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# Characterization of an adhesive molecule from *Bacillus megaterium* ADE-0-1

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ABSTRACT

An adhesive exopolysaccharide (EPS), from a biofilm forming marine strain ADE-0-1, identified as *Bacillus megaterium* using conventional microbiological test and 16S rDNA analysis, contained 75% carbohydrate, 17% uronic acid and 0.00125% pyruvate on dry weight basis as per colorimetric determinations and found anionic in nature by ion exchange chromatography. Paper chromatographic and HPLC analysis of EPS hydrolysate indicated presence of arabinose, glucose, mannose, galacturonic acid and glucuronic acid. Its molecular weight was $0.5 \times 10^6$ Da, by gel permeation chromatography. FT-IR spectroscopic analysis of EPS revealed presence of hydroxyl and carboxyl groups particularly. EPS exhibited an adhesive nature and could glue wood, metals and acrylic plastic. Using this EPS adhesive (10% w/v), maximum lap shear strength observed was 6.12 MPa at pH 7 and 50 °C (curing temperature) for wood to wood specimen as compared to 6.54 MPa obtained with fevicol (48 to 50% w/v).



## 1. Introduction

Currently large quantity of petro-chemical based adhesives is used globally. In 2007, the total world demand for adhesives and sealants was 12 billion Kg, of which natural adhesives (non-microbial origin) were only 0.6 billion Kg (Petrie, 2009). There are significant environmental issues with such adhesives like usage of toxic chemicals, contain volatile organic compounds (toluene, methyl-ethyl ketone, tri-chloro ethane) and poor biodegradability.

Hence in recent past, there is a motivation for exploring biopolymers for adhesive applications (Cha et al., 2009; Combie, Haag, Suci, & Geesey, 2004a; Combie, Steel, & Sweitzer, 2004b; Haag, 2006; Haag, Maier, Combie, & Geesey, 2004; Weimer, Conner, & Lorenz, 2003). In biofilms, exopolymeric substances are involved in the adhesion of cells to surfaces and to each other and often have been reported as exopolysaccharide (EPS) in many of the biofilms. Due to the existence of tremendous structural and chemical diversity in microbial EPS, there is an immense scope for discovery of new and unique bioadhesive molecules.

Bioadhesives produced by barnacales and mussels have been found with excellent adhesive property, particularly for difficult job of underwater adhesion. However, scale-up of such system has proven to be cumbersome (Combie et al., 2004b). Since microbial systems are more suitable for scale-up, EPS (or their chemically modified form) from microorganisms of biofilm origin could be a potential source of ecofriendly/biocompatible adhesive.

Different biopolymers from bacteria are commercially available and have long been exploited as emulsifiers, thickeners, stabilizers and gelling agents (Nwodo, Green, & Okoh, 2012). However none of these polymers is in commercial use as an adhesive. There are only a few reports where microbial EPS have been evaluated as adhesives include: polysaccharide adhesive viscous exopolymer (PAVE), Montana Biotech (MB) adhesive and speciality biopolymer (SB) adhesive (Haag, 2006). In the following study, we have characterized a potential adhesive, having unique chemical compositions and it is the first report of this kind from *Bacillus megaterium*.

## 2. Materials and methods

### 2.1. Identification of strain ADE-0-1

The strain was primarily characterized by morphological, cultural and biochemical tests and identified following Bergey's Manual of Systematic Bacteriology (Sneath, 1986). The identification was also confirmed by routine partial 16S rDNA genes analysis.

*Abbreviations:* EPS, exopolysaccharide; PAVE, polysaccharide adhesive viscous exopolymer; MB, Montana Biotech; SB, speciality biopolymer; TFA, tri-fluroacetic acid; UTM, universal testing machine; MPa, megapascal; ASTM, American Society for testing and Materials; Al, aluminium; Smc, sunmika; ND, no data.

