

## PLANT WALL DEGRADATIVE COMPOUNDS AND SYSTEMS

### CROSS-REFERENCE TO RELATED APPLICATIONS

This patent application is a continuation of U.S. patent application Ser. No. 12/099,653, filed Apr. 8, 2008, which claims priority to U.S. divisional patent application Ser. No. 11/121,154, filed May 4, 2005, issued as U.S. Pat. No. 7,365,180, on Apr. 29, 2008, which claims priority to U.S. Provisional Application No. 60/567,971, filed May 4, 2004, the contents of which are incorporated herein, in their entirety, by reference.

### STATEMENT REGARDING FEDERALLY SPONSORED RESEARCH

This invention was made with government support under Contract Number 5A7528051E awarded by the National Oceanic and Atmospheric Administration (NOAA), and Contract Number DEB0109869 awarded by the National Science Foundation (NSF). The government has certain rights in the invention.

### BACKGROUND OF THE INVENTION

#### 1. Field of the Invention

The invention is generally directed to degradative enzymes and systems. In particular, the present invention is directed to plant cell wall degrading enzymes and associated proteins found in *Microbulbifer degradans* and systems containing such enzymes and/or proteins.

#### 2. Background of the Invention

Cellulases and related enzymes have been utilized in food, beer, wine, animal feeds, textile production and laundering, pulp and paper industry, and agricultural industries. Various such uses are described in the paper "Cellulases and related enzymes in biotechnology" by M. K. Bhat (Biotechnical Advances 18 (2000) 355-383), the subject matter of which is hereby incorporated by reference in its entirety.

*Saccharophaga degradans* strain 2-40 (herein referred to as "*S. degradans* 2-40" or "2-40") is a representative of an emerging group of marine bacteria that degrade complex polysaccharides (CP). *S. degradans* has been deposited at the American Type Culture Collection and bears accession number ATCC 43961. *S. degradans* 2-40, formerly known and referred to synonymously herein as *Microbulbifer degradans* strain 2-40 ("*M. degradans* 2-40"), is a marine  $\gamma$ -proteobacterium that was isolated from decaying *Sparina altemiflora*, a salt marsh cord grass in the Chesapeake Bay watershed. Consistent with its isolation from decaying plant matter, *S. degradans* strain 2-40 is able to degrade many complex polysaccharides, including cellulose, pectin, xylan, and chitin, which are common components of the cell walls of higher plants. *S. degradans* strain 2-40 is also able to depolymerize algal cell wall components, such as agar, agarose, and laminarin, as well as protein, starch, pullulan, and alginate. In addition to degrading this plethora of polymers, *S. degradans* strain 2-40 can utilize each of the polysaccharides as the sole carbon source. Therefore, *S. degradans* strain 2-40 is not only an excellent model of microbial degradation of insoluble complex polysaccharides (ICPs) but can also be used as a paradigm for complete metabolism of these ICPs. ICPs are polymerized saccharides that are used for form and structure in animals and plants. They are insoluble in water and therefore are difficult to break down.

*Microbulbifer degradans* strain 2-40 requires at least 1% sea salts for growth and will tolerate salt concentrations as high as 10%. It is a highly pleomorphic. Gram-negative bacterium that is aerobic, generally rod-shaped, and motile by means of a single polar flagellum. Previous work has determined that 2-40 can degrade at least 10 different carbohydrate polymers (CP), including agar, chitin, alginate, carboxymethylcellulose (CMC), 6-glucon, laminarin, pectin, pullulan, starch and xylan (Ensor, Stotz et al. 1999). In addition, it has been shown to synthesize a true tyrosinase (Kelley, Coyne et al. 1990). 16S rDNA analysis shows that 2-40 is a member of the gamma-subclass of the phylum *Proteobacteria*, related to *Microbulbifer hydrolyticus* (Gonzalez and Weiner 2000) and to *Teridinibacter* sp., (Distel, Morrill et al., 2002) cellulolytic nitrogen-fixing bacteria that are symbionts of shipworms.

The agarase, chitinase and alginate systems have been generally characterized. Zymogram activity gels indicate that all three systems are comprised of multiple depolymerases and multiple lines of evidence suggest that at least some of these depolymerases are attached to the cell surface (Stotz 1994; Whitehead 1997; Chakravorty 1998). Activity assays reveal that the majority of 2-40 enzyme activity resides with the cell fraction during logarithmic growth on CP, while in later growth phases the bulk of the activity is found in the supernatant and cell-bound activity decreases dramatically (Stotz 1994). Growth on CP is also accompanied by dramatic alterations in cell morphology. Glucose-grown cultures of 2-40 are relatively uniform in cell size and shape, with generally smooth and featureless cell surfaces. However when grown on agarose, alginate or chitin, 2-40 cells exhibit novel surface structures and features.

These exo- and extra-cellular structures (ES) include small protuberances, ger bleb-like structures that appear to be released from the cell, fine fimbriae or pill, and a network of fibril-like appendages which may be tubules of some kind. Immunoelectron microscopy has shown that agarases, alginateases and/or chitinases are localized in at least some types of 2-40 ES. The surface topology and pattern of immunolocalization of 2-40 enzymes to surface protuberances are very similar to what is seen with cellulolytic members of the genus *Clostridium*.

There exists a need to identify enzyme systems that use cellulose as a substrate, express the genes encoding the proteins using suitable vectors, identify and isolate the amino acid products (enzymes and non-enzymatic products), and use these products as well as organisms containing these genes for purposes such as those described in the Bhat paper.

### SUMMARY OF THE INVENTION

One aspect of the present invention is directed to systems of plant wallactive carbohydrases and related proteins.

A further aspect of the invention is directed to a method for the degradation of substances comprising cellulose. The method involves contacting the cellulose containing substances with one or more compounds obtained from *Saccharophaga degradans* strain 2-40.

Another aspect of the present invention is directed to groups of enzymes that catalyze reactions involving cellulose.

Another aspect of the present invention is directed to polynucleotides that encode polypeptides with cellulose degrading or cellulose binding activity.

A further aspect of the invention is directed to chimeric genes and vectors comprising genes that encode polypeptides with cellulose depolymerase activity.