Biological Adhesives

A.M. Smith J.A. Callow (Eds.)

Biological Adhesives

With 58 Figures, 3 in Color, and 15 Tables

Springer

Dr. Andrew M. Smith
Associate Professor
Department of Biology
Ithaca College
953 Danby Road
Ithaca, NY 14850
USA

Dr. James A. Callow
Mason Professor of Botany
School of Biosciences
The University of Birmingham
Birmingham B15 2TT
UK

Front cover illustration: Gecko foot, photo taken by Mark Moffett
Back cover illustration: Algal holdfast, photo taken by Luke Miller

Library of Congress Control Number: 2006921554

ISBN-10 3-540-31048-7 Springer Berlin Heidelberg New York
ISBN-13 978-3-540-31048-8 Springer Berlin Heidelberg New York

Springer is a part of Springer Science+Business Media

springer.com

Printed in Germany

Editor: Dr. Sabine Schreck, Heidelberg, Germany
Desk editor: Dr. Andrea Schlitzberger, Heidelberg, Germany
Cover design: Design & Production GmbH, Heidelberg, Germany
Production and typesetting: SPI Publisher Services

Printed on acid-free paper 39/3100 5 4 3 2 1 0

Preface

Many organisms, ranging from microscopic bacteria and fungi through much larger marine algae and terrestrial vertebrates, use adhesive polymers. The performance of these adhesives can be remarkable, and their diversity suggests the potential for developing glues that are markedly different from those currently available. Biological adhesives can vary widely in structure and capabilities, and often perform in ways that differ greatly from conventional man-made adhesives. Many of these adhesives form strong attachments under water–a feat that is greatly complicated by the difficulty in displacing water from the adhesive interface and the problems in dealing with water's ability to weaken many forms of chemical bonds. Another interesting characteristic is that many of these glues are highly deformable and likely to be biocompatible. Finally, unlike most artificial adhesives, they can be remarkably complex, involving a wide range of interactions and components with different functions. While this complexity can be daunting for researchers, it allows a great deal of flexibility in applications.

Another important characteristic that deserves further mention is the non-specific nature of these adhesives. They can often adhere to many different types of surfaces. This book focuses exclusively on such non-specific glues, rather than adhesive molecules more generally. There is a large and important literature on adhesion involving recognition of specific ligands. This information is best reviewed in other books, and will not be covered here. This will allow a more in-depth look at the properties of non-specific glues and their potential for practical applications.

Despite their great potential, many of these adhesives are only recently becoming better understood. There are a number of reasons for this. One of the most important may be the small size of many organisms that adhere strongly. A large number of microbes, fungal and algal spores, microscopic invertebrates, and invertebrate larvae use adhesive polymers to stick to whatever surface they encounter. It is difficult to isolate these glues and perform biochemical analyses, though, due to the miniscule amounts involved. Furthermore, adhesives in general must often be highly insoluble in order to function for extended periods. In many cases they can only be studied effectively during the time immediately after secretion and before they solidify. Another, more subtle issue has been the interdisciplinary nature of the field. To study biological adhesives, it is helpful to know sufficient organismal

diversity to identify potentially interesting glues and model organisms, sufficient biochemistry to analyze the glue, and sufficient engineering and mechanics to interpret the structure and properties of the glue. Because it is difficult to have expertise in these different areas, historically much published information on glues has tended towards untested speculation. A related issue is that published research on glues ranges from basic to applied, biochemical to mechanical, and organismal to molecular. As a result, it tends to be scattered in disparate literatures often housed in different libraries and even different schools. This book should bring these areas together and bridge them so that future scientists can more easily draw on the wealth of information that is now becoming available.

Despite the difficulties, the study of biological adhesives seems to have reached a critical mass. Work is advancing rapidly as modern methods open up new avenues for studying glues, and as we build upon a firmer understanding of the basic biochemical and mechanical principles involved in adhesion.

Recent work has made substantial progress in understanding the glues of microscopic organisms. Polysaccharides and glycoproteins seem to play a large role in the adhesion of bacteria, fungi, and larval and microscopic algae. Often, these organisms secrete a substantial quantity of extracellular polymeric substances to adhere. The size of these organisms, though, has posed unique challenges to the identification and characterization of specific glue polymers. These polymers also may share similarity with cell wall polymers and materials that have other functions. Those challenges are being overcome, and the potential now exists for detailed understanding of these glues. Callow and Callow (Chap. 4) and Epstein and Nicholson (Chap. 3) review work on the adhesives involved in algal and fungal settlement on substrates. This work demonstrates the utility of monoclonal antibodies and molecular genetic techniques in identifying potential adhesive polymers and providing evidence of their function. The use of atomic force microscopy, especially modified versions that do not lead to evaporation of wet adhesives, has also provided insight into the mechanics and curing of these glues (Callow and Callow, Chap. 4; Chiovitti et al. Chap. 5).

Landini et al. (Chap. 2) review work on bacterial adhesives demonstrating the power of molecular genetics to study not only the nature of the adhesive, but the factors controlling its production and secretion. It is worth remembering that these adhesives are complex secretions where the manner, timing and circumstances under which they are laid down can markedly affect the nature of the end product. Furthermore, as Chiovitti et al. (Chap. 5) note in their review of diatom adhesion, there will be considerable variation in structure and properties of the adhesive according to function.

The glues of barnacles and mussels are better understood than most. Collecting sufficient material for biochemistry is not as problematic, and researchers have made great strides in dealing with the natural insolubility of the glues and the difficulty in using standard biochemical techniques

with polymers that are often designed to stick to other polymers and exposed surfaces. Kamino (Chap. 8) reviews what is known of barnacle adhesion. This work highlights the multifunctionality of the glues; there are a number of components, each with an apparently unique function. The structural information available for these proteins is becoming sufficient to begin unraveling structure/function questions. Sagert et al. (Chap. 7) review mussel adhesion, where work has probably advanced furthest. Perhaps it also demonstrates best the complexity and unusual biochemistry of biological glues. There is a variety of interesting mechanisms that may contribute to the cohesive and adhesive interactions of the glue. This diversity contributes to the glue's ability to work so effectively on many different surfaces under a variety of conditions. It is also worth noting that many of these mechanisms depend on post-translational modification of proteins; this is just one more complicating factor in the analysis of biological glues.

There are many other glues that are just recently becoming better understood. Potin and Leblanc (Chap. 6), describe progress in understanding the adhesion of brown algae. The apparent importance of halogen chemistry is an interesting variant of crosslinking chemistry. Echinoderm adhesion has been a subject of interest for many years, and recent work has begun the process of unraveling the physico-chemical characteristics of adhesion (Flammang, Chap. 10). Echinoderms are of particular interest because adhesives are used in different groups for such diverse functions as larval settlement, adult motility, stable attachment, and defense.