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### 2.2. Production and recovery of an adhesive

The isolate was grown by inoculating 10% (v/v) of O.D600 nm = 0.4, into 50 ml medium (pH 7.2 ± 0.2) containing ($g l^{-1}$): Sucrose, 40; $KNO_3$, 1; $K_2HPO_4$, 0.5; $MgSO_4 \cdot 7H_2O$, 0.2; $CaCl_2 \cdot 2H_2O$, 0.1; NaCl, 0.1; and yeast extract 0.1 in 250 ml Erlenmeyer flask, on a rotary shaker (180 rpm) at 30 ± 1 °C for 72 h. Biomass from the culture broth was removed by centrifugation (at 8000 × g for 30 min), the EPS was precipitated from supernatant according to Ashtaputre and Shah (1995), using 3 volumes of chilled acetone, re-dissolved in distilled water, precipitation step was repeated, precipitates were dried in oven (50 °C) for 2 h, ground to powder form and used for adhesion studies.

### 2.3. Chemical characterization

The precipitated EPS solution was dialysed against distilled water for 18 h; freeze dried and was used for chemical analysis. Total carbohydrate content of EPS was estimated by the phenol sulphuric acid method of Dubois, Gilles, Hamilton, Rebers, and Smith (1956). Total uronic acid content was estimated by the method of Bitter and Muir (1962). The presence of protein in EPS was analysed by using the method of Bradford (1976). Pyruvyl and acetyl content in EPS were analysed by the method of Slonekar and Orentas (1962) and Hestrin (1949), respectively.

To know the monosaccharide composition, EPS was hydrolyzed according to Aquino, Grativol, and Mourao (2011) with slight modifications using 2 M tri-fluroacetic acid (TFA) at 100 °C for 2 h and TFA was evaporated *in vacuo*, freeze dried and used for analysis. The monosaccharide composition of hydrolyzed polymer was initially determined by paper chromatography using *n*-butanol/pyridine/water (6:4:3, by vol.) as mobile phase and silver nitrate for visualization of spot. It was also confirmed by HPLC analysis using Waters HPLC model 2410. Authentic monosaccharide standards were also run to determine the identity of the peaks. The ionic nature of EPS was determined by measuring its efficiency of binding to anion (Dowex 1) and cation (Dowex 50) exchange resins as described by Ashtaputre and Shah (1995).

### 2.4. Fourier transform-infrared (FT-IR) spectroscopy

The FT-IR specrum of EPS was obtained as its KBr pellet in the region of 4000–250 $cm^{-1}$, at a resolution of 1 $cm^{-1}$ by using Perkin-Elmer FT-IR spectrometer (Germany).

### 2.5. Molecular weight determination

The molecular weight of the EPS was determined by gel permeation chromatography, performed on sepharose-4B column. Samples (250 μg) were loaded onto the column and eluted with 10 mM phosphate buffer (pH 7.2 ± 0.05) at a flow rate of 0.5 ml $min^{-1}$. Fractions (2 ml) were collected and subjected to total sugar estimation by the phenol sulphuric acid method. Dextrans of different molecular weight ranging from $9 \times 10^3$ to $2 \times 10^6$ Da were used as standards.

### 2.6. Adhesion testing procedure

#### 2.6.1. Lap shear strength

Lap shear strength determines the adhesive's ability to resist forces that attempt to cause internal structure of the adhesive to slide against it. For determination of lap shear strength of the test adhesive, a pair of specimen of wood, iron, steel, aluminium, tin, sunmika and acrylic was joined with the adhesive either individually or in its combinations. An adhesive solution of 10% (w/v in distilled water) having pH 7 was used in this study. Specimen dimensions were 30 × 2.5 × 0.3 cm for wood (*Shorea robusta*) and 30 × 2.5 × 0.1 (metals)/0.2 cm (plastics). Surfaces of adherends were roughed using sand paper, wiped with ethanol and the adhesive (13.33 mg/$cm^2$) was applied to 2.5 × 3 cm corner area of *one of the adherend* and the second adherend overlapped that area (lapped joint). Both adherends were clamped tightly together for 1 h and were pressed under 2.5 kg weights for further 18 h to allow the adhesive to set up. Finally bonded adherends were cured for 7 days at 30 ± 1 °C and 50 ± 5% relative humidity.

