*Review*

# Carbohydrase Systems of *Saccharophagus degradans* Degrading Marine Complex Polysaccharides

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**Abstract:** *Saccharophagus degradans* 2-40 is a γ-subgroup proteobacterium capable of using many of the complex polysaccharides found in the marine environment for growth. To utilize these complex polysaccharides, this bacterium produces a plethora of carbohydrases dedicated to the processing of a carbohydrate class. Aiding in the identification of the contributing genes and enzymes is the known genome sequence for this bacterium. This review catalogs the genes and enzymes of the *S. degradans* genome that are likely to function in the systems for the utilization of agar, alginate, α- and β-glucans, chitin, mannans, pectins, and xylans and discusses the cell biology and genetics of each system as it functions to transfer carbon back to the bacterium.

## 1. Introduction

The marine environment contains a diverse collection of complex polysaccharides (CPs) as all organisms in this environment produce them. For autotrophs and heterotrophs, whether prokaryotic or eukaryotic, these polymers are associated with proteins and membranes, extracellular polysaccharides, structural elements of the cell wall, and/or storage forms of carbon [1]. They can be as simple as starches formed of α-1,4-linked glucose or as complex as the mixed polymers present in what are traditionally known as hemicellulose (e.g., xylans) or pectin. Many of these polymers have industrial

applications. As few of these polymers accumulate in the marine environment, mechanisms must exist in each marine habitat to mineralize them through enzymatic degradation and metabolism.

*Saccharophagus degradans* 2-40 (*Sde*2-40; formerly *Microbulbifer degradans* 2-40) is a rod-shaped bacterium with a salt requirement typical of marine bacteria and capable of processing many CPs to their elemental sugars or sugar derivatives [2]. This aerobic, γ-subgroup proteobacterium of the *Alteromonadales* group was isolated from decaying saltwater marsh grass, *Spartina alternaflora*, in a marine estuary [3–5]. It is a versatile saprophyte that can decompose whole plant material in monoculture and expresses multi-component enzyme systems to degrade at least 10 different CPs [2–7]. *Sde*2-40 is unusual in its ability to utilize CPs of algal, higher plant, fungal and animal origin as sole carbon and energy sources. *Sde*2-40 is able to grow using agar, alginate, cellulose, chitin, α- and β-glucans, galacto-/gluco-mannans, various xylans, citrus pectin or laminarin as the primary carbon and energy source. This bacterium is also able to produce polyalkanoates from these polysaccharides [8,9].

The unusual character of this bacterium was further revealed by the genome sequence [10]. Based upon the genome annotation, this bacterium was predicted to devote a large portion of its genome to the processing of complex polysaccharides. Gene models were identified to produce enzymes containing at least 132 glycoside hydrolase domains spread among 42 families, 37 glycoside transferases, 33 polysaccharide lyases and 13 carbohydrate esterases [11]. In addition, many of the above deduced enzymes carry homologs to carbohydrate binding domains (CBMs) that function in the reversible adsorption of the host enzyme to their substrate or associated carbohydrate polymer [12]. This bacterium is annotated to express 143 homologs of CBMs distributed among 20 families [10]. These catalytic and binding domains are linked in unusual combinations to form the modular carbohydrases expressed by this bacterium. Multiple carbohydrase systems, in turn, were revealed by the genome sequence to match the observed degradative abilities of this bacterium. This review summarizes the properties of many of this bacterium's annotated or verified carbohydrases and extends the observations of Weiner *et al.* [10]. Each system is described from a cell biology and genetic view with new genes and likely or known cellular location of each component described.

## 2. The Agarolytic System

Agar is an agarocolloid gel formed of unsubstituted and substituted agarose polymers [1]. It is a common cell wall constituent of many red algae (Rhodophyta) [13]. Up to 70% of the algal cell wall can be agar polymers. The remaining material consists of other galactans and embedded xylan and cellulose microfibrils. The base polymer of agar is agarose that is composed of repeating neoagarobiose units (3,6-anhydro-L-galactose-α-1,3-D-galactose) joined by β-1,4 bonds that forms a helix in aqueous environments. The galactose moieties of the repeating neoagarobiose units can be methylated, pyruvated, sulfonated or glycosylated to form various substituted derivatives with different gelling and solubility characteristics.

*Sde*2-40 is capable of rapid growth on agars and agarose as the dominant carbon source and produces multiple agarases [7,14]. The mechanism by which *Sde*2-40 degrades agar employs five β-agarases, designated Aga50A, Aga16B, Aga86C, Aga50D and Aga86E [15,16] and a neoagarobiose hydrolase Aga117F [17]. These agarases are modular and retain homologs of glycoside hydrolase families

associated with agarase activity, such as GH16, GH50, GH86 and GH117 (Table 1). Aga16B was unequivocally demonstrated to be a freely secreted *endo*-β-agarase with a GH16 domain that rapidly degraded agar and agarose to neoagarotetraose [15]. The identification of Aga50D as a freely secreted agarase was originally based upon conserved sequence features but directed expression of the cloned gene showed it to be an *exo*-lytic agarase releasing neoagarobiose directly from agar [16]. Aga86E shares sequence similarity to several GH86 agarases and the purified enzyme almost specifically releases neoagarobiose from agarose consistent with exolytic degradation of agarose polymers [15].

**Table 1.** The agarolytic system of *S. degradans*.

| Enzyme | Source gene [1] | Secretion [2] | Catalytic domain [3] | CBM [3] | Size (kDa) | Annotated activity [1] | Confirmed activity |
|---|---|---|---|---|---|---|---|
| Aga16B | 1175 | + | GH16 | 6 (×2) [4] | 64.5 | endoagarase | + [5] |
| Aga50D | 2644 | + | GH50 | | 88.6 | exoagarase | + [6] |
| Aga86E | 2655 | + | GH86 | 6 (×3) | 146.2 | exoagarase | + [5] |
| Aga50A | 1176 | Lipobox | GH50 | | 87.4 | likely exoagarase | + [7] |
| Aga86C | 2650 | Lipobox | GH86 | | 86.2 | endoagarase | + [5] |
| AgaT | 2649 | Inner membrane | | | | putative transporter | |
| Aga117F | 2657 | - | GH117 | | 41.6 | neoagarobiose hydrolase | + [8] |

[1] As described in [10]; [2] As indicated by the presence of a Type II secretion signal or a Lipobox as detected by LipoP 1.0 server; [3] as determined by CAZY [11]; GH, Glycoside hydrolase; CBM, carbohydrate binding domain; [4] As described by [18]; [5] from [15]; [6] from [16]; [7] from [19]; [8] as described in [17].

A feature of Aga16B and Aga86E is the inclusion of multiple CBM6 domains [15]. CBM6 is involved in the binding of the host enzyme to its substrate [20]. The CBM6 modules found in Aga86E and Aga16B form a distinct subclass within the large CBM6 family [15,18]. Five amino acid residues are strictly conserved among CBM6s associated with agarases [20]. As expected from the modularity of these agarases, deletion of the CBM6 domains did not obviously affect the catalytic activity of either enzyme as the catalytic GH16 and GH86 domains are functional independently of other domains. These CBM6 domains did increase the affinity of these enzymes for their substrate and one of the CBM6 modules (Aga16B-CBM6-2) binds to the nonreducing end of agarose polymers [18].

Two cell-associated agarases, Aga86C and Aga50A, are also produced by this bacterium [15,21]. Aga86C is present in an 85 kDa agarolytic fraction of the bacterium. Aga50A, when expressed individually in *E. coli*, enabled the slow pitting of agar [19]. One explanation for the low apparent agarase activity of Aga50A is that this enzyme is also an *exo*-enzyme producing neoagarobiose like Aga50D. Both contain an amino acid sequence at their *N*-termini known as a lipobox [15]. This is significant because lipoboxes are associated with acylation after secretion and subsequent attachment of the host protein in the outer face of the outer membrane [22–25].

The remaining component of the *Sde*2-40 agarolytic system is a neoagarobiose hydrolase that converts the neoagarobiose released by the activity of the β-agarases to galactose and 3,6-anhydro-α-galactose. This activity was predicted to be produced by *Sde*2-40 [15] and shown to be present by metabolic profiling [21,26]. The enzyme was identified as a GH117 enzyme [11,17].

Interestingly this enzyme lacks an obvious secretion signal, suggesting it is cytoplasmic. The source gene for this enzyme is part of an apparent operon that is divergently expressed from Aga86E, suggesting they may be under common regulation.

