LETTER TO THE EDITOR

# Cerato-ulmin, a Toxin Involved in Dutch Elm Disease, Is a Fungal Hydrophobin

Hydrophobins are components of microbial cell walls that contribute to cell surface hydrophobicity. The hydrophobic nature of the surfaces of many microbes, both prokaryotic and eukaryotic, is important to such processes as adhesion of pathogens to host structures and dispersal of aerial spores (Beever and Dempsey, 1978; Doyle and Rosenberg, 1990; Stringer et al., 1991). A class of peptide hydrophobins has recently been discovered and characterized in the filamentous fungi. Wessels (1992) demonstrated that three small hydrophobic peptides from the basidiomycete *Schizophyllum commune* assemble in the walls of aerial structures and that hydrophobic interactions between these peptides are important for their polymerization. Stringer et al. (1991), and more recently Bell-Pedersen et al. (1992) and Lauter et al. (1992), showed that genes encoding similar peptides in the ascomycetes *Aspergillus nidulans* and *Neurospora crassa* are required for the formation of the hydrophobic rodlet layer of asexually produced spores. A gene encoding a fungal hydrophobin of unknown function was also described for the entomopathogenic deuteromycete *Metarhizium anisopliae* (St. Leger et al., 1992).

Although the peptide sequences of fungal hydrophobins are very diverse, they share three common characteristics, as shown in Figures 1 and 2. First, they are small, ranging in length from 96 to 157 amino acid residues. Second, they have eight cysteine residues arranged in a conserved pattern (Figure 1). Finally, they have a similar arrangement of hydrophobic domains (Figure 2). The recently published peptide sequence of the wilt toxin cerato-ulmin (CU) from the filamentous ascomycete *Ophiostoma ulmi*, the causal agent of Dutch elm disease (Stevenson et al., 1979; Yaguchi et al., 1992, as cited in Bolyard and Sticklen, 1992), possesses all three of these structural features. The length of CU, determined by sequenc-

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          Sc1  ......mpFs LAIlALpvLA AAtavp...r .........G .......... ......gask
          Sc4  ......mrFs LAllALpALA AAaPvpgggk .........G .......... ......Agqa
          Sc3  mfarLpvvFl YAfvAFgALv AAlPgghpgt typpstttia .......... ......Aggt
         SSGA  .......mFk alIvALAAvA AAiPtqqps. .........s .......... ......nemn
         RodA  ....Mkfsia aAVvAFAAsv AAlPpahdsq fagngvgnkG nsnvkfpvpe nvtvkqAsdk
          Eas  ....Mqftsv FtIlAiAmtA AAaPaevvpr .......... .......... ..attigpnt
cerato-ulmin  .......... .......... .......... .......... .......... ....sdsydp
    Consensus  ----M---F- LAI-ALAALA AA-P------ ---------G ---------- ------A---

          Sc1  CnS..G.pVQ CCNtlvdtkd kh..qTnivG aLLGldlGsL tGLaGvn... .CS...PvSV
          Sc4  CnS..G.pVQ CCNetttvan aq..kqGLLG GLLGvVvGpi tGLvGln... .CS...PiSV
          Sc3  Ctt..G.sls CCNqvqsass sp..vTaLLG .LLGiVlsDL nvLvGis... .CS...PltV
         SSGA  CdS..G..Vy CCNkv..... aq..nTGivv pidal..... ....sst... .Cg..dtlkl
         RodA  Cgd.qa.qls CCNkatyagd tttvdeGLLs GaLsgliGag sGaeGlglfd qCSkldvavl
          Eas  Csi.ddykpy CCqsmsgpag sp........ GLLnlIpvDL saslG..... .C........
cerato-ulmin  Ctgllqkspq CCNt..... .......... diLGvanlD. .......... .C..hgPpSV
    Consensus  C-S--G--VQ CCN------- -----TGLLG GLLG-V-GDL -GL-G----- -CS---P-SV