Many of these adhesives have high water content, with some being hydrogels. These gels can depend on tangling and crosslinking of large polysaccharides or heavily glycosylated proteins, or they can be primarily proteinaceous. Smith (Chap. 9) outlines the factors affecting the mechanics of such gels and reviews variation and similarity in the structure of adhesive gels from gastropods. With gastropod glues, the ability to identify key proteins and manipulate the components of the glue provides a powerful experimental tool. Graham et al. (Chap. 11) review recent work on the structure and practical potential of protein hydrogels used by certain frogs as defensive secretions. This is another intriguing glue with great potential for guiding biomimetic applications.

In contrast to the adhesive systems described in other chapters, geckos do not secrete a glue, they utilize adhesion between solid surfaces. The mechanics of the arrays of fine hair-like setae on their feet give rise to striking functional properties. Analysis of the mechanical principles has yielded a number of important insights, as reviewed by Autumn (Chap. 12).

The potential for development of practical adhesives using a biomimetic approach exists for all these adhesives. Haag (Chap. 1) reviews the effectiveness of various adhesives extracted or derived from bacterial secretions while Lee et al. (Chap. 13) review work on adhesives derived from research on mussel glues. These chapters highlight the power of a biomimetic approach, with

Lee et al. in particular showing the potential for making synthetic glues when the physico-chemical properties of the natural adhesive are established well enough to provide guidance.

As research in this area progresses, similarities that suggest common underlying principles may emerge, while simultaneously more and more variation in the details becomes clear. It is our hope that bringing together all this information will stimulate further research by providing ideas for experimental approaches and by providing insight into both the common features of glues and the myriad ways that adhesion can be achieved. Ultimately, detailed characterization of the structure of the adhesive polymers and their properties may provide the raw material to inspire a new generation of adhesives for use in medicine, biotechnology and a range of other applications.

February 2006 *Andrew M. Smith and James A. Callow*

List of Contributors

K. Autumn
Department of Biology, Lewis & Clark College, Portland, OR 97219-7899, USA

J.A. Callow
School of Biosciences, The University of Birmingham, Birmingham B15 2TT, UK

M.E. Callow
School of Biosciences, The University of Birmingham, Birmingham B15 2TT, UK

A. Chiovitti
School of Botany, University of Melbourne, Victoria 3010, Australia

J.L. Dalsin
Department of Biomedical Engineering, Northwestern University, 2145 N Sheridan Road, Evanston, IL 60208, USA

C. Dorel-Flamant
Unité de Microbiologie et Génétique UMR CNRS 5122 composante INSA, 10, rue Dubois, 69 622 Villeurbanne Cedex, France

T.M. Dugdale
School of Botany, University of Melbourne, Victoria 3010, Australia

L. Epstein
Department of Plant Pathology, University of California, Davis, CA 95616-8680, USA

P. Flammang
Universite de Mons-Hainaut, Laboratoire de Biologie marine, Academie Universitaire Wallonie-Bruxelles, Mons, Belgium

V. Glattauer
CSIRO Molecular & Health Technologies, Ian Wark Laboratory, Bag 10, Clayton South, Victoria 3169, Australia

L.D. Graham
CSIRO Molecular & Health Technologies, Sydney Laboratory, P.O. Box 184, North Ryde, NSW 1670, Australia

A.P. Haag
Specialty Biopolymers Corporation, 524 Professional Drive, Ste. E, Bozeman, MT 59718, USA

G. Jubelin
Unité de Microbiologie et Génétique UMR CNRS 5122 composante INSA, 10, rue Dubois, 69 622 Villeurbanne Cedex, France

K. Kamino
Marine Biotechnology Institute, 3-75-1 Heita, Kamaishi, Iwate 026-0001, Japan

P. Landini
Dipartimento di Scienze Biomolecolari e Biotecnologie, Università di Milano, Via Celoria 22, 20133 Milano, Italy

C. Leblanc
UMR 7139 CNRS/UPMC, Marine Plants and Biomolecules & LIA-DIAMS, Station Biologique, BP 74, F-29682 Roscoff Cedex, France

B.P. Lee
Department of Biomedical Engineering, Northwestern University, 2145 N Sheridan Road, Evanston, IL 60208, USA

P.B. Messersmith
Department of Biomedical Engineering, Northwestern University, 2145 N Sheridan Road, Evanston, IL 60208, USA

R.L. Nicholson
Department of Botany and Plant Pathology, Purdue University, W. Lafayette, IN 47907, USA

Y.Y. Peng
CSIRO Molecular & Health Technologies, Ian Wark Laboratory, Bag 10, Clayton South, Victoria 3169, Australia

P. Potin
UMR 7139 CNRS/UPMC, Marine Plants and Biomolecules & LIA-DIAMS, Station Biologique, BP 74, F-29682 Roscoff Cedex, France

J.A.M. Ramshaw
CSIRO Molecular & Health Technologies, Ian Wark Laboratory, Bag 10, Clayton South, Victoria 3169, Australia

J. Sagert
Department of Molecular, Cell & Developmental Biology, University of California, Santa Barbara, CA 93106, USA

A.M. Smith
Department of Biology, Ithaca College, Ithaca, NY 14850, USA

C. Sun
Department of Molecular, Cell & Developmental Biology, University of California, Santa Barbara, CA 93106, USA

M.J. Tyler
School of Earth & Environmental Sciences, University of Adelaide, South Australia 5005, Australia

P.R. Vaughan
CSIRO Molecular & Health Technologies, Ian Wark Laboratory, Bag 10, Clayton South, Victoria 3169, Australia

J.H. Waite
Department of Molecular, Cell & Developmental Biology, University of California, Santa Barbara, CA 93106, USA

J.A. Werkmeister
CSIRO Molecular & Health Technologies, Ian Wark Laboratory, Bag 10, Clayton South, Victoria 3169, Australia

R. Wetherbee
School of Botany, University of Melbourne, Victoria 3010, Australia

Contents

1 Mechanical Properties of Bacterial Exopolymeric Adhesives and their Commercial Development

ANTHONY P. HAAG

1.1 Introduction

In industry and society today, there is a need for products which provide environmentally friendly features, such as 1) reduced usage of toxic components and VOCs (volatile organic compounds), 2) reduced dependence on depleting petrochemical resources, 3) safer production processes, and 4) less environmental impact of products after their use (Gross and Kalra 2002). The motivation for exploring biopolymers for use in adhesive applications is to exploit their unique properties to address these needs. Additional incentive for development of biological materials is economics. As the world economy grows and more demand is placed on depleting petrochemical feedstocks, products derived from renewable, biological resources are becoming more cost competitive.

Biopolymers are utilized by all organisms for numerous functions in varied environments and thus have evolved into a diversity of chemical compositions. Biopolymers offer the material scientist a source of unique compositions which are unavailable by synthetic means with which to search for novel properties, such as adhesive strength. The discovery of new compositions and optimization of their production is also becoming easier with new, sophisticated biological methods. In the future, biologically derived materials will increasingly fill needs in commercially important applications.