Using these data, a model for agarose degradation by *Sde*2-40 can be assembled. Irrespective of the activity, these agarases appear to be coordinately expressed as the activities are only observed during growth on agar or agarose [7,14,21,26]. The major secreted endoagarase of *Sde*2-40 appears to Aga16B. The resident CBM6 domains may play a role in attachment of this enzyme to algal cell walls to minimize diffusion of the enzyme and could also function to destabilize the cell wall polymers. The surface-associated Aga86C may function in a similar capacity to produce neoagarooligosaccharides. Both enzymes would increase accessibility of the *exo*-acting enzymes to their substrate. Neoagarobiose would be produced by the activity of secreted Aga86E, Aga50D, and possibly the cell-associated Aga50A. The observation that neoagarobiose hydrolase is cytoplasmic indicates that this bacterium imports the released neoagarobiose. A candidate sugar transporter is divergently expressed from *aga86C*, again suggesting common regulation, and like other co-localized agarase genes, has its strongest homolog in *Pseudoalteromonas atlantica*. This transporter is designated here as AgaT as a candidate neoagarobiose transporter. Once in the cytoplasm, neoagarobiose would be converted to galactose and 3,6-anhydro-α-galactose by the activity of neoagarobiose hydrolase. The released galactose is most likely metabolized by the Leloir pathway as the enzymes for the other metabolic pathways of galactose are missing in the genome annotation [26] (although there are two candidate tagatose 1,6-P aldolases in the genome annotation). The 3,6-anhydro-α-galactose appears to be reduced to fucose and then metabolized to triose phosphate [26].

## 3. The Alginolytic System

Alginic acid is a viscous, high molecular weight polymer composed of β-1,4-linked stretches of β-D-mannuronic acid (M) and α-L-guluronic acid (G) [27]. These sugar derivatives are C5 epimers of each other. Alginic acid is found in the cell wall of the brown seaweeds (Phaeophyceae) [1] where it is believed to function as an intercellular skeletal matrix [28]. Alginate, the salt of alginic acid, comprises about 60% of the cell wall mass of *Fucus distichus* [29]. Alginate is also produced by two bacterial families, *Azotobacteriaceae* and *Pseudomonadacease* [30] as an extracellular polysaccharide and is a major component of many biofilms.

Alginate is degraded by a group of enzymes known as alginases [30–33]. Alginases are usually polysaccharide lyases (EC 4.2.2.-) acting on a wide range of naturally acidic polysaccharides and catalyze the β-elimination of the 4-*O*-linked glycosidic bond forming unsaturated uronic acid-containing oligosaccharides [30,33,34]. This depolymerization of alginate causes the formation of a double bond between the C4 and C5 of the six-carbon ring. Both *endo-* and *exo-*acting alginate lyases have been identified, ultimately releasing 4-deoxy-L-*threo*-5-hexosulose uronate from the non-reducing terminus [30,33].

*Sde*2-40 is able to grow on sodium alginate as a sole carbon source [7,35,36]. Thus, a pathway for degradation, transport and metabolism of alginate must exist. Consistent with this prediction, *Sde*2-40 appears to produce an array of alginate lyases with Alg6F as the key example (Table 2). These enzymes include polysaccharide lyase domains PL6, PL7, PL14, PL18. With the exception of

Alg14M, most are annotated as poly (β-1,4-D-mannuronate) lyases. Many of these enzymes also include CBM16 and CBM32 domains as well. Some of these enzymes carry a FA58C domain that is a less defined CBM. Like other carbohydrase systems, five enzymes also include a lipobox suggestive of a surface localization. The apparent redundancy in the system could explained by: (1) substrate specificities of the enzymes; (2) *endo vs. exo* activity of the enzymes; and (3) possible differential regulation of the source genes by different substrates.

**Table 2.** The predicted alginolytic system of *S. degradans*.

| Enzyme | Source gene [1] | Secretion [2] | Catalytic domain [3] | CBM [3] | Size (kDa) | Annotated activity [1] |
|---|---|---|---|---|---|---|
| Alg2A | 3278 | + | PL2 | 16, FA58C | 58.5 | alginate lyase |
| Alg6B | 3285 | + | PL6 | | 83.2 | same |
| Alg17C | 3284 | + | PL7 | | 81.6 | same |
| Alg7D | 2547 | + | PL7 | 32, FA58C | 65.4 | same |
| Alg6F | 2873 | + | PL6 | | 163.1 | same |
| Alg7G | 1507 | + | PL7 | 32, FA58C | 94.3 | same |
| Alg18J | 3272 | + | PL18 | 16, 32 | 58.5 | same |
| Alg14M | 3918 | + | PL14 | | 44.2 | same |
| Alg7A | 3286 | Lipobox | PL7 | | 37.5 | same |
| Alg7E | 2478 | Lipobox | PL7 | 32, FA58C | 56.3 | same |
| Alg6H | 3275 | Lipobox | PL6 | | 93.6 | same |
| Alg6I | 3274 | Lipobox | PL6 | | 57.4 | same |
| Alg7K | 2839 | Lipobox | PL7 | | 50.5 | same |
| DctM | 1268 | Inner membrane | | | | transporter |
| DctQ | 1267 | Inner membrane | | | | transporter |
| DctP | 1266 | Periplasm | | | | transporter |
| HxuI | 950 | - | | | | 4-deoxy-L-*threo*-5-hexosulose uronate isomerase |
| HxuK | 1280 | - | | | | same |
| | 1269 | - | | | | 2-dehydro-3-deoxygluconate kinase |
| HxuA | 1382 | - | | | | 2-dehydro-3-deoxyphosphogluconate aldolase |
| AlgR | 1270 | - | | | | GntR family regulator |

[1] As described in [10] or here; [2] As indicated by the presence of a Type II secretion signal or a Lipobox; [3] As determined by CAZY [11]; PL, polysaccharide lyase domain.

Degradation of polymeric alginate obviously occurs outside of the bacterium because all alginate lyases thought to be produced by this bacterium have secretion signals and the bacterium lacks a mechanism to import alginate. Thus, the bacterium must have mechanisms to import the released 4-deoxy-L-*threo*-5-hexosulose uronate. After import, the 4-deoxy-L-*threo*-5-hexosulose uronate could be converted to 2-dehydro-3-deoxygluconate by an isomerase, phosphorylated by a kinase and then cleaved to produce pyruvate and triose phosphate [30,33]. Putative enzymes to carry out these

activities have been identified (Table 2). Interestingly, the candidate kinase is in an apparent operon with homologs to dicarboxylate transporters (DctM, DctQ and DctP) that might function in the importation of 4-deoxy-L-*threo*-5-hexosulose uronate. In addition, there is a divergently expressed GntR homolog that could function in the regulation.

## 4. The α-Glucanases

α-Linked glucans are ubiquitous polymers that include starches, glycogens, and pullulans. Starch is formed of amylose which is essentially unbranched α-1,4 glucan, and amylopectin is based upon amylose with an α-1,6 linkage approximately every 30 glycosyl units to initiate a new stretch of amylose. Glycogen is like amylopectin but the frequency of branching via α-1,6 linkages is higher. Pullulan is formed of maltotriose (3 α-1,4-linked glycosyl units) joined by an α-1,6 linkage. α-Glucans are easily utilized for metabolism due to its easily digestible nonplanar structure with α-1,4-linked glycosyl units forming a helix in solution. Branching further disrupts this structure. Starches are commonly found in algae and plants [1]. In addition to animal sources, some bacteria can also produce and accumulate glycogen as a storage product.

Degradation of amylose by bacteria usually involves α-amylases that predominantly release maltotriose, maltose (glucose-α-1,4-glucose) and some glucose. The better-known β-amylases produced by many other organisms specifically release maltose. α-Glucosidases cleave the remaining glycosyl bonds to release glucose. A third family of enzymes that include pullalanases hydrolyzes the α-1,6 bond.

Mixed starches support the growth of *S. degradans* [7]. Thus, like for the other growth-supporting carbohydrates, this bacterium can be predicted to express the enzymes to break down this material. A review of the genome annotation of *Sde*2-40 indicates the presence of a complete system to hydrolyze α-linkages between glucan units (Table 3). Three freely secreted α-amylases were identified in the genome annotation that contained GH13 domains and exhibit end-to-end similarity to known α-amylases. In contrast to many other secreted carbohydrases in this bacterium [10], these enzymes lacked any obvious CBMs. In addition, there is a starch binding protein with a CBM20 domain but no obvious catalytic domain. It is unclear whether this protein forms an association with the amylases or functions independently to destabilize starch. Three secreted α-glucosidases are produced that contain GH97 domains. The role of these enzymes in starch degradation by this bacterium has not been established.

Debranching of amylopectins/glycogen and the release of maltotriose from pullulan appear to involve a surface-associated pullulanase and its helper as homologs are present in the genome. Both proteins include a lipobox consistent with acylation and surface association. In addition, two amylases (Amy13D and GlyNAX) also carry lipoboxes. Only a GH13 α-glucosidase (Gly13E) and a sucrose phosphorylase (Suc13F) appear to be cytoplasmic. The presence of a strong candidate sucrose phosphorylase in the cytoplasm argues that this bacterium must have a mechanism to import dimeric sugars.