After curing, the single lapped joint adherends were pulled apart using universal testing machine (UTM); a lap shear strength measuring instrument [model 223/445, Alfred J. Amsler and Co. Schaffhouse (Switzerland)]. The lap shear strength was measured in kg by UTM and it was further converted into megapascal (MPa) by using a conversion factor 10.197 Kg/$cm^2$ = 1 MPa. Above studies were adaptations of the standard method ASTM D905-03 (ASTM International D905-03, 2003) for wood, sunmika and acrylic specimens and ASTM D1002-10 (ASTM International D1002-10, 2010) for metal specimens. Fevicol, a commercial wood cum multipurpose adhesive, was used as a positive control. Each individual determination of lap shear strength was carried out in triplicate and each complete experiment was carried out twice and average was recorded.

#### 2.6.2. Effect of environmental factors

*2.6.2.1. Temperature.* To know the effect of temperature on lap shear strength, the joined adherends were prepared as described above and cured at different temperatures (4 °C and 50 °C) along with control (curing temperature −30 °C) at 50 ± 5% relative humidity for seven days. After curing at specific temperature, the adherends were pulled apart using UTM and adhesive strength was measured and compared with control, where adhesive-pH and curing temperature of specimens was 7 and 30 °C, respectively. Each individual determination of lap shear strength was carried out in triplicate and each complete experiment was carried out twice and average was recorded.

*2.6.2.2. pH.* To know the effect of pH on adhesive strength, the adhesive (10% w/v) was prepared in distilled water and pH was adjusted to 4 and 8. Such adhesives (having different pH) were applied on different specimens for joining as described previously and cured at 30 °C at 50 ± 5% relative humidity for seven days. After curing, the adherends were pulled apart using UTM, and adhesive strength was measured and compared with control, where adhesive-pH and curing temperature of specimens was 7 and 30 °C, respectively. Each individual determination of lap shear strength was carried out in triplicate and each complete experiment was carried out twice and average was recorded.

## 3. Results and discussion

### 3.1. Identification of the strain ADE-0-1

The morphological, cultural and biochemical characteristics studied of the strain were found similar to those, typical of *B. megaterium* as described by Sneath (1986). The strain was amylase and protease producer too. Thus the strain was identified as *B. megaterium* ADE-0-1. On 16S rDNA analysis, based on nucleotide homology and phylogenetic analysis strain was characterized as *B. megaterium* (GenBank accession no. KF280264.1). *B. megaterium* strain HDDMG02 having Gene Bank accession number EU723818 showed 99% homology with *B. megaterium* ADE-0-1. Henceforth the adhesive from this organism is termed as *BM adhesive*.

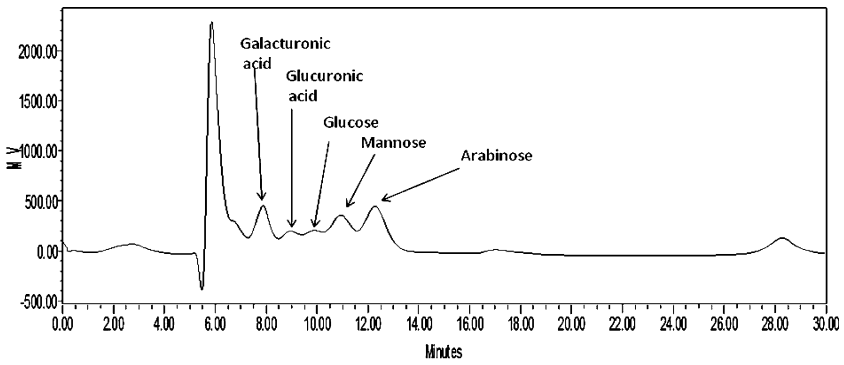


**Fig. 1.** HPLC analysis of the exopolysaccharide (EPS) hydrolysate. EPS was hydrolysed using 2 M TFA for 2 h at 100 °C. HPLC analysis was performed using a Waters 2410 HPLC system. Water containing EDTA (40 mg/l) and $CaCl_2$ (15 mg/l) was used as a mobile phase at a flow rate of 0.4 ml/min and the peaks were detected using RI detector. 30 μl of either authentic standards (20 mg/ml) or hydrolysate (20 mg/ml) was injected.