In biofilms, a widespread form of bacterial existence, cells form highly hydrated cohesive masses that adhere to surfaces. Extracellular polymeric substances are largely responsible for the structure and properties of biofilms, including the adhesion of cells to surfaces and to each other. In addition to facilitating the growth of the bacterial colony, biofilms can be problematic and result in, for example, corrosion and fouling in industrial systems, and resistance to antibiotics on medical devices (Costerton and Stewart 2001; Flemming and Wingender 2001). The mechanical properties of biofilms have been described as those of a viscoelastic fluid in which the biofilm behaves elastically at low shear stress and viscously at higher shear stress (Klapper et al. 2002). Thus, extracellular polymeric substances impart substantial mechanical properties to biofilms in their natural environment.

Specialty Biopolymers Corporation,
524 Professional Drive, Ste. E, Bozeman, MT, USA 59718

Biological Adhesives
(ed. by A.M. Smith and J.A. Callow)

Extracellular polymeric substances in biofilms are composed of polysaccharides, proteins, and nucleic acids (Flemming and Wingender 2001). Protein components are generally attributed to the initial adhesion processes (formation of a conditioning layer) and polysaccharides, in addition to various other biological functions, are largely responsible for the subsequent adhesive interactions and provide cohesive strength. This review will focus primarily on exopolysaccharides which are often the predominant extracellular component in bacterial biofilms and most readily developed for commercialization.

Bacterial exopolysaccharides have found use in other applications such as thickening and gelling agents for aqueous mixtures and have undergone a significant amount of commercial development. Examples are xanthan gum (from *Xanthomonas campestris*), gellan (*Sphingomonas paucimobilis*), curdlan (*Agrobacterium radiobacter*), dextran (*Leuconostoc mesenteroides*), and levan (*Bacillus polymyxa*). Scleroglucan and pullulan, although of fungal origin, show similar behavior. Sodium alginate is produced commercially from algae but structurally similar materials are also produced by the bacteria *Azotobacter vinelandii* and *Pseudomonas aeruginosa*. Structures of some of the polysaccharides described in this chapter are shown in Fig. 1.1. General structural features of polysaccharides which are useful in adhesives include high molecular weight and polar functional groups. Mechanical properties generally improve with molecular weight (Lazaridou et al. 2003) and native bacterial polysaccharides often possess molecular weights greater than 10^6 Da. Lower molecular weights can be produced, if desired, by control of culture conditions or depolymerization of the native product followed by fractionation. The polar and hydrogen-bonding functional groups of polysaccharides, such as ethers, hydroxyls, and carboxylates, impart good adhesion to high energy surfaces such as wood and metal and also strong interchain interaction for cohesive strength. The hydroxyl and carboxylate groups of polysaccharides also offer potential sites for synthetic derivatization and crosslinking which can be utilized to modify the adhesive properties. Tertiary structures, such as helices, are formed by some bacterial polysaccharides and account for their notable mechanical properties. Other distinguishing properties of bacterial exopolysaccharides are hydrophilicity, ability to form hydrogels (structure dependent), biodegradability, production from renewable resources, and generally low toxicity.

Xanthan gum, gellan, and dextran are produced commercially at volumes greater than 1 million lb/year. Production of most bacterial exopolysaccharides is accomplished by batch-wise fermentation in stirred tanks equipped with efficient agitation for the resulting highly viscous mixtures. Glucose and sucrose are commonly used as the carbon and energy sources. Raw materials account for a significant fraction of the product cost so substitution of cheaper carbon sources, such as agricultural waste products is advantageous. In the production of xanthan gum, which is the most successful industrial biopolymer produced by fermentation, yields of 30 g/L and productivities of 0.7 g/l-h are reported (Born et al. 2002). After the fermentation operation, the mixture is sterilized, and the biopolymer is isolated by precipitation into

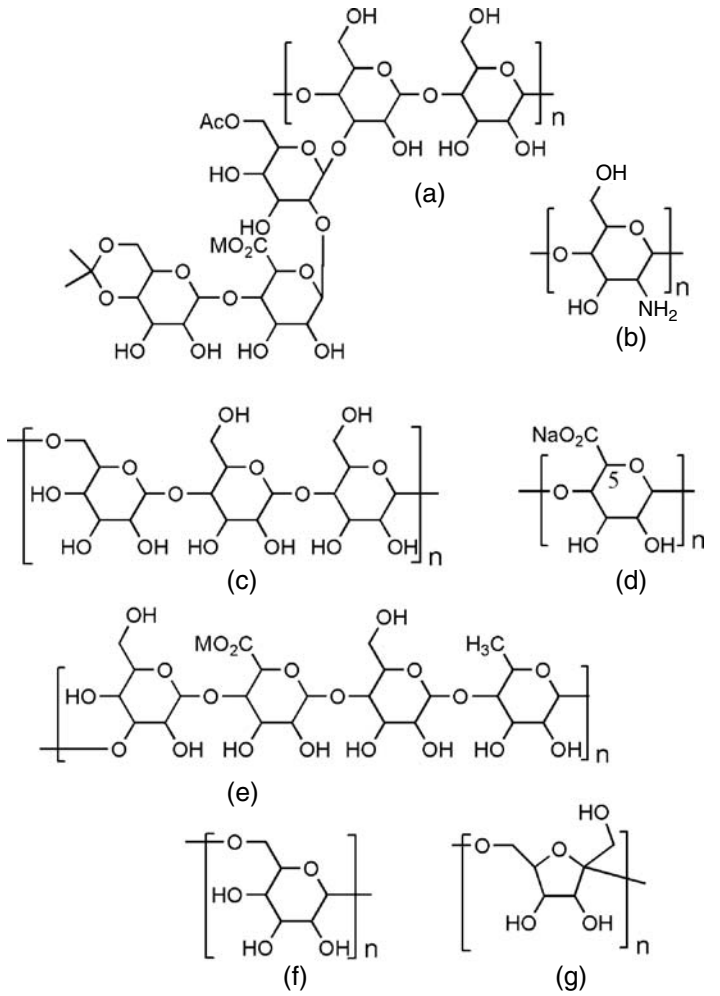

Fig. 1.1. Structures of some of the polysaccharides referred to in this chapter. (a) Xanthan gum (M=metal), (b) chitosan, (c) pullulan, (d) sodium alginate (note mixture of epimers at C-5), (e) gellan, (f) dextran, and (g) levan

isopropanol and then dried. Cell biomass is not separated for industrial grades of xanthan; higher purity grades are obtained by removal of the biomass by filtration or absorption. Xanthan gum is currently produced globally at a volume of 40 million lb/year (Sutherland 2002) and sells for $4.5/lb (Chemical Market Reporter 2005).

