Structure Elucidation of Bacterial Polysaccharides by NMR and Mass Spectrometry – A Review

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The article highlights the use of NMR and Mass Spectrometry in the structural elucidation of bacterial polysaccharides.

Introduction

It was observed in twenties that the filtrate of culture of pathogenic pneumococci contains a substance which belongs to the important class of compounds called polysaccharides. Subsequently, it was discovered that the type of specificity and the virulence of pneumococci was associated with the presence of polysaccharides which were the main components of the capsule surrounding the organism2,4. Similar bio-active polysaccharides have been obtained from other pathogenic bacteria, such as, Vibrio vulnificus. It produces a capsular polysaccharide which is reported5 to be essential for virulence in septicemia. The observations gave a great impetus to researchers to explore the structure of polysaccharides derived from bacterial sources (see, Table I).

The article reviews structural studies carried out on the bacterial polysaccharides and covers the literature up to January 2000. Included in the article are the polysaccharides derived from nitrogen fixing bacteria (for example, Rhizobium fredii HH 103) which invade the roots of leguminous plants and trigger the formation of nodules that contain the nitrogen fixing microsymbiont.

The main theme of the article is to highlight the use of physical methods, with particular reference to the non-destructive techniques – nuclear magnetic resonance spectroscopy (H, 13C, 2D) and mass spectrometry (EI, Cl, MALDI, FAB) in the structural elucidation of bacterial polysaccharides. These methods in conjunction with the chemical methods (such as compositional analyses, partial degradation, methylation analysis, etc.) have made it possible to assign unambiguous three dimensional structures to bacterial polysaccharides (see, Table 2). Introductory remarks have been incorporated about each method of spectroscopic analysis followed by examples of its application. The polysaccharides are often subjected to chemical or enzymatic depolymerization to yield oligosaccharides which are readily amenable to structural analysis. A few illustrative examples have been included in the article to show the way bacteria can be cultivated in the laboratory, including the methods employed for the isolation and purification (involving centrifugation, lyophilisation, gel-permeation chromatography, high performance liquid chromatography, etc.). The homogeneity of the isolated polysaccharides can be gauged by taking recourse to thin-layer chromatography, ultra-centrifugation and gel-electrophoresis (in borate buffer).

Cultivation of Microorganisms

Streak Plate Method

The streak plate method is usually employed for the isolation and cultivation of the microorganisms; for example, the plant pathogen – Erwinia stewartii, is reported to have been grown on plates with CPG-agar. Similarly, the pathogens – Vibrio cholerae sero type 0139 (NRCC # 4740) are reported to have been grown on sheep’s blood agar plates.

Gel Permeation Chromatography

It is a purification technique employed for an effective separation of molecules with low molecular weights (100-2,000) from molecules of larger molecular weights.
In GPC, the largest molecules emerge out of the column first, followed by the molecules of lower molecular weights. The column material employed for GPC of bacterial oligo, polysaccharides are: Sephadex G-10, G-15 (buffer: Pyr-HOAc; pH 5.6), G-2510, G-5011,12, Superdex-3013, Bio-Gel P-21, P-416, P-617, P-1019 (Buffer: sodium acetate, pH 5.2).

**High Performance Liquid Chromatography (HPLC)**

HPLC has taken an important place in the armamentarium of organic chemists. It is the technique wherein the size and thermal lability of an organic compound is not a liability. It is an extremely rapid, sensitive and convenient method of analysis and offers a high resolution and excellent reproducibility. HPLC is an invaluable tool and best suited to the separation of complex oligosaccharides18,20 and yields constituents in a high degree of purity.

**Reverse Phase-HPLC**

It is employed to separate low molecular weight compounds according to their hydrophobicity. In this type of chromatography, silica gel particles, covered with chemically bonded hydrocarbon chains (C-C) represent the lipophilic phase. In reverse phase chromatography, the hydrophilic compounds will move faster than hydrophobic, since the mobile phase is always more hydrophilic than the stationary phase.