### 3.2. Chemical analysis of BM adhesive

Based on colorimetric analysis, EPS contained 75% total carbohydrates, 17% uronic acid and 0.00125% pyruvate. Amino sugars, proteins and acetyl content were not detected. Comparison of retention times of the peaks, obtained in HPLC analysis (Fig. 1), with those of the authentic compounds, revealed presence of galacturonic acid, glucuronic acid, glucose, mannose and arabinose in the EPS hydrolysate.

Compared to BM adhesive, MB EPS adhesive contained 95% carbohydrates, 1 to 3% protein and only 1% uronic acid (Combie et al., 2004a). While EPS of *B. megaterium* (Strain 98TH11316) isolated from sea water, capable of biofilm formation on glass surface, was composed of glucose, mannose, galactose and glucuronic acid (Kwon et al., 2002). Capsular material, chemically poly-γ-glutamate (PGA), has been reported as a biodegradable 'bionylon' from *B. megaterium* WH320 (Shimizu, Nakamura, & Ashiuchi, 2007).

Hence, It can be concluded that BM adhesive is chemically unique because of its very high *i.e.* 17% (w/w) uronic acid content which could have a significant role to play in adhesion process per se during both biofilm formation and adhesive applications. Binding of 91.6% of EPS to Dowex 50 resin indicated the anionic nature of the EPS which is understandable looking to its high uronic acid content. It has been reported that uronic acids and other moieties including pyruvates and acyl groups provide an overall negative surface charge to polymer, thereby imparting binding properties with positively charged surfaces (Bhaskar & Bhosle, 2005; Balestrino, Ghigo, Charbonnel, Haagensen, & Forestier, 2008; Nichols & Nichols, 2008).

### 3.3. FTIR analysis

Polysaccharides contain a significant number of hydroxyl groups. An intense broad stretching peak at 3445 $cm^{-1}$ was typical of hydroxyl groups (Fig. 2). Two weak C—H stretching peaks at 2929 and 2857 $cm^{-1}$ corresponded to methyl as well as methylene groups. While strong absorption at 1638 $cm^{-1}$ and very weak absorption at 1350 $cm^{-1}$ indicated C=O stretch of the $COO^-$ group. A broad but weak peak at 1159 $cm^{-1}$ suggested presence of ketal (pyruvate) (Silverstein & Webster, 2006). Thus described gross chemical composition and results of FTIR analysis were found in accordance with each other.

The polar hydroxyl groups in the adhesive promote adhesion to polar surfaces like aluminium (Al) but they are also hydrophilic and lead to low water resistance. The presence of carboxyl groups revealed may serve as a binding site for divalent cations such as aluminium and iron.

### 3.4. Molecular weight determination

Molecular weight of BM adhesive was $0.5 \times 10^6$ Da. The molecular weight of exopolysaccharides isolated from eight biofilm forming strains from glass surface ranged from 0.38 to $25.19 \times 10^4$ Da and EPS of *B. megaterium* strain (98TH11316) had