Their performance in nature, unique chemical compositions, economics of production from renewable resources, and biodegradability make bacterial exopolysaccharides attractive candidates for development as adhesive materials. This review summarizes recent efforts to identify adhesive materials

derived from bacterial exopolysaccharides and evaluates their mechanical properties for adhesive applications. Comparisons include descriptions of adhesive composition and preparation, testing procedures, and testing results. In addition to the bacterial products, selected structurally related polysaccharides from plant and animal sources are briefly compared.

1.2 Adhesive Development

1.2.1 Mechanical Testing of Adhesive Bonds

The search for practical adhesives relies on evaluation of mechanical properties. Fundamental studies of the physical and mechanical properties of bacterial exopolysaccharides on the nanoscopic, molecular level in their native, aqueous environments have been accomplished with atomic force microscopy (AFM) (Dufrene 2002; Kawakami et al. 2004). In addition to the fact that mechanical properties measured on the molecular level are not directly relatable to adhesive strengths observed on the macroscopic level, the test conditions are also different than those in most industrial adhesive applications in which the adhesive is in a dry state and subject to varying levels of humidity and temperature. Thus, evaluation of new adhesives should employ conditions simulating expected uses. Standard methods have been developed by professional testing organizations such as the American Society for Testing and Materials (ASTM) for specific applications. The three forces to which adhesives are subjected are shear, peel, and cleavage (Fig. 1.2) (Pocius 1991). Shear strengths can be measured under tension or compression. Peel and cleavage tests measure similar forces, but the former is used with deformable substrates. Preliminary testing of adhesives for metal bonding applications generally employ single-lap shear test specimens with 3.2 cm^2 bond area (ASTM D 1002) or butt-joined bar specimens (ASTM D 2095). Preliminary testing of wood adhesives generally employ two methods: three-ply plywood specimens assembled from 1.6 mm-thick veneers with a 6.4 cm^2 bond area are sheared under tension (ASTM D 906); and two 19 mm-thick wood blocks with 19.4 cm^2 bond area are sheared under compression (ASTM D 905). Results are expressed in terms of stress or pressure, that is, the force required to break the bond divided by the bond area. Analysis of the bond failure mode is also commonly reported and indicates whether the bond failed at the adhesive-substrate interface (adhesive failure), within the adhesive itself (cohesive failure), or in the substrate (substrate failure).

Testing methods for bonding biological materials, such as tissue and skin, have been designed for these unique substrates, for example, ASTM F 2255-03 (“Test Method for Strength Properties of Tissue Adhesives in Lap-Shear by Tension Loading”) (McDermott et al. 2004). In vivo testing of adhesive films on human skin has also been developed (Repka and McGinity 2001).

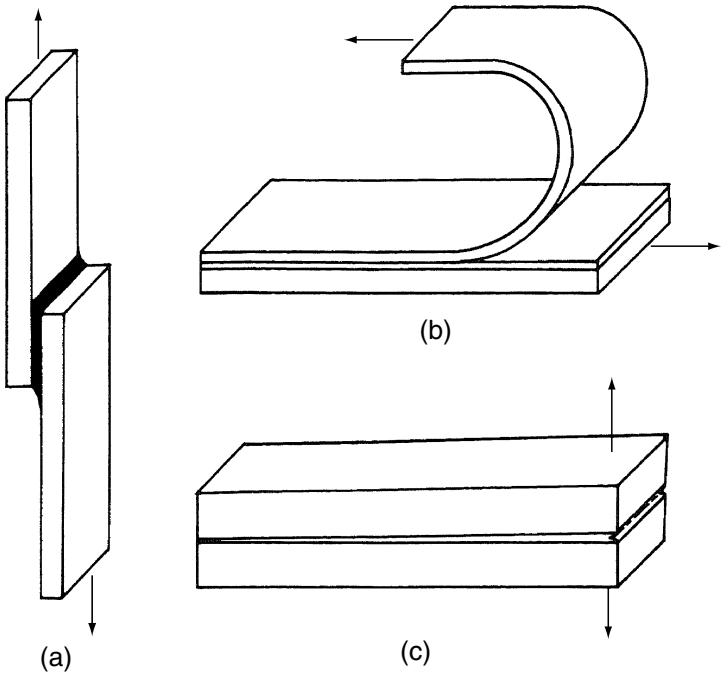

Fig. 1.2. Illustrations of forces to which adhesive bonds are subjected: (a) a standard lap shear specimen where the black area shows the adhesive. The adherends are usually 25 mm wide and the lap area is 312.5 mm2. The *arrows* show the direction of the normal application of load; (b) a peel test where the loading configuration, shown by the *arrows*, is for a 180 ° peel test; (c) a double cantilever beam test specimen used in the evaluation of the resistance to crack propagation of an adhesive. The normal application of load is shown by the *arrows*. This load is applied by a tensile testing machine or other mechanical means of holding open the end of the specimen (Pocius 1991). Reprinted with permission of John Wiley & Sons, Inc., copyright ©1991

1.2.2 Bacterial Exopolymer Adhesives

1.2.2.1 Polysaccharide Adhesive Viscous Exopolymer

The marine bacterium *Alteromonas colwelliana* LST produces an exopolysaccharide which it uses to adhere strongly to surfaces under severe conditions in its natural environment. It also synthesizes tyrosinase, dihydroxyphenylalanine (DOPA), and related quinones which participate in water-resistant adhesive production in higher organisms (Yamada et al. 2000). Therefore, the mechanical properties of "polysaccharide adhesive viscous exopolysaccharide" (PAVE) isolated from several strains of this bacterium were evaluated on commercially relevant substrates (Labare et al. 1989). An efficient process for production of PAVE from one strain of bacteria was demonstrated (5–11 g/l). Depending on culture conditions, the carbohydrate/protein ratio in PAVE as

determined by classical colorimetric methods ranged from 1 to 14. The adhesives used for screening of shear strengths were prepared from mucoid exopolymers of nine bacterial strains scraped from agar surfaces prepared with various media. No further chemical characterization was described. The PAVE material was formulated for use in adhesive testing in three ways: as isolated in concentrated form, or diluted with acetone 10 or 100 times.

Adhesive testing utilized lap-shear specimens. Substrates composed of glass, aluminum, brass, stainless, hot-rolled steel, wood (unspecified species or conditioning), and acrylic plastic were bonded with 2.5 cm^2 overlap area using adhesives from nine bacterial strains. Two shearing test configurations were used–the first employed application of a torque force (applied 5 cm from midjoint) and the second method applied a tensile force. The authors state that the two methods reflect strengths of adhesion and cohesion, respectively.

