Arginine metabolism is central to life. Arginine is the enzyme which not only controls the hydrolysis of arginine to ornithine in the urea cycle but has been implicated in the regulation of nitric oxide signalling. No structure for arginase currently exists. Arginase has been isolated from a variety of species ranging from bacteria and fungi to plants and animals. The physiological cofactor is Mn\(^{2+}\) but activation by a variety of divalent cations, such as VO\(^{2+}\), Fe\(^{2+}\), Co\(^{2+}\) and Cd\(^{2+}\), has also been reported, making arginase an attractive target for the study of metal-ion mediated catalysis, and also of the stabilisation of quaternary and tertiary structure by metal-ions.

The thermostable arginase from the thermophilic bacteria, 'Bacillus caldoventosus' has been cloned, sequenced, expressed in E. coli and purified utilising the thermostable character of the protein. The amino acid sequence shows limited similarity to other arginases, but the putative metal ion binding motif (L/I)GGDDS-(14X)-DAH has been conserved.

X-ray quality crystals have been obtained which diffract to 2.2 Å resolution and crystallise in an orthorhombic cell \((a=89.7\text{ Å}, b=146.1\text{ Å}, c=154.9\text{ Å})\) with six molecules in the asymmetric unit. Heavy atom derivative screening is currently in progress, the results will be reported.

**PS04.02.10 HIGH RESOLUTION CRYSTAL STRUCTURES OF 2,3-DIHYDROXYBIPHENYL 1,2-DIOXYGENASE FROM A PCB-DEGRADING BACTERIUM COMPLEXED WITH SUBSTRATE** Jeffrey T. Bolin, Seungil Han, Lindsay D. Ellis, Biological Sciences/ILLY, Purdue University, W. Lafayette, IN 47907, U.S.A.; Biochemistry Dept., Université Laval, Ste-Foy, Quebec, Canada

2,3-dihydroxybiphenyl 1,2-dioxygenase (DHBD) catalyses the ring cleavage step in the aerobic degradation of biphenyl and PCBs using an Fe(II)-dependent, extradiol cleavage mechanism. Crystal structures of DHBD in complexes with 2,3-dihydroxybiphenyl, 3-methylcatechol, and catechol have been determined at 1.9 Å, 1.9 Å, and 1.9 Å resolution, respectively. The structure of the free enzyme has also been determined at 1.9 Å resolution\(^1\).

These studies target the DHBD from *Pseudomonas* LB400, an organism that transforms a broad range of PCBs. The enzyme is a D4 symmetric homoolomer of 298 residue subunits and contains one Fe per subunit. Crystallization and diffraction studies were performed under anaerobic conditions so as to maintain the active, Fe(II) form of the enzyme.

These are the first structures of any extradiol dioxygenase in the Fe(II) form. As such they provide a structural framework for discussion of the mechanism of extradiol ring cleavage reactions as well as the potential adaptation of this enzyme to the biphenyl degradation pathway to achieve bioremediation of PCBs.

The structures of substrate complexes show bidentate binding via the vicinal hydroxyl oxygens. However, the two Fe-O bond lengths are markedly different, suggesting mononuclear binding and deprotonation of the 2-hydroxyl in the case of 2,3-dihydroxybiphenyl. The active site structure in the complexes is similar to that of the free enzyme, but there are several important differences including a > 0.5 Å shift of the Fe and its ligands, and subtle changes in the hydrogen bonding pattern that may be important for the mechanism.