The reverse phase HPLC has been exploited for purifying the Nod factor of *Rhizobium fredii*, *Rhizobium* species21 NGR234. Likewise the monophosphoryl lipid A was secured from the lipo-poly saccharide of *Rhodoseudomonas sphaeroides*22 ATCC 17023 and *Chlamydia trachomatis*23, respectively by the aforementioned technique.

**Ultracentrifugation**

The range of ultracentrifugation could vary from 10,000 (rpm) to 154,000 rpm and duration of spinning from 4 h to 16 h, at 20 °C.7,15,16,24,25

**Dialysis**

The biological material is extensively dialysed against distilled water (48 h). This is the technique used for removing low molecular weight materials.7,15,25,26

**Freez Drying**

The dialysed material is then freeze-dried (lyophilized).7,11-18,24,25,26,90,190. The homogeneity is tested by any of the following methods: TLC, gel electrophoresis, exclusion chromatography, and ultra-centrifugation.

**Gel Electrophoresis**24

The lipopolysaccharide of *Coxiella burnetii* strain Nine Mile is avirulent Phase II and is reported to give a single band on sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) in the presence of 4 M urea. On the basis of this, the high degree of homogeneity of the material is established.

There is another report27 about the isolation of polysaccharides from the mushroom, *Astraeus hygrometricus* and whose homogeneity was confirmed by high voltage electrophoresis in borate buffer. The same objective has been achieved by taking recourse to exclusion chromatography and/or ultracentrifugal analysis28.

**Different Spectroscopic Studies on Bacterial Polysaccharides**

**Proton Nuclear Magnetic Resonance Spectroscopy (1H-NMR)**

The 1H-NMR spectrum has been a source of valuable structural information of polysaccharides29. For example, about the number and the configuration of anomeric protons. The signals arising due to anomeric protons usually appear in the range: 4.3-5.9 ppm, and also the protons of α-glycosides usually resonate 0.3-0.5 ppm downfield from those of the corresponding β-glycosides. The proton NMR spectrum also gives an idea about the constituent monosaccharides, based on chemical shifts and vicinal coupling constants (J). It may, however, be noted that the limitation of 1H-NMR spectroscopy is that it is difficult to determine the configuration of furanosides30,31.

**Example:** 1H-NMR analysis of the major fraction of lipo-chitin oligosaccharide (obtained from *Rhizobium fredii* HH103) has been shown2 to have structure I. The NMR signals (ppm) corresponding to anomeric protons in the oligosaccharide are given in Table 2.

The proton NMR spectroscopic data of the extra-cellular polysaccharide (derived from *Sphingomonas paucimobilis* strain 1-886) indicate that it consist of repeating units of six sugars (vide infra). This is arrived at by counting the number of proton signals in the anomeric region (5.7-4.5 ppm)32,33 (vide infra).
Nuclear Overhauser Effect (NOE)

The NOE experiments are helpful in determining the linkages and the sequence of monosaccharides. The complete conformation of the individual monosaccharides can also be deduced from these experiments.

Example: The oligosaccharide (derived from *Shigella dysenteriae* Type 7, strain 408, O-specific polysaccharide) on NaBH₄ reduction is reported to yield another oligosaccharide with the structure:

The sequencing of [A], [B], [C] (structure 2) has been arrived at by NOE experiments. Thus, the irradiation of anomeric proton resonance of [A] at 5.13 ppm caused a NOE of the signal for H-4 of [B] at 4.49 ppm; whereas irradiation of the anomeric proton resonance of [B] caused a NOE of the signal for one of the protons of the [C] at 4.19 ppm. Conclusion was, therefore, drawn that [A] consequently is terminal sugar and 4-linked to [B].

Another example which illustrates the use of NOE experiments in elucidating (in part) the structure of bacterial polysaccharide is that of O-polysaccharide derived from *Hafnia alvei* Strain 1216. The constituents of the polysaccharide back-bone are sequenced as shown in structure 3.