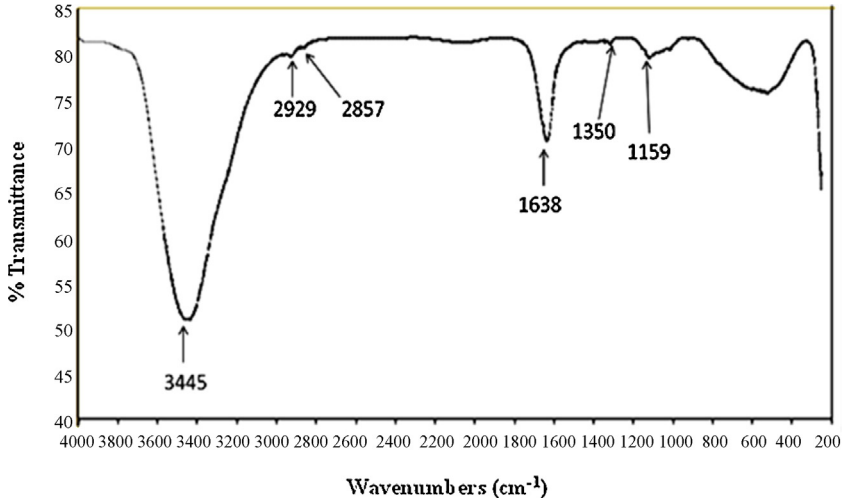


**Fig. 2.** FT-IR spectrum of the exopolysaccharide. The pellets for analysis were obtained by grinding a mixture of 1.2 mg of purified EPS with 150 mg of KBr powder followed by pressing the mixture into a mold. The FT-IR specrum was recorded in the region of 4000–250 $cm^{-1}$, at a resolution of 1 $cm^{-1}$ by using Perkin-Elmer FT-IR spectrometer.

**Table 1**
Lap shear strengths (MPa) of different specimens bonded with adhesive.

| Samples | Wood | Smc | Acrylic | Iron | Steel | Al | Tin |
|---|---|---|---|---|---|---|---|
| Wood | 5.46 ± 0.04 | 1.99* | 0.79 ± 0.05 | 0.26 ± 0.01 | 0.79 ± 0.02 | 0.53 ± 0.01 | 0.66 ± 0.02 |
| Smc | | 0.93 ± 0.04 | 0.39 ± 0.05 | 1.06 ± 0.04 | 0.66 ± 0.02 | 0.79 ± 0.05 | 0.39 ± 0.02 |
| Acrylic | | | 0.93 ± 0.07 | 0.39 ± 0.05 | 1.06 ± 0.06 | 0.26 ± 0.04 | 0.26 ± 0.01 |
| Iron | | | | 1.59 ± 0.01 | 0.53 ± 0.03 | 2.79 ± 0.08 | 1.33 ± 0.01 |
| Steel | | | | | 2.26 ± 0.12 | 0.53 ± 0.03 | 0.39 ± 0.02 |
| Al | | | | | | 1.59 ± 0.02 | 1.06 ± 0.04 |
| Tin | | | | | | | 0.79 ± 0.02 |

An adhesive solution of 10% (w/v) and having pH 7 was used to join 28 unique combinations of specimens. Adhesive (13.33 mg/cm$^2$) was applied to 2.5 × 3 cm corner area of *one of the adherend* and the second adherend overlapped that area. Both adherends were clamped tightly together for 1 h and were pressed under 2.5 kg weights for further 18 h to allow the adhesive to set up. Finally bonded adherends were cured for 7 days at 30 ± 1 °C and 50 ± 5% relative humidity and lap shear strength (MPa) was determined using universal testing machine at ambient temperature. Each individual determination was carried out in triplicate and each complete experiment was carried out twice.
* In this case substrate failure (in Smc) had occurred.

molecular weight 2.1 × 10$^4$ Da (Kwon et al., 2002). While molecular weight of MB adhesive and Speciality Biopolymer (SB) adhesive were 4 × 10$^4$ Da (Haag et al., 2004) and 5 × 10$^5$ Da (Haag, 2006), respectively. High molecular weight is one of the desirable structural features useful in adhesive as mechanical properties generally improve with high molecular weight (Lazaridou, Biliaderis, & Kontogiorgos, 2003).

### 3.5. Adhesion studies

Out of 28 combinations of specimens analysed, maximum lap shear strength observed was 5.46 ± 0.04 MPa in the case of wood–wood specimen (Table 1). Among the metals, lap shear strength was found in the order of Al–Iron (2.79 ± 0.08 MPa) > steel–steel (2.26 ± 0.12 MPa) > Iron–Iron (1.59 ± 0.01 MPa) and Al–Al (1.59 ± 0.02 MPa) > Tin–Iron (1.33 ± 0.01 MPa). With the exception of steel, it was observed that when specimen was prepared using two metal surfaces, higher adhesive ability was observed than that of specimen prepared using one metal surface and one non-metal surface. These indicate that ionic interaction is more involved in the case of metal–metal bonding. Ionic interaction has also been reported to be involved in bacterial adhesion to metals (Sheng, Ting, & Pehkonen, 2008).