Testing results using the torque method on glass substrates showed strengths of 1.0–4.4 kg/cm torque for the nine adhesives. Cure times of seven days gave higher strengths than one day. PAVE prepared from a tyrosinase and DOPA producing strain (TAC-1) gave higher results (100%) than the corresponding DOPA-negative variant strain (TAC-2). Adhesive strengths varied for some PAVE preparations when formulated in water or acetone. In the tensile shear tests, adhesive strengths of PAVE prepared from nine bacterial strains were evaluated on seven substrate materials. Each adhesive showed unique surface dependent shear strengths (up to 0.5 MPa, Tables 1.1 and 1.2). In this series, PAVE prepared from the DOPA producing strain again showed

Table 1.1. Relative shear strength of crude PAVE preparation from marine bacteria on metal surfaces[a]. Numbers in parentheses are standard deviations (Labare et al. 1989). Reprinted with permission of the authors and Brill NV

Strain[b]	Surfaces Aluminum		Brass		Stainless steel		Hot-rolled steel	
MPF-1	8.7	(5.4)	17.5	(7.3)	8.2	(6.8)	7.6	(5.1)
RAM-1	7.6	(0.8)	8.4	(3.9)	8.0	(4.8)	17.3	(5.7)
KAN-1	10.0	(0.9)	10.8	(6.8)	7.6	(3.8)	22.1	(0.6)
TAC-1	0.0	(0.0)	0.5	(0.2)	2.9	(2.5)	14.2	(6.7)
TAC-2	6.8	(1.4)	12.9	(6.6)	4.1	(0.9)	11.5	(3.1)
PAS-1	12.8	(3.1)	25.6	(17.0)	12.2	(6.7)	5.3	(4.2)
MPL-1	12.5	(3.4)	22.5	(0.2)	7.1	(3.1)	6.0	(1.0)
DLS-T	4.3	(2.1)	9.5	(3.6)	2.9	(0.2)	31.2	(9.5)
DPR-8	18.1	(16.7)	ND[c]	-	15.6	(9.8)	17.1	(6.8)

[a] Crude PAVE was extracted with acetone, stored for seven days at 25 °C, spread on a 2.5 × 2.5 cm area, clamped, cured for seven days, and tested for shear force (kg) using Instron

[b] Bacteria were grown in brain-heart infusion + 2.3% NaCl medium for 24 h except MPL-1, TAC-2, and DPR-8, which were grown in BHI, BHI + extra salts, and marine agar + casein hydrolysate, respectively

[c] ND=no data

Table 1.2. Relative shear strength of crude PAVE preparations from marine bacteria on non-metal surfaces[a]

Strain[b]	Surfaces Acrylic		Glass		Wood	
MPF-1	3.7	(1.7)	6.7	(0.3)	25.2	(1.5)
RAM-1	6.5	(0.5)	6.5	(0.5)	13.8	(6.7)
KAN-1	9.6	(1.3)	6.1	(2.7)	17.5	(2.6)
TAC-1	3.0	(0.7)	6.1	(2.5)	9.8	(1.9)
TAC-2	2.9	(1.5)	3.1	(1.9)	3.7	(0.6)
PAS-1	10.8	(7.9)	9.9	(2.8)	36.4	(8.3)
MPL-1	4.8	(0.6)	4.0	(0.6)	13.0	(1.4)
DLS-T	3.2	(1.8)	11.5	(1.0)	16.8	(0.8)
DPR-8	10.0	(8.6)	11.6	(0.9)	19.4	(2.3)

[a] Crude PAVE was extracted with acetone, stored for seven days at 25 °C, spread on a 2.5 × 2.5 cm area, clamped, cured for seven days, and tested for shear force (kg) using Instron
[b] Bacteria were grown in brain-heart infusion + 2.3% NaCl medium for 24 h except MPL-1, TAC-2, and DPR-8, which were grown in BHI, BHI + extra salts, and marine agar + casein hydrolysate, respectively

a 100% higher shear strength on glass compared to the DOPA-negative strain, but the effect was not consistent for other substrate materials. Overall, the PAVE products showed encouraging but relatively low adhesive strengths. The highest shear strengths were on wood, hot-rolled steel, and glass. Although no commercial adhesive benchmarks were tested for comparison during this study, adhesive shear strengths of 1–50 MPa (3.2 cm^2 bond area) are common (Pocius 1991) in similar tests. Further analysis of the value of these adhesives should include testing of the effects of moisture and application rate, and determination of adhesive composition and polymer structure.

The DOPA-based crosslinking concept continues to be a productive approach to improve mechanical properties, in particular moisture resistance of adhesives derived from the amine-functionalized polysaccharide, chitosan (see Sect. 1.2.3.3).

1.2.2.2 Biomass Fermentation Residue

Economical viability of a bioconversion process for ethanol production from cellulosic biomass requires the development of other valuable coproducts. The fermentation residues have been found to be potentially useful as wood adhesives. Ruminal cellulolytic bacteria ferment cellulose, hemicelluloses, and pectin to ethanol. During the degradation process, adherence to the cellulose substrate is mediated by adhesins. The fermentation residue (FR) consists of incompletely fermented biomass, adherent bacterial cells, and associated exopolymer adhesins.

Initial studies focused on fermentation of purified cellulose with the anaerobic bacteria *Ruminococcus albus* and *Ruminococcus flavefaciens* (Weimer et al. 2003). After incubation, the fermentation residue was separated from the liquid phase and lyophilized. Characterization of the FR showed 14–23% alkali-soluble carbohydrate and 0.4–5% protein. Monosaccharide composition was similar in the exopolysaccharide from three bacterial strains (approximately 0.7 glucose/0.02 galactose/0.08 mannose/0.18 xylose [mol fraction]). Adhesives were formulated by mixing lyophilized FR with water (33 wt%) and commercial phenol-formaldehyde (PF) adhesive. The method used to test adhesive strength employed aspen veneers which were conditioned at 30% relative humidity (RH) and 27 °C, bonded with adhesive, assembled into three-ply panels, pressed at 1.1 MPa/180 °C/10 min, and later cut to testing size. Half of the plywood specimens were tested for shear strength at 30% RH and half were tested after a vacuum-pressure water soak treatment. Percentage of wood failure was also measured.

Shear strengths of plywood panels bonded with FR alone were half that of PF (1.8 and 3.4 MPa, respectively) under dry conditions and one-fifth that of PF under wet conditions. However, substitution of up to 73% of PF with FR produced shear strengths and % wood failure nearly equal to that of PF alone under dry conditions. The FR/PF blends were also nearly as strong as PF alone under the wet conditions. The FR from *R. albus* gave superior shear strengths to two strains of *R. flavefaciens* although the fermentations with the latter were less complete (greater residue dry weight).

Subsequent work explored residues derived from fermentation of a more practical biomass substrate, lucerne (*Medicago sativa* L., alfalfa) fiber, with *Ruminococcus flavefaciens* and *Clostridium thermocellum* (Weimer et al. 2005). The preparation of FRs and adhesives, and the adhesive testing method were the same as in the earlier work. The composition of the FR from both bacteria (alkaline extract) was 4.2–2.8% protein, 7.7–7.8% carbohydrate, 0.8–1.2% uronic acid, with the remainder being fiber, lignin, and ash.