Overall, it would appear that since most enzymes to convert starch to glucose are secreted or surface associated, the activities of these enzymes combine to form glucose outside of the cell. Assuming they are expressed and functional, these enzymes would release glucose for importation.

Import of external glucose most likely involves a sugar transporter and glucokinase. Genes 904 and 1018 are annotated to encode homologs of a glucokinase. In addition gene 1017 is divergent expressed from 1018 and encodes a glucose/galactose transporter. Metabolism of the imported glucose is predicted to occur by the Entner-Douderoff pathway as genes for the diagnostic enzymes of this pathway are present in the genome and the physiology of this bacterium is similar to pseudomonads that typically use this pathway.

**Table 3.** The predicted α-glucan degradation system of *S. degradans*.

| Enzyme | Source gene [1] | Secretion [2] | Catalytic domain [3] | CBM [3] | Size (kDa) | Annotated activity [1] |
|--------|-----------------|---------------|----------------------|---------|------------|------------------------|
| Amy13A | 556 | + | GH13 | | 70.1 | α-amylase |
| Amy13B | 563 | + | GH13 | | 81.6 | α-amylase |
| Amy13C | 573 | + | GH13 | | 64.6 | α-amylase |
| Gly97A | 590 | + | GH97 | | 76.6 | α-glucosidase |
| Gly97D | 2360 | + | GH97 | | 76.4 | α-glucosidase |
| Gly97B | 2499 | + | GH97 | | 77.0 | α-glucosidase |
| Cbm20A | 314 | + | | 20 | 71.0 | Starch binding |
| PulA | 560 | Lipobox | pul | | 151.3 | pullananase |
| PulB | 589 | Lipobox | | | 77.1 | pullananase helper |
| Amy13D | 2938 | Lipobox | GH13 | 20 | 37.5 | α-amylase |
| GlyNAX | 600 | Lipobox | | | 87.4 | glucan 1,4-α-glucosidase |
| GlgT | 1017 | Inner membrane | | | | Glc/Gal transporter |
| GlkA | 1018 | - | | | | glucose kinase |
| Gly13E | 601 | - | GH13 | | 61.3 | α-glucosidase |
| Suc13F | 3210 | - | GH13 | | 68.3 | sucrose phosphorylase |

[1] As described in [10]; [2] As indicated by the presence of a Type II secretion signal or a Lipobox; [3] As determined by CAZY [11].

## 5. The Cellulolytic System

Globally abundant cellulose is formed of linear β-1,4-glucan that assembles into paracrystalline structures in water [1,37]. It is formed by autotrophs of many taxonomic classifications as a component of their cell wall. Oomycetes can also form cellulose as well as several groups of bacteria. Degradation of cellulose involves *endo-* and *exo*-glucanases that hydrolytically form cellobiose and cellodextrins that are converted to glucose by the activity of β-glucosidases.

*S. degradans* is well established as a cellulolytic bacterium and the enzymes produced by this bacterium are described in Table 4 [2,6,7,10,38–41]. Analysis of the genome led to the demonstration that *Sde*2-40 secretes at least 15 β-1,4-endoglucanases, three of which have been reported to be processive endoglucanases (Cel5G, Cel5H and Cel5J) that appear to substitute for the cellobiohydrolases apparently absent in this system [39]. This, however, has not been independently verified and an alternative activity for Cel5H, and presumably Cel5G and Cel5J, has been proposed [42]. Three other enzymes of the system are likely processive enzymes [2,40]. These include Cel5A in which one of the GH5 domains is in the same phylogenetic clade as those of the processive GH5 enzymes and the GH9 enzymes that are processive in some other bacteria [41]. Additional enzymes to

what was originally proposed [10,38], such as Gly5L and Gly5M, also appear to be part of the cellulolytic system of this bacterium [41]. These enzymes are glucanases and their parent genes are induced by cellulose. Thus, it would appear that the 12 GH5 enzymes, the GH6 enzyme and two GH9 enzymes are endoglucanases. As reported previously, most of the secreted enzymes include one or more CBM6 or CBM2/10 [38]. Some of the endoglucanases carry lipoboxes indicative of cell surface association. All of these secreted glucanases have pH optima near neutrality and are salt tolerant [39,40].

**Table 4.** The cellulolytic system of *S. degradans*.

| Enzyme | Source gene [1] | Secretion [2] | Catalytic domain [3] | CBM [3] | Size (kDa) | Annotated activity [1] | Confirmed activity [4] |
|---|---|---|---|---|---|---|---|
| Cel5A | 3003 | + | GH5 (×2) | 6 (×3) | 127.2 | endoglucanase | + |
| Cel5D | 2636 | + | GH5 | 2a, 10 | 65.9 | endoglucanase | + |
| Cel5E | 2929 | + | GH5 | 6 (×2) | 65.4 | endoglucanase | + |
| Cel5F | 1572 | + | GH5 | | 42.0 | endoglucanase | + |
| Cel5G | 3239 | + | GH5 | 6 | 67.9 | endoglucanase | + |
| Cel5H | 3237 | + | GH5 | 6 | 66.9 | endoglucanase | + |
| Cel5I | 3420 | + | GH5 | 2a, 10 | 77.2 | endoglucanase | + |
| Cel5J | 2494 | + | GH5 | 6 (×2) | 65.2 | endoglucanase | + |
| Cel6A | 2272 | + | GH6 | 2 (×2) | 81.2 | endoglucanase | + |
| Cel9A | 636 | + | GH9 | | 62.7 | endoglucanase | + |
| Cel9B | 649 | + | GH9 | 2a, 10 | 89.5 | endoglucanase | + |
| Cel5B | 2490 | Lipobox | GH5 | 6 | 60.8 | endoglucanase | + |
| Cel5C | 0325 | Lipobox | GH5 | | 49.1 | endoglucanase | + |
| Gly5L | 2996 | Lipobox | GH5 | 6 | 93.3 | endoglucanase | + [5] |
| Gly5M | 3023 | Lipobox | GH5 | 6 | 94.9 | endoglucanase | + [5] |
| Ced3A | 2497 | Lipobox | GH3 | | 116.0 | β-glucosidase | + |
| Ced3B | 0245 | Lipobox | GH3 | | 92.9 | β-glucosidase | + |
| Bgl3C | 2674 | Lipobox | GH3 | | 95.4 | β-glucosidase | + |
| Bgl1A | 3603 | - | GH1 | | 52.8 | β-glucosidase | + |
| Bgl1B | 1394 | - | GH1 | | 49.8 | β-glucosidase | + |
| Cep94A | 1318 | - | GH94 | | 91.7 | cellobiose phosphorolase | + |

[1] As described in [10]; [2] As indicated by the presence of a Type II secretion signal or a Lipobox; [3] As determined by the CAZY team [11]; [4] see [38–40]; [5] see [41].

Cellobiose produced by the activity of the classical and/or processive endoglucanases appears to be metabolized by two pathways [40]. Cytoplasmic Cep94A catalyzes the phosphorolytic cleavage of cellobiose to form glucose 1-phosphate and glucose. The resulting glucose 1-phosphate would be converted to glucose 6-phosphate by the activity of a phosphoglucomutase. This is likely an energy conservation step during periods of nutrient limitation as an ATP is not consumed by phosphorolysis or in the subsequent isomerization of the glucose 1-phosphate to glucose 6-phosphate. The rate of cellobiose phosphorolysis appears to be 25% the rate of hydrolysis during rapid growth on cellulose. Hydrolysis involves five β-glucosidases. All enzymes functioning in the conversion of cellobiose to glucose or glucose phosphate were cell-associated with Cep94A, Bgl1A and Bgl1B cytoplasmic and Ced3A, Ced3B and Bgl3C exported and attached to the outer membrane, presumably by acylation.

Since the cell contains a cytoplasmic cellobiose phosphorolase that is predicted to account for a large fraction of the cellobiase activity in *Sde*2-40 [40], presence of a cellobiose transporter in the system seems likely. Cep94A is produced from the *cep94A* gene in an apparent operon with a putative sugar transporter, but there is insufficient information at the present time to predict function of this apparent transporter.

Genes of the cellulolytic system are regulated by their substrate. For selected genes of the cellulolytic system, qRT-PCR has been used to identify gene sets with similar patterns of expression. As predicted from previous biochemical studies on this bacterium [7], there was a high degree of specificity to the gene induction observed. Presence of microcrystalline cellulose in glucose-deficient growth media induced expression of all of the annotated cellulase genes. Three distinct expression patterns were detected [41]. The expression of the genes for some cellulases, such as Cel5A, was induced 2–10 fold within 2 h and then expression remained relatively constant thereafter. A larger subset with Cel5H as the example was induced (>500-fold) 4–10 h after the nutritional shift but then expression was reduced by an order of magnitude at 24 h. A third group that includes Cel5I exhibited the highest average induction but only after 24 h. These distinct patterns of expression indicate that at least part of the apparent redundancy in enzymes (e.g., the 15 endoglucanases) may be due to their independent regulation by distinct transcriptional factors [2]. Each pattern of expression would represent a set of co-regulated genes responsive to a specific cellulose-linked regulatory system. If verified, it will be interesting to see what other activities are part of each regulon.