Nuclear Overhauser Effect Spectroscopy (NOESY)

The NOESY experiments, the two dimensional analogues of NOE experiments, provide information about the spatial structure of the molecule. NOESY cross peaks have been observed between proton pairs, for example, between H-1 and H-3/H-5 for β-glucopyranosyl residue and between H-1 and H-2 for α-glucosylpyranosyl configuration. It is also used in the assignment of sequence and for determining the site of glycosidic linkages in the oligo-/poly-saccharides. It may be noted, however, that NOESY experiments are superior to normal ID difference NOE experiments.
Table 2 – NMR signals of anomeric protons in the oligosaccharide

<table>
<thead>
<tr>
<th>NMR signals (ppm)</th>
<th>Coupling Constants (J, Hz)</th>
<th>Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td>5.50</td>
<td>(J_{H:1}, J_{H:2}) or (J_{1:2})</td>
<td>β-D-glucopyranose residue</td>
</tr>
<tr>
<td>5.42</td>
<td>7.8</td>
<td></td>
</tr>
<tr>
<td>5.27</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5.04</td>
<td>3.5</td>
<td>β-D-glucopyranose residue</td>
</tr>
<tr>
<td>4.83</td>
<td>1.7</td>
<td>L-Rhamnopyranose residue</td>
</tr>
<tr>
<td>5.19</td>
<td>8.9</td>
<td>Methyl (Rhamnose)</td>
</tr>
<tr>
<td>8.67</td>
<td></td>
<td>Methylen</td>
</tr>
<tr>
<td>8.38</td>
<td></td>
<td></td>
</tr>
<tr>
<td>7.65</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Example: The structure 4 has been suggested for the repeating unit of *Hafnia alvei* 32 O specifici polysaccharide.

\[
\text{II} \quad 1 \quad V \quad IV
\]

\[
\rightarrow 4)-α-D-Galp-(1\rightarrow 2)-α-L-Rhap-(1\rightarrow 4)-β-D-Galp-(1\rightarrow 3)-β-D-GalpNAC-2,3\quad OAc
\]

\[
\text{III} \quad (1\rightarrow 4)-\text{GlcNAC-(1}\rightarrow 4\quad 4
\]

\^[13]C-Nuclear Magnetic Resonance(CMR) Spectroscopy

The CMR spectroscopy has several advantages over the 'H-NMR spectroscopy of polysaccharides, such as

(i) Greater chemical shift dispersion and lack of complexities arising due to spin-spin coupling.

(ii) The proton noise decoupled is well resolved and easy to interpret, and

(iii) CMR spectral data provide almost all the structural information like, for example, the number of sugar residues, constituent monosaccharides,
anomeric configuration, including the position of the appended groups.

Example: The oligosaccharide part of *Vibrio salmonicida* (Strain NCMB 2262) lipo-polysaccharide has been reported\(^\text{13}\) to have the structure 5.

\[
\begin{array}{c|c|c|c|c}
\alpha-D-Fucp & 4NBA & (1 \rightarrow 4) & \alpha-NeopA & (2 \rightarrow 6) \\
\beta-D-Glc & (1 \rightarrow 4) & \alpha-D-Hepp & (1 \rightarrow 5) \\
\end{array}
\]

where:

- L-α-D-Hep = L-glycero-a-D-manno-heptopyranose
- D-α-D-Hep = D-glycero-a-D-manno-heptopyranose
- α-NonA = 5-Acetamidino-7-acetamido-3,5,7,9-tetradeoxy-L-glycero-a-D-galactonulosonic acid
- Kdo = 3-deoxy-D-manno-oct-2-ulosonic acid
- BA = (R)-3-hydroxybutanoyl
- PEA = phosphoethanolamine
- P = Phosphorylated

The above structure has been, in part, inferred\(^\text{15}\) from \(^{13}\)C-NMR spectroscopic data which is recorded in the Table 3.