FR was blended with PF and used to bond plywood panels. Shear strengths of specimens bonded with 30% FR/PF were 2.5–2.8 MPa under dry conditions, equivalent to specimens bonded with PF containing 30% of a common extender additive (30% RH) (Fig. 1.3). The shear strength of 30% FR/PF was significantly higher than that of a 30% blend of unfermented fiber and PF, and also twofold higher than a 45% blend of FR/PF. Wood failure of specimens bonded with the 30% FR/PF blend was nearly 100% under dry conditions. Under wet conditions, shear strengths of specimens bonded with 30% FR/PF were 1.7–2.0 MPa, significantly higher than specimens bonded with either 30% commercial extender/PF or 30% unfermented fiber/PF. Wood failure of specimens bonded with the 30% FR/PF blend was nearly 100% under wet conditions. Note that the adhesives prepared from the two bacterial sources gave similar results. The composition of the FRs showed considerable differences but the alkali-extractable carbohydrate content was the same. The monosaccharide compositions were also very similar suggesting that observed shear strengths correlate with the carbohydrate content and structure.

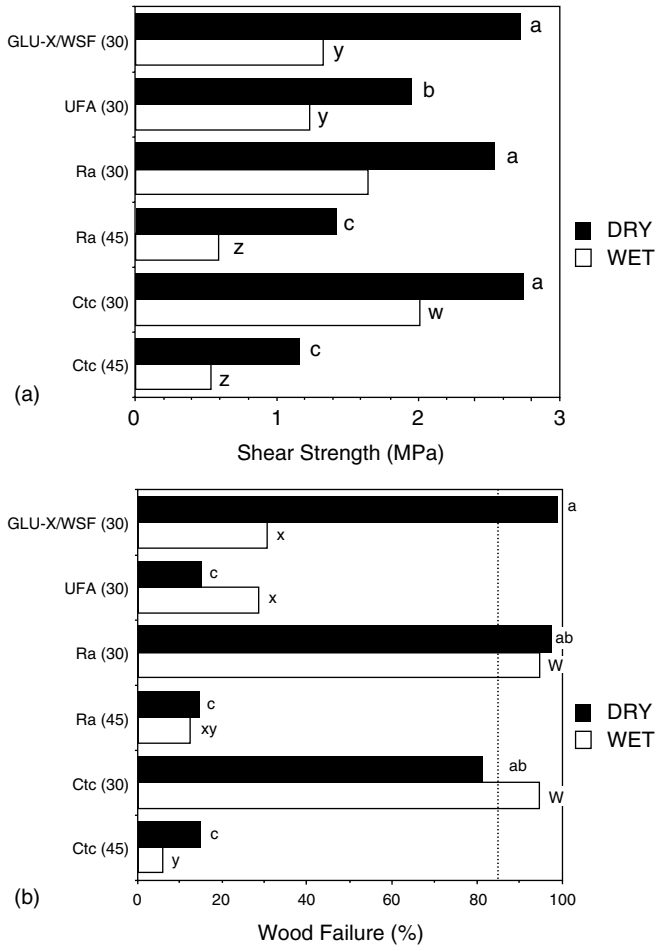

Fig. 1.3. (a) Shear strength. (b) Wood failure data, both for plywood adhesives formulated with PF resin and the indicated co-adhesive. GLU-X/WSF=GLU-X and walnut shell flour extenders, UFA=unfermented lucerne fiber, Ra=residue from *R. albus* 7 lucerne fiber fermentation, Ctc=residue from C. *thermocellum* ATCC 27405 lucerne fiber fermentation. Values in *parentheses on the vertical axis labels* indicate percentages (dry weight basis) of additive, with the remainder being PF (see reference for formulations). In the case of GLU-X/WSF, each was added at 15% by dry weight for a total of 30%. Different a, b, c letters indicate significant differences ($P<0.01$) in shear strength or wood failure among the panels under dry conditions. Different w, x, y, z letters indicate significant differences ($P<0.01$) in shear strength or wood failure among the panels under wet (VPS) conditions. The vertical line in B indicates the industry standard for wood failure (85%). Reprinted with permission of the authors (Weimer et al. 2005)

The sources of adherence during degradation by ruminal cellulolytic bacteria are cellulose-binding domains of cellulosome components, pilin-like proteins in fimbrae, and exopolymer materials synthesized following irreversible adsorption to cellulose fibers. That is, the intrinsic affinity of

adhesins for cellulose is likely to contribute, in part, to adhesive strength compared to other bio-based adhesives for bonding wood. Overall, the proposed use of FR as an adhesive alters the bioconversion strategy: production of FR with an estimated value similar to ethanol obviates the need for high substrate conversion and requisite costly pretreatments.

1.2.2.3 Montana Biotech Adhesive

A water-based bacterial exopolymer adhesive was developed as an alternative to VOC-containing adhesives (Combie et al. 2004). Testing of adhesive strength was performed on aluminum and coated aluminum substrates. Bacterial isolates from the culture collection of Montana Biotech SE, Inc. (Rock Hill, SC, USA) were screened for production of adhesive exopolymers. The adhesive material which gave the best adhesive testing results in the initial screening was then selected for the following studies.

MB (Montana Biotech) adhesive was produced by an unidentified organism and isolated from the liquid medium, after separation of cells, by precipitation with alcohol. Its molecular weight was 10^6 Da and it was composed of >95% carbohydrate (dry weight basis). Other structural information was not disclosed. More hydrophobic derivatives were also prepared in order to increase moisture resistance. Partially methylated or acetylated derivatives of dried MB adhesive were prepared by reaction with methyl iodide or acetic anhydride, respectively. Water solubility decreased as degree of substitution increased.

Flatwise tensile strengths were determined using two butt-joined cylindrical aluminum specimens (alloy 2024, 1.56 cm^2 cross-sectional area). After pressing for 1 h at ambient temperature and RH, the specimens were set at 35 °C and 23% RH for seven days. Tensile strength was measured at ambient temperature with a manually operated Mark 10 BGI force measuring instrument. Shear strengths were determined using aluminum 2024 coupons which were either anodized with sulfuric acid and hot water sealed; anodized and epoxy coated; or anodized, epoxy undercoated and topcoated with "chemical agent resistant coating", CARC. The coupons were bonded in a single lap shear configuration with 3.2 cm^2 bond area, pressed for 1 h, and after setting, tested on an Instron universal testing machine.