## 6. The Chitinolytic System

The second most abundant polysaccharide in the environment is chitin formed of poly β-1,4-*N*-acetylglucosamine [43]. It is found in the cell walls of fungi, the exoskeletons of arthropods and diatoms, and the feeding structures of some mollusks and cephalopods. Metabolism of chitin can be similar to that of cellulose with external degradation of the polymer to soluble chitooligosaccharides and subsequent processing to *N*-acetyl-glucosamine [44]. A chitin binding protein is essential for the process [45]. *N*-acetyl-β-glucosamine is then imported, deacetylated and deaminated to form fructose 6-phosphate. Alternatively the polymer can be deacetylated externally to form chitosan and then cleaved by chitosanases.

*S. degradans* produces a chitinolytic system that has been partially characterized by genome annotation, molecular cloning, and biochemical characterization of purified products [44,46,47]. This bacterium secretes the endochitinases Chi18A and Chi18C, the chitodextrinase Cdx18A as well as a chitin binding protein (Table 5). The released chitodextrins can be converted to chitobiose by the secreted Cdx18A and the surface-associated Chi18B. Chi18B is an interesting enzyme in that it has two GH18 domains that are separated from a lipobox and each other by polyserine domains [47,48]. With the apparent cell surface attachment as a reference point, the distal GH18 domain is an *endo*-acting domain whereas the proximal GH18 is an *exo*-acting enzyme. The juxtaposition of these domains would place production of chitobiose by this enzyme directly at the surface of the cell where it could enter the periplasm via by outer membrane porins. There is also one surface-associated *N*-acetyl-glucosaminidase (Hex20A) that could convert the externally produced chitobiose and chitodextrins to *N*-acetyl-β-glucosamine as well. In addition, there is an apparent periplasmic form of

this enzyme that could convert periplasmic chitobiose and chitodextrins to *N*-acetyl-β-glucosamine. The *N*-acetyl-β-glucosamine produced by the activity of either enzyme would be imported into the cytoplasm by a NagE homolog, an inner membrane transporter, and converted to fructose 6-phosphate by the remaining Nag system (Table 5).

**Table 5.** The chitinolytic system of *S. degradans*.

| Enzyme | Source gene [1] | Secretion [2] | Catalytic domain [3] | CBM [3] | Size (kDa) | Annotated activity [1] | Confirmed activity |
|---|---|---|---|---|---|---|---|
| Chi18A | 1704 | + | GH18 | 5 | 56.3 | endochitinase | + [4] |
| Chi18C | 3605 | + | GH18 | 5 | 82.5 | endochitinase | + [4] |
| Cdx18A | 3902 | + | GH18 | 5 | 122.1 | chitodextrinase | + [4] |
| CbpA | 633 | + | | 2, 33 | 46.1 | chitin binding | |
| Chi18B | 3870 | Lipobox | GH18 (×2) | | 135.2 | endo/exochitinase | + [5] |
| Hex20A | 3037 | Lipobox | GH20 | FA58C | 88.5 | *N*-acetylglucosaminidase | + [4] |
| Hex20B | 3271 | Periplasm | GH20 | FA58C | 98.4 | *N*-acetylglucosaminidase | + [4] |
| NagE | 3038 | Inner membrane | | | 47.5 | NAG transporter | |
| Hex3C | 1790 | - | GH3 | | 37.5 | *N*-acetylglucosaminidase | + [4] |
| NagF | 3036 | - | | | 31.8 | *N*-acetylglucosamine kinase | |
| NagC | 3047 | - | | | 41.4 | LacI homolog | |
| NagB | 3041 | - | | | 37.0 | fructose 6P transaminase | |
| NagA | 3040 | - | | | 41.4 | *N*-acetylglucosamine deacetylase | |

[1] As described in [10]; [2] As indicated by the presence of a Type II secretion signal or a Lipobox; [3] As determined by CAZY [11]; [4] As described in [41]; [5] As described in [47].

## 7. The Laminarinase System

Laminarin is a storage polysaccharide found in brown algae [1]. It is primarily composed of β-1,3-linked glucosyl units with occasional β-1,6 linkages. Overall, laminarin is considered to be similar in structure to amylopectin. Mannitol has also been reported in this polymer.

*Sde*2-40 grows on laminarin and both laminarinase and amylase activity can be detected under these conditions [7]. The bacterium is annotated to produce 8 candidate laminarinases [10]. With the exception of Lam81A, all carry GH16 domains (Table 6). Six of the laminarinases appear to be freely secreted. Three of these carry CBM6 domains. Gly16H, a likely laminarinase, has a CBM32 domain. Lam16B, Lam16D and Gly16H all carry at least one CBM-like FA58C domain. The laminarinodextrins produced by the activity of these enzymes are likely to be converted to glucose by the activity of one or more β-glucosidases described as part of the cellulolytic system. Thus further metabolism of laminarin is would be similar to that of cellulose. Like each of the previous systems, three enzymes were found to carry lipoboxes at their amino termini, suggesting they are surface-associated through acylation.

Degradation of the β-1,6 branches in laminarin and the laminarin-associated mannitol by this bacterium has not been established. There are genes for two apparently acylated β-1,6-glucanases in the genome of this bacterium (Table 6). Two cytoplasmic mannitol dehydrogenases are also annotated in the 2-40 genome (941 and 1241). Genes for both dehydrogenases are located within apparent operons with genes predicted to encode glucuronate isomerases. There is also a mannitol/fructose type PTS system produced by this bacterium as well (genes 3180–3182). Thus, this bacterium is predicted to have a mechanism to debranch laminarin and to metabolize whatever mannitol might be associated with laminarin.

**Table 6.** The predicted laminarinase system of *S. degradans*.

| Enzyme | Source gene [1] | Secretion [2] | Catalytic domain [3] | CBM [3] | Size (kDa) | Annotated activity [1] |
|---|---|---|---|---|---|---|
| Lam16A | 1393 | + | GH16 | 6 (×2), 56 | 183.2 | β-1,3(4)-endoglucanase |
| Lam16B | 2927 | + | GH16 | 6, FA58C (×2) | 158.6 | β-1,3(4)-endoglucanase |
| Lam16C | 1444 | + | GH16 | 4, 32 | 129.1 | β-1,3(4)-endoglucanase |
| Lam16D | 3021 | + | | FA58C | 77.7 | β-1,3(4)-endoglucanase |
| Lam16E | 0652 | + | GH16 | 6 (×2) | 61.4 | β-1,3(4)-endoglucanase |
| Gly16H | 2878 | + | GH16 | 32, FA58C | 107.2 | β-1,3(4)-endoglucanase |
| Lam16F | 3141 | Lipobox | GH16 | | 80.2 | β-1,3(4)-endoglucanase |
| Lam16G | 2832 | Lipobox | GH16 | 6 (×2) | 94.2 | β-1,3(4)-endoglucanase |
| Lam81A | 2834 | Lipobox | GH16 | 56 | 133.1 | β-1,3-endoglucanase |
| Gly30A | 2992 | Lipobox | GH30 | 6 (×2), 13 | 107.4 | β-1,6-glucosidase |
| Gly30B | 2994 | Lipobox | GH30 | | 52.8 | β-1,6-glucosidase |
| Lam55A | 54 | - | GH55 | FA58C | 58.5 | β-1,3-endo/exoglucanase |

[1] As described in [10]; [2] As indicated by the presence of a Type II secretion signal or a Lipobox; [3] As determined by CAZY [11].

## 8. The Mannanase System

Glucomannan is β-1,4-mannose mixed with β-1,4-glucose with slightly more mannose content than glucose. Galactomannan is β-1,(3)4-mannose with at least 5% α-1,6-galactose. Mannans are primarily found as glucomannans that can be a constituent of red algal cell walls and as galactomannans found in some leguminous seeds and fungi [1].

*Sde*2-40 is able to utilize both glucomannan and galactomannan as the primary carbon source for growth [49], thus indicating that this bacterium produces the enzymes to metabolize this polymer. The bacterium is annotated to secrete an *endo*-acting β-1,4-mannanase (Man5O) and an *exo*-acting β-1,4-mannosidase (Man5N) ([10]; Table 7). The endomannanases Man5P and ManR and the exomannosidases Man5Q and Man26A are predicted to be on the cell surface due to the presence of a lipobox. Both a mannanase and a mannosidase appear to be cytoplasmic. The reason for this is not clear. The glucan component of mannan is likely degraded by the cellulolytic system.