          Sc1  IGV.G.GN.S CstQTV.... CC.EgtQfNG ...LVNVG.. CT.PInVgl   109
          Sc4  VGVlt.GN.S CtAQTV.... CC.DhVtqNG ...LVNVG.. CT.PIsl..   111
          Sc3  IGV.G.Gs.g CsAQTV.... CC.ENtQfNG ...LINIG.. CT.PInIl.   125
         SSGA  VtVdalnd.k CtsQTV.... CC.nNVQqNG ...LVNVa.. CT.PIdV..    96
         RodA  IGIqdlvNqk Ckqnia.... CC.qNspssa dgnLIgVGlp Cv.algsil   157
          Eas  ..VvGvigsq CgAsvk.... CCkDdVtntG nsfLIinaan Cv.a.....   108
cerato-ulmin  ptspsqfqaS CvAdggrsar CCtls..... ...Llglalv CTdPVgI..    75
    Consensus  IGV-G-GN-S C-AQTV---- CC--NVQ-NG ---L-NVG-- CT-PI----
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**Figure 1.** Alignment of Cerato-ulmin with Fungal Hydrophobins.

Sc1, Sc4, and Sc3 are peptides from *S. commune* (Wessels, 1992). SSGA, RodA, and Eas are translated from the genes in *M. anisopliae* (St. Leger et al., 1992), *A. nidulans* (Stringer et al., 1991), and *N. crassa* (Bell-Pedersen et al., 1992; Lauter et al., 1992). Cysteine, asparagine, and leucine residues that are nearly completely conserved are shaded. Highly conserved residues are shown as a consensus sequence.

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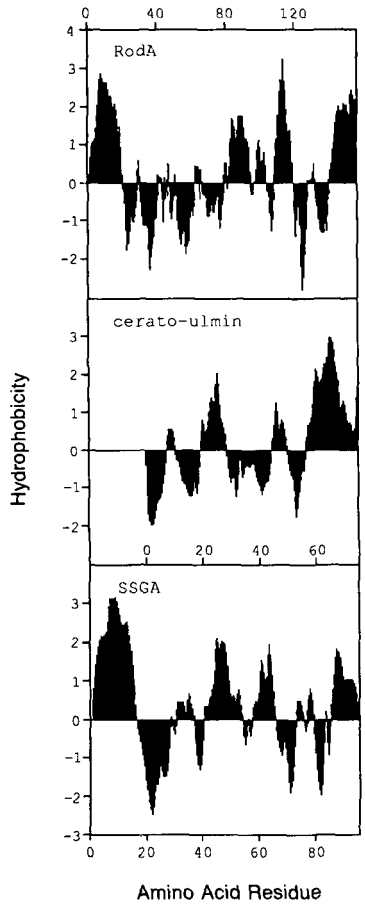


**Figure 2.** Hydrophobicity Plots of Fungal Hydrophobins.

The hydrophobicity plot of CU (Bolyard and Sticklen, 1992) is compared to those of RodA (Stringer et al., 1991) and SSGA (St. Leger et al., 1992). The plot for CU is offset because we assume that the sequence derived from the mature peptide lacks the hydrophobic secretion signal.

ing the native peptide, is 75 amino acid residues, which falls within the known length range for fungal hydrophobins, given that previously reported lengths are thought to include signal sequences of approximately 20 amino acid residues (Wessels, 1992). CU contains eight cysteine residues arranged in the pattern characteristic of hydrophobins, as well as conserved asparagine and leucine residues (Figure 1). The array of hydrophobic domains in CU is also similar to arrays in other fungal hydrophobins (Figure 2). These similarities lead us to conclude that CU is a fungal hydrophobin.

It has been demonstrated that CU is capable of producing the same disease symptoms as the fungus itself (Bolyard and Sticklen, 1992). Thus, the biological activity of CU is not related to a structural role in the fungal cell wall. However, a structural role for CU is not excluded. Some fungal hydrophobins are secreted from submerged hyphae but accumulate in the walls of cells exposed to an air interface (Wessels, 1992). It is possible that the role of CU as a virulence factor is a fortuitous consequence of secretion or diffusion of a cell wall component.

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