Tensile strengths of approximately 7 MPa were reported for MB adhesive-bonded Al 2024. The adhesive was resistant to organic solvents (48 h soak) and showed superior strength over other, commercially available polysaccharides. The MB adhesive showed a low moisture resistance. After initially setting for seven days at 35 °C, the bonded specimens were soaked in water for 2 h at ambient temperature and retained only 5% of their initial shear strength. A partially acetylated derivative when set under the same conditions showed the same shear strength as the nonderivatized MB adhesive, but after a 2 h water soak retained 37% of its initial strength. Another test for

moisture resistance employed exposure to 75% RH conditions for seven days after an initial set at 35 °C for seven days at low RH. Under these conditions, MB adhesive retained 10% of its initial tensile strength while the partially acetylated derivative retained 66%. The shear strength of MB adhesive-bonded aluminum and aluminum coated with epoxy and CARC ranged from 4.5–5.6 MPa.

In addition to the initial studies with aluminum substrates and organic coatings, MB adhesive was also evaluated as a wood adhesive (Haag et al. 2004). Many wood adhesives have VOC and/or toxic components such as formaldehyde which is now classified as a carcinogen. In addition most adhesives are based on depleting petrochemical resources. The water-based MB adhesive was well suited to applications with porous substrates such as wood which allow for facile absorption and evaporation of the water solvent.

The exopolysaccharide used in these studies was prepared as described above but a lower molecular weight product was obtained, approximately 40 KDa as measured by size exclusion chromatography. A partially acetylated derivative, MB-OAc, was also prepared from this material by reaction with acetic anhydride. The adhesive strengths were determined using a standard method for initial evaluations of wood adhesives, ASTM D 905, "Standard Test Method for Strength Properties of Adhesive Bonds in Shear by Compression Loading". In this method, two wood boards (1.9 cm thick × 6.4 cm wide × 30.5 cm long) are bonded, pressed overnight, allowed to set for one week at 53% RH and 22 °C, cut to size for testing (19.4 cm^2 bond area), and then shear strength was measured. Experiments to determine moisture resistance utilized setting conditions of 53% (moderate) RH for one week followed by an additional week at either 53% RH or 94% (high) RH.

The shear strength of MB adhesive was initially evaluated on substrates of two wood species (sugar maple, a hardwood, and Douglas fir, a softwood) and two wood composites (particleboard and medium density fiberboard). It was also compared to a commercial polyvinylacetate (PVA)-based wood adhesive. MB adhesive showed a shear strength of 12.5 MPa on maple, 73% of that observed with PVA adhesive. Substrates bonded with MB adhesive showed cohesive bond failure in contrast to the PVA adhesive which failed largely in the adhesive mode. Shear strengths observed with the other, softer substrates were 12.2, 2.1, and 2.5 MPa for Douglas fir, medium density fiberboard, and particleboard, respectively, which were not significantly different than that of the PVA adhesive. These all showed high percentages of substrate failure, that is, the wood or composite failed before the adhesive bond.

The setting rate (shear strength vs time) of maple substrates bonded with MB adhesive was measured at 53% RH at 22 °C. Half of the maximum shear strength was obtained within an 8-h period while full strength was reached in 48 h.

To determine the uniqueness of the MB adhesive polysaccharide composition, the shear strengths of maple substrates bonded with aqueous mixtures of some commercially available polysaccharides were measured at 53% RH. Sodium carboxymethylcellulose, a synthetic cellulose derivative, and pullulan

showed shear strengths that were not significantly different than MB adhesive. Dextrin, a starch derivative, and sodium alginate were both significantly lower.

Long term performance, or durability, of adhesive joints is affected by environmental conditions such as humidity and temperature. Adhesives that resist these effects have more applications and are of greater value. Evaluation of the moisture resistance of MB adhesive-bonded maple substrates was performed after setting for one week at 53% RH and then an additional week at either 53% RH or 94% RH. Specimens subjected to the high humidity conditions gave shear strengths that were only 1% (0.2 MPa) of specimens subjected to the moderate humidity conditions (Table 1.3). In contrast, when subjected to the high humidity conditions, the PVA-based benchmark adhesive retained 72% of its moderate humidity strength. However, the partially acetylated MB-OAc adhesive showed a substantial improvement in moisture resistance under the high RH conditions and retained 35% of its shear strength (5 MPa) obtained at moderate humidity. The shear strength of MB-OAc at moderate RH was not significantly different than that of the native, nonderivatized MB adhesive. These results demonstrated that moisture resistance can be significantly improved through manipulation of polysaccharide structure.

1.2.2.4 Specialty Biopolymers Adhesive

Another bacterial exopolymer-based adhesive has been developed by Specialty Biopolymers Corporation (Bozeman, MT, USA) and has shown improved properties over the previously described MB adhesive for bonding wood substrates. The Specialty Biopolymers (SB) adhesive is composed of a polysaccharide of undisclosed structure with a molecular weight of 500 KDa

Table 1.3. Influence of relative humidity on shear strength of MB adhesive and its partially acetylated derivative. Reprinted from Int. J. Adhesion and Adhesives (Haag et al. 2004) with permission from Elsevier

Adhesive	Relative humidity (%)	Mean strength (MPa)	Percentage of strength achieved at 53% RH	Number of replicates	CV (%)	p-Value at 95% confidence level[a]
PVA	53	24.0		8	19	0.0004
PVA	53→94	17.2	72	10	6	
MB	53	16.3		8	23	
MB	53→94	0.2	1	3	12	
MB-OAc	53	14.4		8	24	0.29
MB-OAc	53→94	5.1	35	9	34	

[a] Probability based on two-tail student t-test that shear strength of candidate adhesive is significantly different from that of MB adhesive

and formulated as a 33% solution in water. A partially acetylated derivative of SB adhesive (SB-OAc) was also prepared by reaction with acetic anhydride and formulated in aqueous ethanol. Testing of shear strength of bonded wood blocks under compression was performed as described above in Sect. 1.2.2.3 using ASTM method D 905.

The shear strength of SB adhesive was first evaluated in a survey with two wood species and two composites at 53% RH and 22 °C. Maple substrates bonded with SB adhesive had a shear strength of 14.6 MPa which was 79% of that of a benchmark, interior grade PVA-based adhesive. The SB adhesive bond failed in the cohesive mode and also showed 18% wood failure. The corresponding PVA bond failed in the adhesive mode with 17% wood failure. Douglas fir bonded with SB adhesive showed a shear strength of 12.5 MPa which was 93% of the shear strength of the PVA benchmark adhesive. The SB adhesive-bonded specimens showed 79% wood failure. SB adhesive-bonded particleboard and medium density fiberboard showed 88 and 55% of the shear strength of the PVA adhesive, respectively. Shear strengths of the bonded composites were improved by initially performing a light surface sanding and resulted in no significant difference from that obtained with the PVA adhesive.