Example: The \(^{13}\)C-NMR spectrum\(^\text{42}\) of the polysaccharide isolated from *Escherichia coli* 0126 lipopolysaccharide exhibited four anomeric carbons located at 99.9, 102.3, 102.8 and 105.4 ppm having \(\text{J}_{\text{CH}}\) values of 173, 162, 160, 163 Hz. These data point to the presence of one glycosidic linkage, in the repeating unit of the O-polysaccharide, with an α-configuration, while the remaining three have β-configuration. The signals at 17.9 ppm are indicative of methyl group of fucose and the signals appearing at 63.1-63.6 ppm arise due to three hydroxymethyl groups (C-6 of galactose, mannose and glucosamine). The C-2 atom of the amino-sugar shows a signal at 55.8 ppm. The other sugar carbons also show up in the region 69.3-81.3 ppm as well as acetamido group (CH at 24.6 ppm and CO at 179.2 ppm). The above data (in conjunction with the other physico-chemical results) point to the fact that the polysaccharide is built up of a tetrasaccharide repeating unit of the structure 6.

\[
\begin{array}{c|c|c|c|c}
\beta-L-Fucp & \text{4} & \beta-D-Manp & (1 \rightarrow 3) & \alpha-D-Galp & (1 \rightarrow 3) & \beta-D-Glc & \text{NAcp} & (1 \rightarrow 2) \\
\end{array}
\]

**Example** The chemical depolymerization by HF of stewartan – the acidic exopolysaccharide of *Eriwinia stewartii*, is reported\(^\text{7}\) to yield a pentasaccharide. The structure 7 for the same has been arrived at by chemical and mass spectrometric analyses.

\[
\begin{array}{c|c|c|c|c|c|c|c|c}
\alpha-D-Glc & \alpha- & \beta-D-Glc & \text{p} & \alpha-D-Glc & \text{p} & \beta-D-Glc & \text{p} & \alpha-D-Glc & \text{p} \\
\end{array}
\]

The structure 7 has been confirmed by NMR spectroscopy (viz., 2D COSY, TOCSY and 2D ROESY).

In the structure 7, the four pyranose (three gluco and one galacto) monosaccharide spin systems, all with β-anomeric configuration were completely or partially assigned, using 2D COSY and TOCSY NMR spectra (vide data given above). For the anomeric proton of the fifth residue D (a galactopyranose with α-anomeric con-
Table 3 – ¹³C-NMR spectral data

<table>
<thead>
<tr>
<th>¹³C Resonance (ppm)</th>
<th>Interpretation</th>
</tr>
</thead>
<tbody>
<tr>
<td>16.4, 17.4, 19.1, 19.7, 22.7, 22.9</td>
<td>Six methyl carbons</td>
</tr>
<tr>
<td>54.3, 54.5, 55.0</td>
<td>Three carbons linked to nitrogen</td>
</tr>
<tr>
<td>37.5, 45.5</td>
<td>CH₂ carbons</td>
</tr>
<tr>
<td>41.1, 41.2 (doublets)</td>
<td>Nitrogen bearing carbons of two PEA groups</td>
</tr>
<tr>
<td>[(J_{CP} = 7) Hz each]</td>
<td></td>
</tr>
<tr>
<td>60.9-66.2</td>
<td>Methylene groups</td>
</tr>
<tr>
<td>62.6, 62.7</td>
<td>Hydroxymethyl of two PEA groups</td>
</tr>
<tr>
<td>[(J_{CP} = 5) Hz each]</td>
<td></td>
</tr>
<tr>
<td>95.6 ((J_{CH} 173) Hz)</td>
<td>Anomic carbons</td>
</tr>
<tr>
<td>101.3 ((J_{CH} 174) Hz)</td>
<td></td>
</tr>
<tr>
<td>101.6 ((J_{CH} 172) Hz)</td>
<td></td>
</tr>
<tr>
<td>103.5 ((J_{CH} 162) Hz)</td>
<td></td>
</tr>
<tr>
<td>~98 and 101 (small signals)</td>
<td></td>
</tr>
<tr>
<td>167.6, 173.3, 174.5, 176.1</td>
<td>Carbonyl carbons</td>
</tr>
</tbody>
</table>

A characteristic signal with a large geminal coupling constant to fluorine (54.3 Hz) has been reported. The inter-residual cross peaks in the 2D-ROESY spectrum have been exploited to indicate the linkages between the monosaccharide residues, as shown in structure 7.