**Table 7.** The predicted mannanase system of *S. degradans*.

| Enzyme | Source gene [1] | Secretion [2] | Catalytic domain [3] | CBM [3] | Size (kDa) | Annotated activity [1] |
|---|---|---|---|---|---|---|
| Man5O | 656 | + | GH5 | 10 | 52.6 | mannanase |
| Man5N | 64 | + | GH5 | 2, 10 | 57.6 | mannosidase |
| Gly5K | 2993 | + | GH5 | 6 (×2), 13 | 94.6 | |
| Man5P | 509 | Lipobox | GH5 | | 50.9 | mannanase |
| ManR | 2285 | Lipobox | | | 42.8 | mannanase |
| Man5Q | 2541 | Lipobox | GH5 | | 92.3 | mannanase |
| Man26A | 3691 | Lipobox | GH26 | 10, cbm [4] | 54.9 | mannosidase |
| Man2A | 169 | - | GH2 | | 91.7 | mannosidase |
| Man2B (Gly5R) | 1121 | - | GH2 | | 59.2 | mannanase |

[1] As described in [10]; [2] As indicated by the presence of a Type II secretion signal or a Lipobox; [3] As determined by CAZY [11]; [4] not currently classified CBM.

## 9. The Pectinolytic System

Pectic components are primarily composed of α-1,4-galacturonan with dispersed α-1,2-rhamnose residues [50]. Side chain polymers composed arabinan or arabinogalactan can be present. Three pectic polysaccharides, homogalacturonan (HG), rhamnogalacturonan-I (RG-I) and substituted galacturonans (rhamnogalacturonan-II), have been identified. HG is methylated α-1,4-D-galacturonate. RG-I has α-1,2-rhamnose alternating with α-1,4-D-galacturonate in the backbone polymer. Some of the rhamnose residues can be substituted at C-4 with linear and branched α-L-arabinofuranosyl and/or β-D-galactopyranosyl residues. RG-II is composed of 1,4-linked α-D-galacturonate substituted at C2 with non-saccharide or octasaccharide side chains and different disaccharides can be attached at C-3. These pectic compounds can comprise a major fraction of algal cell walls [1].

Degradation of pectic polymers requires pectin esterases to remove methyl groups creating methanol in the process. Pectate/pectin lyases catalyze elimination reactions at the non-reducing end of the polymer to release 4-deoxy-α-D-mann-4-enuronosyl residues. Polygalacturonase and rhamnogalacturonanase cleave the polymer hydrolytically producing galacturonic acid and rhamnose. Both lyase and hydrolytic mechanisms can be employed by the same strain for degrading pectins [51].

*Sde*2-40 is highly pectinolytic and is able to utilize neutralized citrus pectin as the primary carbon source during growth [7]. Pitting is observed on pectin-based gels consistent with the secretion of enzymes to degrade pectins and the utilization of released sugar derivatives. As with all other CPs, processing of pectin occurs external to the cell. This bacterium secretes a number of PL1, PL3, PL10 and PL11 lyases to degrade pectin polymers (Table 8). Many of these probable lyases carry CBM6 and CBM35 domains and some carry CBM2 domains. Complementing the lyases are GH105 hydrolases. In addition, there are a number of arabinofuranosidases and galactosidases that could function in the degradation of pectin as well (Table 9). All annotated pectin esterases (pectin methyl esterases) appear to be surface-associated as they carry lipoboxes at their amino termini. This suggests that the secreted lyases act on methylated pectin to release pectin fragments. There are additional lyases and hydrolases with lipoboxes as well, indicating that conversion of released pectin fragments to their constituent

sugars and sugar derivatives likely occurs at the cell surface. Clearly this bacterium produces many pectinolytic enzymes. Differences in substrate specificity (HG/RG-I/RG-II)) and regulation as well as localization may account for the deduced redundancies in enzyme activities.

**Table 8.** The probable pectinase system of *S. degradans*.

| Enzyme | Source gene [1] | Secretion [2] | Catalytic domain [3] | CBM [3] | Size (kDa) | Annotated activity [1] |
|---|---|---|---|---|---|---|
| Pel1B | 937 | + | PL1 | | 46.1 | pectate lyase |
| Pel1C | 942 | + | PL1 | 6, 35 | 78.9 | pectate lyase |
| Pel1A | 943 | + | PL1 | 6, 35 | 136.4 | pectate lyase |
| Pel1F | 2311 | + | PL1 | 2, 6, 35 | 81.5 | pectate lyase |
| Pel3B | 608 | + | PL3 | | 46.1 | pectate lyase |
| Pel3D | 1703 | + | PL3 | 13 | 41.6 | pectate lyase |
| Pel3A | 2308 | + | PL3 | 2, 6 | 52.3 | pectate lyase |
| Pel3C | 3007 | + | PL3 | | 42.9 | pectate lyase |
| Pel10A | 1051 | + | PL10 | 2, 6, 35 | 73.3 | pectate lyase |
| Pel11A | 1650 | + | PL11 | 35 | 97.6 | rhamnogalacturon lyase |
| Rgh105B | 2808 | + | GH105 | | 45.5 | rhamogalacturon hydrolase |
| Rgh105C | 3946 | + | GH105 | | 44.3 | rhamnogalacturon hydrolase |
| Rgh105A | 951 | Lipobox | GH105 | | 92.4 | rhamnogalacturon hydrolase |
| PelG | 3881 | Lipobox | Pel | 6 | 78.2 | pectate lyase |
| PmeA | 890 | Lipobox | | | 75.9 | pectinesterase |
| Pme12A | 3094 | Lipobox | CE12 | | 30.2 | pectinesterase |
| Pel43X | 944 | Lipobox | GH43 | | 42.4 | pectinesterase |
| PmeB | 3447 | Lipobox | | | 115.1 | pectinesterase |
| Pel1D | 3448 | Lipobox | PL1 | | 63.3 | pectinesterase |
| Pel1E | 2307 | Lipobox | PL1 | | 45.4 | pectate lyase |
| Pel9A | 2946 | Lipobox | PL9 | | 75.6 | exopectate lyase |
| Pel10B | 2947 | Lipobox | PL10 | 6, 35 | 60.8 | pectate lyase |

[1] As described in [10]; [2] As indicated by the presence of a Type II secretion signal or a Lipobox; [3] As determined by CAZY [11].

## 10. The Xylanolytic System

Xylans are β-1,3- or β-1,4-linked xylose that can be modified to include acetyl groups, arabinose and methylated glucuronate. Included with this polysaccharide can be arabitans composed arabinose with various linkages and arabinogalactans with α- and β-linkages [1]. In addition to their structural roles in the hemicellulose component of higher plant cell walls as would be found in marine and estuarine grasses [52], xylans can substitute for cellulose in the cell walls of some siphonous green algae [53,54] and red algae [55].

The variation among the constituent linkages in the polymers of the xylans, arabitans and galactoarabitans requires a diverse collection of enzymes to release the constituent sugars and sugar derivatives, such endoxylanases and xylosidases to produce xylose. Galactosidases, glucosidases, glucuronidases and arabinofuranosides would release galactose, glucose, glucuronate and arabinose, respectively. Acetoxylan esterase would deacetylate xylan backbones.

*Sde*2-40 can utilize xylans from terrestrial sources as the primary carbon source for energy and growth [7]. This bacterium, however, does not seem to be able to utilize arabinogalactans well [49], but it can grow on the constituent sugars [2]. A review of the genome annotation, metabolic profiling and the biochemical activities of selected genes indicates that this bacterium produces all of the enzymes to degrade and utilize xylan and arabinogalactan of marine and terrestrial origin [6,10,49,56]. This bacterium produces GH10 and GH11 xylanases [19,56] as well as the enzymes to remove backbone modifications (Table 9). In these cases, the xylanases appear to be freely secreted as >90% of the activity produced by this bacterium is present in culture filtrates [49]. Most of the enzymes to remove backbone modifications have lipoboxes suggestive of surface attachment. Only a few hypothetic esterases together with a candidate arabinofuranoside and glucuronidase have the properties of cytoplasmic enzymes.