The porous nature of the wood surface, facilitate good penetration of the adhesive into the wood, enabling very intimate wood adhesive interaction which results into high bond strength (Frihart, 2005). Further high wood surface roughness due to its cellular structure and multi-polymer (cellulose, hemicelluolose and lignin) composition is an additional factor which enhances good wood bonding compared to other surfaces (Frihart, 2005). Also the polar and H-bonding functional groups of polysaccharides such as ethers, hydroxyls and carboxylates impart good adhesion to high energy surfaces such as wood and metals and also strong inter-chain interaction for cohesive strength (Haag, 2006).

#### 3.5.1. Effect of environmental factors on adhesive ability

Since, characterization of adhesive performance under a range of environmental conditions is significant in determining the potential applications and market value of an adhesive. Hence, effect of different environmental parameters like curing temperature and pH on adhesive ability was studied. As compared to pH 4, adhesive ability was higher at pH 8 and this increase was in the range of 30 to 75% depending upon the combinations of surfaces in the specimens (Table 2). This is probably because ionization of carboxyl groups (present in uronic acids) increases under alkaline condition and consequently increased ionic interaction involved in adhesion makes bond stronger. Whereas at pH 4 (in acidic condition) ionization of carboxylic groups decreases and consequently decreased ionic interaction involved in adhesion makes bond weaker. It has been reported that an increase in ionic strength in the solution enhanced the bacterial adhesion to surface due to electrostatic attraction force (Bowen, Lovitt, & Wright, 2000; Sheng et al., 2008). However non-ionic interactions are also involved in adhesion process so even at pH 4 considerable adhesive strength of 3.3 ± 0.4 MPa (for wood specimen) was observed (Table 2).

Increase in curing temperature (from 4 °C to 50 °C) of specimens improved the lap shear strength in the range of 9.6 to 25%, respectively, as compared to control (Table 2). BM adhesive showed a low resistance at 4 °C (curing temperature) and the integrity of adhesive bond was compromized by rehydration due to high moisture prevailing around the bonded area as water solvents of adhesive could not evaporate completely at such a low curing temperature. Thus, weaken the adhesive strength and as a result adherends got separated during curing. Like BM adhesive, sensitivity of adhesive bond to moisture has been also reported in the case of Montana Biotech (MB) as well as speciality biopolymers (SB) adhesive and less hydrophilic, chemically modified derivatives of both showed improvement in shear strength during submersion in water and

**Table 2**
Effect of environmental factors on lap shear strength (MPa) of different specimens bonded with adhesive.

| Samples | pH 4[a] | pH 8[a] | % Change as compared to pH 4 | Control[c] | 4 °C[b] | 50 °C[b] | % Change as compared to control[c] |
|---|---|---|---|---|---|---|---|
| Wood–wood | 3.3 ± 0.4 | 5.79 ± 0.03 | 75.45 | 5.46 ± 0.04 | ND | 6.12 ± 0.03 | 12.08 |
| Iron–Al | 2.13 ± 0.15 | 2.92 ± 0.03 | 37.09 | 2.79 ± 0.08 | ND | 3.06 ± 0.04 | 9.67 |
| Smc–wood | 1.46 ± 0.10 | 1.99* | * | 1.99* | ND | 1.99* | * |
| Steel–steel | 1.86 ± 0.07 | 2.53 ± 0.02 | 36.02 | 2.26 ± 0.12 | ND | 2.66 ± 0.04 | 17.7 |
| Iron–iron | 1.33 ± 0.07 | 1.73 ± 0.01 | 30.07 | 1.59 ± 0.01 | ND | 1.99 ± 0.06 | 25.15 |
| Al–Al | 1.2 ± 0.11 | 1.73 ± 0.01 | 44.16 | 1.59 ± 0.02 | ND | 1.99 ± 0.05 | 25.15 |

An adhesive solution of 10% (w/v) was used.
[a] Adhesive solution used had pH 4 or 8.
[b] Bonded specimens were prepared and cured at 4 or 50 °C for 7 days, along with control (curing temperature 30 °C). The lap shear strength (MPa) was determined using universal testing machine at ambient temperature. Each individual determination was carried out in triplicate and each complete experiment was carried out twice. ND- no data as adherends got separated during curing at 4 °C.
[c] Adhesive solution having pH 7 was used and specimens were cured at 30 °C.
* In this case substrate failure (in Smc) had occurred.