**Heteronuclear Multiple Quantum Coherence (HMQC)**

Example: HF depolymerized amylovan – the acidic exopolysaccharide of *Erwinia amylovora* is reported to yield a tetrasaccharide, which has the structure 8. This structure has been confirmed by NMR spectroscopy, for example, by using 2D COSY, TOCSY and 2D ROESY experiments. In addition, the ¹³C chemical shifts of all carbon atoms in the tetrasaccharide have been determined from a HMQC spectrum. The HMQC spectrum is reported to show the expected long range ¹³C-¹H correlations for the proposed structure. Thus, H-1 (d, 4.90 ppm) of residue B showed correlation to C-4 of residue C (α) at 75.8 and C (β) at 75.0 ppm; H-1 (d, 4.66 ppm) of residue E showed correlation to C-3 of residue C(α) at 79.7 and C(β) at 82.2 ppm. H-1 (α, 4.62 ppm) of residue D showed a correlation to C-3 of residue E at 82.8 ppm.

**Homonuclear Hartman-Hahn Spectroscopy (HOHAHA)**

Example: The analysis of the partially hydrolysed (i.e., O-deacetylated) exocellular polysaccharide from *Sphingomonas paucimobilis* strain I-886, with one-dimensional HOHAHA and various 2D NMR experiments yielded most of the ¹H NMR chemical shifts. Residue A, B, C and D were assigned to the D-glucose, E to the
rhamnose residue, and F to deoxyglucuronic acid. Further, the spectral data show that one of the β-D-Glc residues is terminal. It also shows that the α-glucose residue is linked to the 6-position of another glucose residue. The repeating units of the polysaccharide have the structure 9.

\[
\begin{align*}
&\text{C E B} \\
&\rightarrow4)-\beta-D-\text{Glc}-p-(1\rightarrow4)-\alpha-L-\text{Rhap}-(1\rightarrow3)-\beta-D-\text{Glc}-p-(1\rightarrow4)-2-
\end{align*}
\]

deoxy-β-D-arabino-HexA.

\[
\begin{align*}
&6 \\
&1 \\
&\beta-D-\text{Glc}-(1\rightarrow6)-\alpha-D-\text{Glc}
\end{align*}
\]

**Mass Spectrometry (MS)**

In characterization of molecular structure of organic compounds (for example, polysaccharides), mass spectrometry has become an ubiquitous research tool. In a mass spectrometer, the compound under investigation is bombarded with a beam of electrons. The positive ion (molecular ion) and the fragments derived therefrom are quantitatively recorded as what is known as a mass spectrum.

**Electron Impact Ionization Mass Spectrometry (EI-MS)**

The technique of EI-MS is an old one and is applicable to a wide range of organic compounds (gas, liquid or solid) volatilized at 400°C. Electron beam (70 ev) is made to impinge on the organic molecule in the gas phase. In this process molecular ion and a large number of small fragments ions are formed, which provide useful structural information. The limitation of EI-MS, however, is that the molecular ion may be weak or absent for some compounds. Moreover, thermally labile compounds are not suitable candidates for electron impact ionization mass spectrometry.

The oligosaccharides, obtained from *Legionella pneumophila* were subjected to GLC-MS (vide infra) using both EI and CI (with ammonia) modes.

When EI-MS on a particular organic compound fails to produce a molecular ion, CI-MS is then the method of choice. In CI, the so called "soft" ionization technique, an electron beam is impinged on to a reagent gas (methane, isobutane or ammonia) which ionizes and gently transfers protons to the sample, usually producing (M+H)+ quasimolecular ion. Fewer fragmentation ions result in the process, and hence a simple mass spectrum is obtained. The fragmentation pattern, however, is not as much informative as that obtained by EI-MS.