**Table 9.** The xylanolytic system of *S. degradans*.

| Enzyme | Source gene [1] | Secretion [2] | Catalytic domain [3] | CBM [3] | Size (kDa) | Annotated activity [1] | Confirmed activity |
|---|---|---|---|---|---|---|---|
| Xyn10A | 181 | + | GH10 | 5, 7 | 61.7 | endoxylanase | + |
| Xyn10B | 2934 | + | GH10 | 2,10 | 65.0 | endoxylanase | + |
| Xyn10C | 2633 | + | GH10 | | 92.3 | endoxylanase | + |
| Xyn10D | 3612 | + | GH10, GH43 | 2, 6, 22 | 129.6 | endoxylanase | +[4] |
| Xyn11A | 701 | + | GH11 | | | endoxylanase | + |
| Xyn11B | 3061 | + | GH11, CE11 | 2, 10, 60 | 80.8 | endoxylanase | + |
| Xyl43J (Arg43J) | 789 | + | GH43 | | 35.1 | xylosidase | |
| Xyl43L | 946 | + | GH43 | | 36.1 | xylosidase | |
| Gal31A | 1593 | + | GH31 | | 46.5 | α-galactosidase | |
| Glu115A | 1755 | + | GH115 | | 110.0 | α-glucuronidase | |
| Glu2G | 2632 | + | GH2 | | 68.8 | β-glucuronidase | |
| Arb51A | 1767 | + | GH51 | | 59.3 | arabinofuranosidase | |
| Arb43A | 2809 | + | GH43 | 6, 13, 35 | 85.0 | arabinofuranosidase | |
| Arb43D | 1014 | + | GH43 | 13 | 92.4 | arabitan *endo* 1,5-α-arabinosidase | |
| Arg53B | 3710 | + | GH53 | 13 | 52.6 | arabinogalactan *endo* 1,4-β-galactosidase | |
| Axe2A | 2370 | + | CE2 | | 40.9 | acetoxylan esterase | |
| Gal2B | 1177 | + | GH2 | | 97.1 | β-galactosidase | |
| Gal2F | 3882 | + | GH2 | 35 | 107.1 | β-galactosidase | |
| Xyn10E | 323 | Lipobox | GH10 | | 75.2 | endoxylanase | + |
| Arb43H | 598 | Lipobox | GH43 | | 63.8 | arabinofuranosidase | |
| Arb43B | 787 | Lipobox | GH43 | | 40.7 | arabinofuranosidase | |
| Gly97C | 790 | Lipobox | GH97 | | 73.3 | α-glucosidase | |
| Arb43I | 1655 | Lipobox | GH43 | | 62.6 | arabinofuranosidase | |
| Arb43J | 791 | Lipobox | GH43 | 13 | 67.2 | arabinofuranosidase | |
| Arb43K | 822 | Lipobox | GH43 | | 42.6 | xylosidase | |

**Table 9.** *Cont.*

| | | | | | | |
|---|---|---|---|---|---|---|
| Xyl31A | 2500 | Lipobox | GH31 | | 110.2 | xylosidase |
| Arg53A | 683 | Lipobox | GH53 | | | arabinogalactan *endo* 1,4-β-galactosidase |
| Arg53C | 2827 | Lipobox | GH53 | Ricin | 71.1 | arabinogalactan *endo* 1,4-β-galactosidase |
| Gly43M | 3317 | Lipobox | GH43 | | 127.2 | arabinofuranosidase |
| Arb43E | 786 | Lipobox | GH43 | | | arabitan *endo* 1,5-α-arabinosidase |
| Axe1C | 3746 | Lipobox | CE1 | | 37.0 | acetoxylan esterase |
| Axe2C | 3143 | Lipobox | CE2 | | 40.4 | acetoxylan esterase |
| Axe3A | 3994 | Lipobox | CE3 | | 27.8 | acetoxylan esterase |
| Gal2A | 684 | Lipobox | GH2 | | 88.1 | β-galactosidase |
| Gal2C | 1285 | Lipobox | GH2 | | 91.8 | β-galactosidase |
| Gal2E | 2936 | Lipobox | GH2 | | 34.0 | β-galactosidase |
| Gal2D | 2935 | - | GH2 | | 65.6 | β-galactosidase |
| Arb43C | 777 | - | GH43 | | 35.9 | arabinofuranosidase |
| Agu67A | 1025 | - | GH67 | | 89.1 | α-glucuronidase |
| | 51 | - | CE1 | | 30.5 | carboxyl esterase |
| | 2890 | - | CE1 | | 30.6 | carboxyl esterase |
| | 139 | - | CE4 | | 34.3 | carboxyl esterase |
| | 653 | - | CE4 | | 41.2 | carboxyl esterase |

[1] As described in [10]; [2] As indicated by the presence of a Type II secretion signal or a Lipobox; [3] As determined by CAZY [11]; [4] As shown by Ko *et al.* [56].

Thus it can be predicted that depolymerization of xylans occurs external to the cell, mostly through the activity of freely secreted enzymes. Removal of modifications can occur at the source or on the cell surface. Ultimately, the released xylose, glucose, glucuronate, galactose, and arabinose are imported into the cell. This presumably involves outer membrane porins and cell membrane transporters for these sugars that have yet to be identified.

## 11. Concluding Remarks

Although the native habitat for *S. degradans* has not been established, its physiology is consistent with this bacterium being of marine origin. As such, this bacterium faces the issue of how to obtain subsistence in light of two problems. The bacterium does not import any of these minimally soluble CPs. Instead it secretes enzymes to solubilize the CP first. Any enzyme that it secretes, however, has the potential to diffuse away in the marine environment, and therefore, be lost. Similarly, recovery of any solubilized CPs faces the same difficulty. Solubilized CPs have the potential to diffuse away as well. Thus, the bacterium would needs to minimize the loss of the carbohydrases it secretes and to maximize the recovery of sugars and sugar derivatives to use as carbon and energy sources. Three features of the carbohydrase systems of *S. degradans* appear to address these problems: (1) Expression of the genes for a carbohydrase system are specifically induced by contact with their substrate; (2) the structural properties of the secreted carbohydrases favor adsorption to their substrate; and

(3) the sugars or sugar derivatives derived from CPs are generated at the cell surface to maximize their uptake. This insures the maximum economy of use for each substrate CP.

In this bacterium, carbohydrases are produced on a "per need" basis as the carbohydrase systems are subject to tight genetic regulation. The degradative system for a specific polymer is only expressed in the presence of that polymer [7]. This regulation occurs at the transcriptional level [41]. As the CPs are insoluble and generally found in cell walls or as carbon storage granules, it seems reasonable to predict that the bacterium first adsorbs to the surface of these materials. Surface-associated cadherin domains might be an example of this capacity [57,58], but colloidal activity could function this way as well. To sense adsorption to a suitable substrate, a mechanism must exist to produce a signal molecule specific to that polymer. The simplest way to do this is through the activity of basally expressed sentinel enzymes. The cellodextrins produced by the activity of basally expressed glucanases and activate production of cellulases are one example of this phenomenon [41].

The bacterium must also have at least one mechanism to perceive the signal molecule. This argues for the involvement of a regulatory system to activate expression of contributing genes. For example, the cellodextrins produced by the basally expressed glucanases induce transcription of the genes in the cellulolytic system of this bacterium. Interestingly, as many as three regulatory systems could function in this process. Each regulatory system would have its own transcriptional factor. Understanding what genes are co-expressed in this bacterium as part of the regulon for each cognate transcriptional factor could help understand how to digest specific carbohydrates and raw biomass.

The localization of the carbohydrases of each system provides a plausible explanation for how CPs are used as food. Each system involves secreted enzymes to depolymerize their target CPs as the enzymes have easily recognized type II secretion signal sequences. These secreted enzymes appear to be adapted to the marine environment as they do not function well at the acidic pH's typical of many terrestrial systems, but instead, require the more neutral environment of seawater. In addition, the activities of these secreted enzymes seem to be tolerant to salt concentrations as high as 5%.

Another unifying feature of these secreted carbohydrases is the inclusion of CBMs. The secreted enzymes many times carry one or more CBMs joined to the catalytic domains by flexible linkers. These CBMs likely assist in the adsorption of secreted enzymes to their substrate. Thus, the adsorption modules function to minimize the loss of the host enzyme through diffusion. Since adsorption occurs independently of catalytic activity, these enzymes would still be able to solubilize their substrate polymer. Loss of secreted carbohydrases would be minimal as they would only be produced when the bacterium is adsorbed to that substrate and the secreted carbohydrases would be bound to their substrate through their CBMs to limit diffusion. For those enzymes that lack obvious CBMs, it will be interesting to see if they interact with proteins that do carry CBMs as part of multimeric complexes.

Interestingly, the secreted carbohydrases are likely to only partially depolymerize their substrate CPs to form soluble oligosaccharides. Degradation of the oligosaccharides to their constituent sugars or sugar derivatives appears, in many cases, to occur at the cell surface using secreted enzymes with lipoboxes. In those cases where it has been examined, the enzymes with lipoboxes are cell-associated as predicted (e.g., [40]). In this way, most diffusible sugars or sugar derivatives from the CPs would be produced at the cell surface. This argues that the specificity and affinity of the cognate transport systems in the cell membrane would be critical to the ability of this bacterium to utilize these CPs. Little is known of these transport systems.

An example of this strategy is found in the Chi18B chitinase produced by this bacterium. Expression of the source gene is induced by chitin. This dual domain enzyme appears to be secreted and anchored to the outer membrane due to the presence of a lipobox. The catalytic domains are separated from this lipobox by a long polyserine domain (~110 residues) that would place the enzyme at the outer membrane surface. This placement is augmented by the position of the distinct catalytic domains in which the more distant domain is *endo*-acting forming chitooligosaccharides and the closer domain is *exo*-acting producing chitobiose. Thus the most diffusible products of chitin are formed at the cell surface [47].