**Table 3**
Comparison of experimental conditions during evaluation of adhesive strength of BM adhesive with other EPS-adhesives with wood.

| Adhesives | Bond area ($cm^2$) | % Solids used (w/v) | Adhesive Application to one adherend/both adherends | Effective concentration of polymer ($mg/cm^2$) | Lap shear Strength (MPa) |
|---|---|---|---|---|---|
| MB | 19.4 | 31 | On both the adherends | 13–20 | 12.5 |
| SB | 19.4 | 33 | On both the adherands | 13–20 | 14.6 |
| BM (present work) | 7.5 | 10 | On single adherand | 13.33 | 6.12 |

under humid atmospheric conditions (Haag, 2006; Haag et al., 2004).

In contrast, high temperature of curing lead to decrease in water solvent at the bonded region and this in turn would increase bond strength under such conditions. Maximum lap shear strength observed was $6.12 \pm 0.03$ MPa with the wood to wood specimen when cured at 50 °C.

Previously evaluation of "polysaccharide adhesive viscous exopolysaccharide" (PAVE) material, isolated from nine marine strains of *Alteromonas colwelliana* LST, for seven different substrates using lap shear specimens and 6.25 $cm^2$ bond area showed maximum shear strength upto 0.57 MPa (with Aluminium—0.28 MPa, stainless steel—0.24 MPa, Acrylic—0.17 MPa and wood—0.57 MPa) (Labare, Guthrie, & Weiner, 1989).

Evaluation of two well investigated bacterially derived MB and SB adhesives, composed of exopolysaccharides, primarily as a wood adhesive using maple wood specimen, have shown 12.5 and 14.6 MPa shear strength (bond area 19.4 $cm^2$) respectively (Haag, 2006; Haag et al., 2004). Further MB adhesive showed shear strength of 5.6 to 6.2 MPa with bare aluminium (3.22 $cm^2$ bond areas) and epoxy glass. Of the six samples tested, diallyl phthalate and acrylonitrile butadiene styrene were the only plastic that exhibited shear strength above 0.69 MPa. Against this 0.93 MPa shear strength observed with sunmika and acrylic plastic glued with the *BM adhesive (present work)* reported here.

As shown in Table 3, while comparing shear strengths of MB/SB adhesives with *BM adhesive (present work)*, it should be noted that in above cases adhesive preparations used had 31% (w/v) solids (MB adhesive) and 33% (w/v) solids (SB adhesive) against only 10% (w/v) solids used in the case of *BM adhesive*. Further application of MB and SB adhesive was in the range of 13–20 $mg/cm^2$ of *both the adherends* compared to 13.33 $mg/cm^2$ bond area of *only one adherend* as in the case of *BM adhesive*. Also with wood specimen total bond area was 19.4 $cm^2$ (MB/SB adhesive) against 7.5 $cm^2$ in the case of *BM adhesive*. Taking above mentioned differences into consideration (*i.e.* % solid used, total bond area and adhesive applied on one adherend only), MB/SB adhesives were tested at approximately *5 to 8 time's* higher concentration than that of *BM adhesive* and thus *BM adhesive* appeared superior to MB and SB adhesive.

## 4. Conclusions

This is the first report, where EPS produced by *B. megaterium* has demonstrated good adhesive property with variety of surfaces. The adhesive has high molecular weight and unique chemical compositions. Increase in pH and curing temperature, significantly improved the adhesive ability. Lap shear strength of the adhesive was comparable to commercial wood adhesive and appeared superior to previously reported bacterial adhesives.

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