Zool.* **2**, 321–454 (1951).
- Sansom, I. J. *et al. Nature* **379**, 628–630 (1996).
- Peignoux-Deville, J., Lallier, F. & Vidal, B. *Cell Tissue Res.* **222**, 605–614 (1982).
- Smith, M. M. *et al. Proc. R. Soc. Lond. B* **256**, 137–145 (1994).

Anoxic bioremediation of hydrocarbons

The contamination of soils and sediments by petroleum is a matter of international concern because of the toxicity and refractory character of the aromatic components in the absence of oxygen¹. Gaseous oxygen can be injected into the anaerobic zone of a contaminated environment² to stimulate biodegradation, but this is costly and inefficient. Other more soluble electron acceptors, such as nitrate or sulphate, can be used instead, but oxidation is slow and hydrocarbon degradation is incomplete³. Here we describe how chlorite dismutation by perchlorate-reducing bacteria can be used as an alternative source of oxygen for degrading contaminants. This dismutation

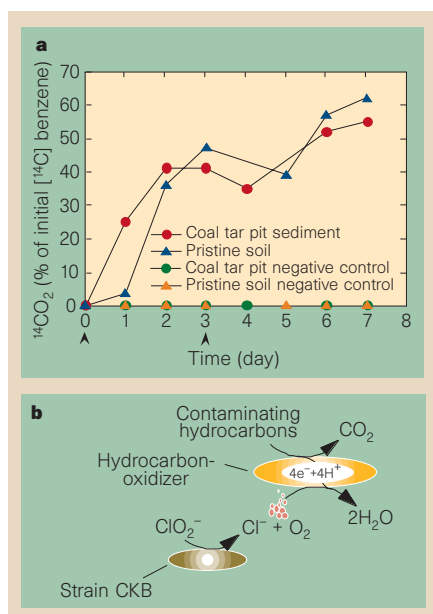


Figure 1 Stimulation of aromatic hydrocarbon oxidation by chlorite dismutation. **a**, Oxidation of [¹⁴C]-benzene to ¹⁴CO₂ in anoxic contaminated and pristine soil and sediment samples amended with chlorite and inoculated with strain CKB. Arrowheads, points of addition of 1 mM chlorite. **b**, Mechanism by which chlorite dismutation might stimulate hydrocarbon oxidation in anoxic sediments.

of chlorite into molecular oxygen and chloride is an intermediate step in the microbial reduction of perchlorate or chlorate⁴.

As part of a study on microbial perchlorate reduction, we isolated a new microorganism, strain CKB, which grows anaerobically by reducing perchlorate or chlorate, from waste sludge from a paper mill in Pennsylvania. When strain CKB is inoculated with chlorite into anoxic, petroleum-contaminated soil samples, [¹⁴C]-benzene is rapidly oxidized to ¹⁴CO₂, with about 40% of the original ¹⁴C being recovered in this form after two days of incubation (Fig. 1a). If the sediments are further amended with 1 mM chlorite on day 3, about 60% of the ¹⁴C can be recovered as ¹⁴CO₂ by day 6 (Fig. 1a). No ¹⁴CO₂ is produced in samples that are not amended with chlorite or strain CKB.

Similar results are obtained with anoxic soil samples that have had no previous exposure to hydrocarbons (Fig. 1a). However, there is a slight lag phase of 24 hours, which is consistent with adaptation to benzene by the microbial population. The stimulatory effects are not significantly altered when lower chlorite concentrations are used: 1 μM chlorite resulted in more than half the level of benzene degradation observed with 1 mM chlorite.

This stimulatory effect also occurs in defined mixed cultures in the absence of soil. When an anaerobic, washed-cell suspension of strain CKB is combined with the aerobic hydrocarbon-oxidizing *Pseudomonas*

strain JS-150 and amended with chlorite under anaerobic conditions, [¹⁴C]naphthalene is rapidly oxidized to ¹⁴CO₂ (data not shown). No ¹⁴CO₂ is produced if either of the organisms or chlorite is omitted, unless O₂ is added to the headspace. The degradation of naphthalene is therefore directly dependent on the combined presence of both strain CKB and chlorite.

Because strain CKB cannot degrade aromatic hydrocarbons in pure culture, we considered the possibility that the stimulation of hydrocarbon degradation could be the result of chlorite dismutation into chloride and O₂ by strain CKB. The resultant O₂ is used by indigenous, aerobic hydrocarbon-oxidizing bacteria that are otherwise inhibited by the anoxic condition of the soil (Fig. 1b). In support of this, when chlorite is amended to an anaerobic, washed whole-cell suspension of strain CKB, O₂ is rapidly and proportionally produced. There is no O₂ production if the cells are omitted or killed by heat.

Our results show that the dismutation of chlorite by perchlorate-reducing bacteria in anaerobic environments can produce extracellular O₂. This O₂ can be used by hydrocarbon-oxidizing bacteria to degrade hydrocarbons, such as benzene, which is a particularly important environmental contaminant owing to its toxicity and relative solubility. Little is known about perchlorate-reducing bacteria, although they are ubiquitous in a wide range of environments, including pristine soils and petroleum-contaminated sediments⁵.

High concentrations of chlorite may be toxic to many microbial species, but our results indicate that significant degradation of hydrocarbons can be stimulated at chlorite concentrations (1 μM is 90 μg per litre) well below the limits imposed by the World Health Organization (200 μg per litre) and the US Environmental Protection Agency (1 mg per litre)^{6,7}. As a bioremediative strategy, the application of chlorite dismutation to stimulate hydrocarbon oxidation in contaminated environments offers a new alternative to other injection processes.

John D. Coates, Royce A. Bruce,

John D. Haddock

Department of Microbiology,

Southern Illinois University,

Carbondale, Illinois 62901, USA

e-mail: jcoates@micro.siu.edu

- Anderson, R. T. & Lovley, D. R. *Adv. Microbiol. Ecol.* **15**, 289–350 (1997).
- Crocetti, C. A., Head, C. L. & Ricciardelli, A. J. *Aeration-Enhanced Bioremediation of Oil-Contaminated Soils: A Laboratory Treatability Study* (National Groundwater Association, Houston, 1992).
- Coates, J. D., Anderson, R. T. & Lovley, D. R. *Appl. Environ. Microbiol.* **62**, 1099–1101 (1996).
- Rikken, G., Kroon, A. & van Ginkel, C. *Appl. Microbiol. Biotechnol.* **45**, 420–426 (1996).
- Michaelidou, U., Bruce, R., Achenbach, L. & Coates, J. D. in *Abstr. 98th Gen. Meeting Am. Soc. Microbiol.* 313 (American Society for Microbiology, Atlanta, 1998).
- Foundation for Water Research *Research Report FR0390* (1993).
- US Environmental Protection Agency *Fed. Reg.* **59**, 6331–6444 (1994).