Other systems are more complicated as the core polymers are modified. Removal of the modifications (e.g., pectin esterases, acetoxylan esterases, *etc.*) appears to occur at the cell surface in some cases as these enzymes have lipoboxes. This was not anticipated as homologs of many secreted depolymerases only act on the core polymer lacking modifications. This predicts that the depolymerases of this bacterium may lack specificity in their substrate preference (e.g., have the ability to degrade acetylated substrates). Alternatively, the secreted enzymes may be limited in their activity until the surface-associated enzymes act on the material. Thus, close proximity to the adsorbed bacterium would be necessary for these secreted enzymes to be active.

This bacterium has the potential to produce a large number of carbohydrases to degrade the CPs found in the marine environment. Most other bacteria produce substantially fewer carbohydrases and tend to degrade less types of CPs [10]. With such a plethora of enzymes, where did these genes come from? Most likely, these genes were acquired by horizontal gene transfer as their codon usage and the third position nucleotide in codons differ from that used by the core housekeeping genes [10]. The mechanism of acquisition, however, is unclear. Originally, the genes associated with each system were largely thought to be dispersed in the genome [10], and thus, acquired independently. As shown in the Tables, however, genes of several systems are often clustered to a degree. For example, genes encoding components of the agarase system are clustered around genes 1175 and 2650. Similarly, some of the alginolytic system clusters near genes 1270 and 3274. The amylase system is concentrated near gene 556 and the pectinolytic system has a focus near gene 937. These areas of concentration may reflect the evolution of the genes as blocks of genes could have been acquired through horizontal gene transfer. For example, the agarolytic systems genes between 2649 and 2657 show transcriptional and translational similarity to their counterparts in *Pseudoalteromonas atlantica*, and thus, are a candidate to have been acquired as a genetic unit. Alternatively, more classic bacterial evolution could have occurred in which acquired genes are duplicated at nearby locations [59]. On the other hand, it may be that gene fragments representing a domain are acquired or duplicated and then recombined with other fragments to form novel enzymes. This could explain some of the unusual apparent enzyme structures of this bacterium and the mixed phylogenies of domains in enzymes like Chi18C and Chi18B [60].

In conclusion, the carbohydrase systems of this bacterium are providing new insights into the degradation of CPs. In some cases, the enzymes of a system seem to be similar to those of other bacteria. In other cases, new activities are identified to explain oddities in the system. This bacterium, thus, can serve as a paradigm for processing of complex polysaccharides in the marine environment and offers the opportunity for comparative studies with terrestrial systems.

**References**

1. McCandless, E. Polysaccharides of the seaweeds. In *The Biology of Seaweeds*; Lobban, C., Wynne, M., Eds.; University of California Press: Berkeley, CA, USA, 1981; pp. 559–588.
2. Suvorov, M.; Kumar, R.; Zhang, H.; Hutcheson, S. Novelties of the cellulolytic system of a marine bacterium applicable to cellulosic sugar production. *Biofuels* **2011**, *2*, 59–70.
3. Andrykovitch, G.; Marx, I. Isolation of a new polysaccharide-digesting bacterium from a salt marsh. *Appl. Environ. Microbiol.* **1988**, *54*, 3–4.
4. Gonzalez, J.; Weiner, R. Phylogenetic characterization of marine bacterium strain 2-40, a degrader of complex polysaccharides. *Int. J. Syst. Evol. Microbiol.* **2000**, *50*, 831–834.
5. Ekborg, N.; Gonzalez, J.; Howard, M.; Taylor, E.; Hutcheson, S.; Weiner, R. *Saccharophagus degradans* gen nov., sp. nov., a versatile marine degrader of complex polysaccharides. *Int. J. Syst. Evol. Microbiol.* **2005**, *55*, 1545–1549.
6. Shin, M.; Lee, D.; Skogerson, K.; Wohlgenuth, G.; Choi, I.-G.; Fiehn, O.; Kim, K. Global metabolic profiling of plant cell wall polysaccharide degradation by *Saccharophagus degradans*. *Biotechnol. Bioeng.* **2009**, *105*, 477–488.
7. Ensor, L.; Stotz, S.K.; Weiner, R.M. Expression of multiple insoluble complex polysaccharide degrading enzyme systems by a marine bacterium. *J. Ind. Microbiol. Biotechnol.* **1999**, *23*, 123–126.
8. Gonzalez-Garcia, Y.; Nungaray, J.; Cordova, J.; Gonzalez-Reynoso, O.; Koller, M.; Atlic, A.; Braunegg, G. Biosynthesis and characterization of polyhydroxyalkanoates in the polysaccharide-degrading marine bacterium *Saccharophagus degradans* ATCC43961. *J. Ind. Microbiol. Biotechnol.* **2008**, *35*, 629–633.
9. Alva Munoz, L.; Riley, M. Utilization of cellulosic waste from tequila bagasse and production of polyhydroxyalkanoate (PHA) bioplastics by *Saccharophagus degradans*. *Biotechnol. Bioeng.* **2008**, *100*, 882–888.
10. Weiner, R.; Taylor, L.; Henrissat, B.; Hauser, L.; Land, M.; Coutinho, P.; Rancurel, C.; Saunders, E.; Longmire, A.; Zhang, H.; *et al.* Complete genome sequence of the complex carbohydrate-degrading marine bacterium, *Saccharophagus degradans* Strain 2-40. *PLoS Genet.* **2008**, *4*, e1000087.
11. Henrissat, B.; Davies, G. Structural and sequence-based classification of glycoside hydrolases. *Curr. Opin. Struct. Biol.* **1997**, *7*, 637–644. Available online: http://www.cazy.org/b369.html (accessed on 1 October 2010).
12. Boraston, A.; Bolam, D.; Gilbert, H.; Davies, G. Carbohydrate-binding modules: Fine-tuning polysaccharide recognition. *Biochem. J.* **2004**, *382*, 769–781.
13. Fu, X.; Kim, S. Agarases: Review of major sources, categories, purification method, enzyme characteristics and application. *Mar. Drugs* **2010**, *8*, 200–218.

14. Whitehead, L.A.; Stosz, S.K.; Weiner, R.M. Characterization of the agarase system of a multiple carbohydrate degrading marine bacterium. *Cytobios* **2001**, *106* (Suppl. 1), 99–117.

15. Ekborg, N.; Taylor, L.; Weiner, R.; Hutcheson, S. Genomic and proteomic analysis of the agarolytic system of *Saccharophagus degradans* strain 2-40. *Appl. Environ. Microbiol.* **2006**, *72*, 3396–3405.

16. Kim, H.; Lee, S.; Lee, D.; Kim, H.-S.; Bang, W.-G.; Kim, K.; Choi, I.-G. Overexpression and molecular characterization of Aga50D from *Saccharophagus degradans* 2-40: An *exo*-type β-agarase producing neoagarobiose. *Appl. Microbiol. Biotechnol.* **2010**, *86*, 227–234.

17. Lee, S.; Lee, J.; Ha, S.; Shin, D.; Kim, K.; Choi, I.-G. Crystallization and preliminary X-ray analysis of neoagarobiose hydrolase from *Saccharophagus degradans*. *Acta Crystallogr.* **2009**, *F65*, 1299–1301.

18. Henshaw, J.; Money, V.; Bolam, D.; Czijek, M.; Ekborg, N.; Weiner, R.; Hutcheson, S.; Davies, G.; Gilbert, H.; Boraston, A. Family 6 carbohydrate binding modules in β-agarases display exquisite selectivity for the non-reducing termini of agarose. *J. Biol. Chem.* **2006**, *281*, 17099–17107.

19. Hutcheson, S. University of Maryland, College Park, MD, USA. 2005, unpublished work.

20. Michel, G.; Barbeyron, T.; Kloareg, B.; Czjzek, M. The family 6 carbohydrate-binding modules have coevolved with their appended catalytic modules toward similar substrate specificity. *Glycobiology* **2009**, *19*, 615–623.

21. Yun, E.; Shin, M.; Yoon, J.-J.; Kim, Y.; Choi, I.-G.; Kim, K. Production of 3,6-anhydro-L-galactose from agarose by agarolytic enzymes of *Saccharophagus degradans* 2-40. *Process Biochem.* **2011**, *46*, 88–93.

22. d'Enfert, C.; Ryter, A.; Pugsley, A.P. Cloning and expression in *Escherichia coli* of the *Klebsiella pneumoniae* genes for production, surface localization and secretion of the lipoprotein pullulanase. *EMBO J.* **1987**, *6*, 3531–3538.