The set rate (shear strength vs time) was determined at two humidities, 53 and 23% RH, and at 22 °C. Shear strength reached a maximum in 48 h and half of maximum in 8 h at both RHs. However, the maximum shear strength at 23% RH was 50% higher than at 53% RH (20 and 14 MPa, respectively).

Moisture resistance of SB adhesive was studied in comparison to the PVA adhesive. After a one-week set at 53% (moderate) RH, bonded maple specimens were exposed to 94% (high) RH for increased times. The PVA adhesive retained 69% of its shear strength after four weeks exposure to high RH as compared to its initial shear strength at moderate RH (Fig. 1.4). SB adhesive retained only 9% of its shear strength after three weeks exposure to high RH as compared to its initial shear strength measured at moderate RH. The loss in bond strength of SB adhesive-bonded specimens upon exposure to high RH could not be avoided by performing the initial set at a lower RH (23%). These results are consistent with a reversible setting mechanism in which the shear strength is dependent on the water concentration in the adhesive bond. During the setting process, removal of water and increase in strength is driven by evaporation and absorption into the wood until equilibrium is established. Subsequently, moisture content in the bond and shear strength changes with relative humidity. The rate of that change depends on the mass and dimensions of the wood substrates which also absorb moisture and affect its mass transfer to the bond.

Based on the evidence that the bond strength of SB adhesive is limited by its interaction with water at high RH, the SB adhesive was chemically modified to impart a more hydrophobic character. Partial acetylation afforded a water-insoluble product, SB-OAc, which could be dissolved in aqueous ethanol for application. SB-OAc-bonded maple specimens which were set

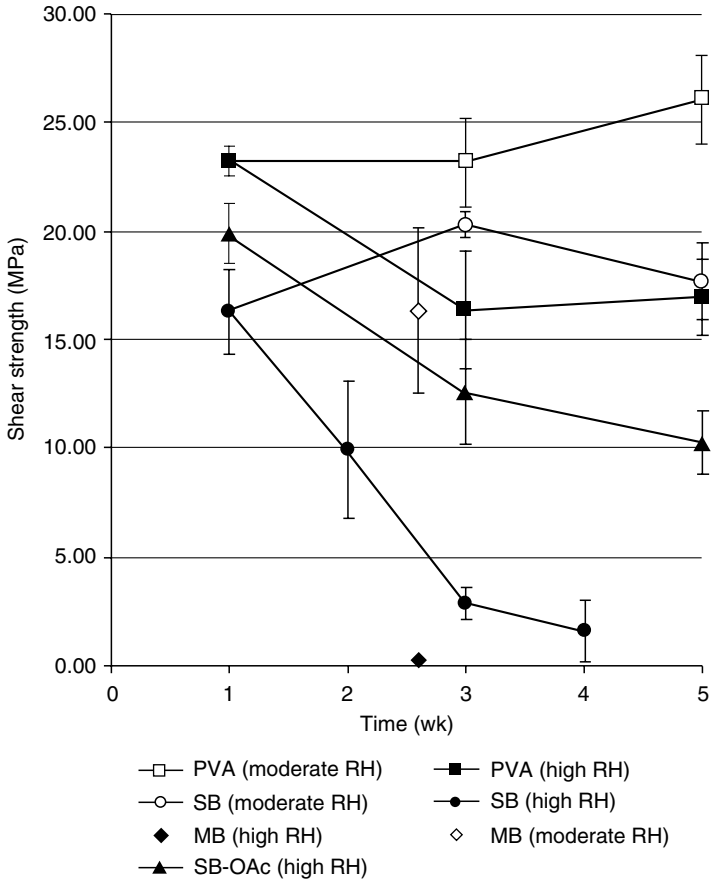

Fig. 1.4. The effect on shear strength of prolonged exposure of bonded maple substrates to high or moderate RH after an initial one-week set period at moderate RH. *Error bars* represent ±1 standard deviation. To facilitate comparison, MB data from Haag et al. (2004) is included. Reprinted from Int J Adhesion Adhesives (Haag et al. 2006), with permission from Elsevier

for one week at 53% RH showed a slightly higher shear strength than SB adhesive and 86% of that obtained with the PVA adhesive. At high RH, the SB-OAc bonded specimens retained 51% (10 MPa) of their initial strength at 53% RH (Fig. 1.4). Thus, these results support earlier observations in which partial acetylation effectively improved moisture resistance. Both of the acetylated derivatives of SB and MB adhesives, with the same degrees of acetylation (58–57%, respectively), showed substantial improvement in moisture resistance. However SB-OAc showed a significantly higher shear strength than MB-OAc at both moderate and high RH. This indicates that the extent to which acetylation affects moisture resistance is dependent on polysaccharide structure.

Overall, the performance of SB adhesive demonstrates that bacterially derived adhesives can possess shear strengths and setting rates at low to moderate humidities which may be suitable for construction of indoor wooden furniture and cabinetry.

1.2.3 Related Polysaccharide-based Adhesives

Starch is a polysaccharide that is widely used in paper-bonding applications. Cellulose derivatives (e.g., nitro- and ethylcellulose) have also been used for many years in organic solvent-based adhesives. It is useful to compare the mechanical properties of bacterial adhesives with those of polysaccharides from other sources in order to discern relationships between structure and performance.

1.2.3.1 Pullulan and Chitosan

Pullulan is a commercially available, high molecular weight, water soluble exopolysaccharide produced by the fungus *Aureobasidium pullulans*. It has been developed for various applications which exploit its mechanical and rheological properties, for example, films, fibers, coatings, and aqueous thickeners (Leathers 2003). Chitosan is an amine-functional polysaccharide soluble in dilute acid which is produced by deacetylation of chitin, a major component in crustacean exoskeletons, and is also found in the cell walls of the fungus *Mucor rouxii*. It is available commercially in large quantities from the former source.

Pullulan and chitosan were very briefly examined as wood adhesives by Mayer et al. (1990). Pullulan was prepared as a 50 wt% solution in water and chitosan was prepared as a 20 wt% solution in 2% aqueous acetic acid. Pine wood blocks were bonded with each adhesive, pressed, and allowed to dry. Shear strengths were 5 and 3 MPa for pullulan and chitosan, respectively, although setting conditions (time, temperature and relative humidity) were unspecified. The shear strength of a commercial PVA-based adhesive measured 5 MPa under the same conditions. These preliminary results indicate that pullulan, chitosan, and possibly other polysaccharides possess mechanical properties that may allow them to be effective adhesives although additional information such as moisture resistance is needed. These results are consistent with observations with other polysaccharides described in Sect. 1.2.2.3.

1.2.3.2 Konjac Glucomannan

Konjac glucomannan (KGM) is a polysaccharide extracted from the tuber of *Amorphohallus Konjac*, K Koch. It is a copolymer of glucose and mannose with β-1,4-linkages. An acetyl group is attached to 1 per 19 sugar units. KGM forms a gel in the presence of alkaline coagulant which causes deacetylation.