23. Pugsley, A.P. The complete general secretory pathway in gram negative bacteria. *Microbiol. Rev.* **1993**, *57*, 50–108.

24. Pugsley, A.P.; Chapon, C.; Schwartz, M. Extracellular pullulanase of *Klebsiella pneumoniae* is a lipoprotein. *J. Bacteriol.* **1986**, *166*, 1083–1088.

25. Seydel, A.; Gounon, P.; Pugsley, A.P. Testing the "+2 rule" for lipoprotein sorting in the *Escherichia coli* cell envelope with a new genetic selection. *Mol. Microbiol.* **1999**, *34*, 810–821.

26. Shin, M.; Lee, D.; Wohlgemuth, G.; Choi, I.-G.; Fiehn, O.; Kim, K. Global metabolite profiling of agarose degradation by *Saccharophagus degradans* 2-40. *N. Biotechnol.* **2010**, *27*, 156–168.

27. Gacesa, P. Alginates. *Carbohydr. Polym.* **1988**, *8*, 161–182.

28. Ertesvag, H.; Hoidal, H.K.; Hals, I.K.; Rian, A.; Doseth, B.; Valla, S. A family of modular type mannuronan C-5 epimerase genes controls alginate structure in *Azotobacter vinelandii*. *Mol. Microbiol.* **1995**, *16*, 719–731.

29. Doubet, R.; Quatrano, R. Isolation of marine bacteria capable of producing specific lyases for alginate degradation. *Appl. Environ. Microbiol.* **1982**, *47*, 704–709.

30. Wong, T.; Preston, L.; Schiller, N. Alginate Lyase: Review of major sources and enzyme characteristics, structure-function analysis, biological roles, and applications. *Annu. Rev. Microbiol.* **2000**, *54*, 289–340.

31. Preiss, J.; Ashwell, G. Alginic acid metabolism in bacteria: I. Enzymatic formation of unsaturated oligosaccharides and 4-deoxy-L-erythro-5-hexoseulose uronic acid. *J. Biol. Chem.* **1962**, *237*, 309–316.

32. Preiss, J.; Ashwell, G. Alginic acid metabolism in bacteria: II. The enzymatic reduction of 4-deoxy-L-erythro-5-hexoseulose uronic acid to 2-keto-3-deoxy-D-gluconic acid. *J. Biol. Chem.* **1962**, *237*, 317–321.

33. Gacesa, P. Enzymic degradation of alginates. *Int. J. Biochem.* **1992**, *24*, 545–552.

34. Linhardt, R.J.; Galliher, P.M.; Cooney, C.L. Polysaccharide Lyases. *Appl. Biochem. Biotechnol.* **1986**, *12*, 135–176.

35. Chakravorty, D. Cell Biology of Alginic acid Degradation by Marine Bacterium 2-40. M.S. Thesis, University of Maryland, College Park, MD, USA, 1998.

36. Abdel-Hafez, A.; Weiner, R. University of Maryland, College Park, MD, USA. 2005, unpublished work.

37. Brett, C. Cellulose microfibrils in plants: Biosynthesis, deposition, and integration into the cell wall. *Int. Rev. Cytol.* **2000**, *199*, 161–199.

38. Taylor, L.; Henrissat, B.; Coutinho, P.; Ekborg, N.; Howard, M.; Hutcheson, S.; Weiner, R. A complete cellulase system in the marine bacterium *Saccharophagus degradans* strain 2-40. *J. Bacteriol.* **2006**, *188*, 3849–3861.

39. Watson, B.; Zhang, H.; Longmire, A.; Moon, Y.-H.; Hutcheson, S. Processive endoglucanases mediate degradation of cellulose by *Saccharophagus degradans* 2-40. *J. Bacteriol.* **2009**, *191*, 5697–5705.

40. Zhang, H.; Moon, Y.-H.; Watson, B.; Suvorov, M.; Santos, E.; Sinnott, C.; Hutcheson, S. Hydrolytic and phosphorolytic metabolism of cellobiose by the marine aerobic bacterium *Saccharophagus degradans* 2-40$^{\text{T}}$. *J. Ind. Microbiol. Biotechnol.* **2011**, doi:10.1007/s10295-011-0945-4.

41. Zhang, H.; Hutcheson, S. Complex expression of the cellulolytic transcriptome of *Saccharophagus degradans*. 2011, unpublished work.

42. Zhang, J. Structure-Function Studies of Biomass Degrading Enzymes: Crystallization of EndoT from *Hypocrea jecorina* and Cel5H from *Saccharophagus degradans*, and Enzyme Characterization of Cel5H. M.S. Thesis, Swedish University of Agricultural Science, Uppsala, Sweden, 2009.

43. Muzzarelli, R. Native, industrial, and fossil chitins. In *Chitin and Chitinases*; Jolles, P., Muzzarelli, R.A.A., Eds.; Birkhauser: Basel, Switzerland, 1999.

44. Howard, M.B.; Ekborg, N.A.; Weiner, R.M.; Hutcheson, S.W. Detection and characterization of chitinases and other chitin-modifying enzymes. *J. Ind. Microbiol. Biotechnol.* **2003**, *30*, 627–635.

45. Vaage-Kolstad, G.; Horn, S.; van Aalten, D.; Syntad, B.; Eijsink, V. Non-catalytic chitin-binding protein CBP21 from *Serratia marcescens* is essential for chitin degradation. *J. Biol. Chem.* **2005**, *280*, 28492–28497.

46. Howard, M.B.; Ekborg, N.A.; Taylor, L.E.; Weiner, R.M.; Hutcheson, S.W. Genomic analysis and initial characterization of the chitinolytic system of *Microbulbifer degradans* strain 2-40. *J. Bacteriol.* **2003**, *185*, 3352–3360.

47. Howard, M.B.; Ekborg, N.A.; Taylor, L.E., II; Weiner, R.M.; Hutcheson, S.W. Chitinase B of "*Microbulbifer degradans*" 2-40 contains two catalytic domains with different chitinolytic activities. *J. Bacteriol.* **2004**, *186*, 1297–1303.

48. Howard, M.B.; Ekborg, N.A.; Taylor, L.E.; Hutcheson, S.W.; Weiner, R.M. Identification and analysis of polyserine linker domains in prokaryotic proteins with emphasis on the marine bacterium *Microbulbifer degradans*. *Protein Sci.* **2004**, *13*, 1422–1425.

49. Suvorov, M.; Kumar, R.; Santos, E.; Hutcheson, S. Zymetis, Inc. College Park, MD, USA. 2008, unpublished work.

50. Buchanan, B.; Gruissem, W.; Jones, R. *Biochemistry and Molecular Biology of Plants*; Wiley-VCH: London, UK, 2000.

51. Lombard, V.; Bernard, T.; Rancurel, C.; Brumer, H.; Coutinho, P.; Henrissat, B. A hierarchical classification of polysaccharide lyases for glycogenomics. *Biochem. J.* **2010**, *432*, 437–444.

52. Carpita, N.C. Structure and biogenesis of the cell walls of grasses. *Annu. Rev. Plant Physiol. Plant Mol. Biol.* **1996**, *47*, 445–476.

53. Craigie, J. Cell Walls. In *Biology of the Red Algae*; Cole, K., Sheath, R., Eds.; University of Cambridge Press: Cambridge, UK, 1990; pp. 221–258.

54. Frei, E.; Preston, R. Non-cellulosic structural polysaccharides in algal cell walls. I. Xylan in siphoneous green algae. *Proc. R. Soc. Lond. B Biol. Sci.* **1964**, *160*, 293–353.

55. Turvey, J.; Williams, E. The structures of some xylans from red algae. *Phytochemistry* **1970**, *9*, 2383–2388.

56. Ko, J.K.; Jung, M.W.; Kim, K.H.; Choi, I.G. Optimal production of a novel *endo*-acting beta-1,4-xylanase cloned from *Saccharophagus degradans* 2-40 into *Escherichia coli* BL21(DE3). *N. Biotechnol.* **2009**, *26*, 157–164.

57. Vincent, F.; Molin, D.; Weiner, R.; Bourne, Y.; Herissat, B. Structure of a polyisoprenoid binding domain from *Saccharophagus degradans* implicated in plant cell wall breakdown. *FEBS Lett.* **2010**, *584*, 1577–1584.

58. Fraiberg, M.; Borovok, I.; Bayer, E.; Weiner, R.; Lamed, R. Cadherin domains in the polysaccharide-degradng marine bacterium *Saccharophagus degradans* 2-40 are carbohydrate-binding modules. *J. Bacteriol.* **2011**, *193*, 283–285.

59. Conant, G.; Wolfe, K. Turning a hobby into a job: How duplicated genes find new functions. *Nat. Rev. Genet.* **2008**, *9*, 938–950.

60. Longmire, A.G.; Hutcheson, S. University of Maryland, College Park, MD, USA. 2005, unpublished work.