Mass Spectrometry/Mass spectrometry (MS-MS) Tandem Mass Spectrometry

In tandem mass-spectrometry, two mass spectrometers are used in series. The first spectrometer is employed to produce the molecular ion and its fragment ions. These ions are selectively subjected to collision induced dissociation (CID) promoted by helium, under high pressure, followed by analysis of the resulting product ion. MS/MS is particularly useful for obtaining structural information about the bacterial polysaccharide. There are reports about the structural investigations involving the use of MS-MS, on the oligosaccharides derived from *Salmonella enteritidis* and *Rhizobium etli* strain CE 358 and CE 359.

Gas Chromatography-Mass Spectrometry (GC-MS)

A powerful technique GC-MS (gas chromatograph directly coupled to a mass spectrometer) finds wide use, amongst other applications, in the structural investigations related to bacterial polysaccharides. The oligosaccharide (obtained by depolymerization of polysaccharide) is required to be derivatized prior to GC-MS analysis.

Example: The following example would illustrate the application of GC-MS employing both the ionization techniques, viz. CI and EI for investigating the structure of major polysaccharide (PSI) derived from the acidic exopolysaccharide produced by mucoid strain of *Burkholderia cepacia*.

The GC-MS analysis of the trimethyl silylated methyl glycoside derivative (obtained by the methanalysis of PSI) showed the abundant ions in the CI mass spectrum, at m/z 440 (M+NH)+, 408 (M-MeOH+NH)+ which points to the molecular mass of 422 (structure 10) corresponding to the trimethyl silyl derivative of a methyl-(1-carboxyethylidene methyl ester)-hexo-pyranoside.

Further structural information was provided by EI-MS. Thus, m/z 363 (M-COOMe)+ and m/z 243 clearly
point to 4,6-O(1-carboxyethylidene)-D-galactose structure. The 4,6-linkage of the carboxyethylidene group is also deduced by the occurrence (in the EI-MS) of an ion at m/z 204, requiring an adjacent trimethyl-silyl groups at positions 2 and 3, and establishing a pyranose form for the galactose unit.

Fast-Atom-Bombardment Mass Spectrometry (FAB-MS)

A simple and rapid ionization technique employed in organic mass spectrometry, for a large variety of compounds (including polar and/or thermally labile) is the FAB-MS, by high energy beam of neutral xenon atoms. FAB-MS has been employed for the structural investigations of several lipooligosaccharides obtained from various bacteria. Example: Smith degradation of O-specific polysaccharide, derived from Hafnia alvei strain 32 is reported\(^a\) to yield two core oligosaccharides. One of the oligosaccharides on positive FAB-MS analysis yielded an (M+H\(^+\))\(^a\) ion at m/z 676. The high energy collision induced decomposition tandem-MS of (M+H\(^+\))\(^a\) ion showed that the trisaccharide was linked to a glycerol group (structure 11).

Another example is the determination of structure of the liberated oligosaccharide, OS (derived from lipooligosaccharide of Campylobacter jejuni strain PC 637) is, in part, based on the data obtained from FAB-MS. Thus, permethylated OS is reported\(^b\) to yield a pseudo-molecular ion (M+Na\(^+\))\(^b\) at 2555 amu corresponding to a composition shown in structure 12.

Matrix-Assisted Laser Desorption/Ionization (MALDI)

The MALDI is an ionization technique used in the mass analysis of large and/or labile molecules. Example: The MALDI-MS of Smith oligosaccharide of Vibrio vulnificus ATCC 27562 is reported\(^c\) to exhibit two types of ions corresponding to m/z, 870.5 (M+Na\(^+\))\(^c\) and m/z, 892.6 (M+2Na-H\(^+\))\(^c\). The former ion was chosen to probe the structure of this molecule by high energy collision induced dissociation (CID) in the first field region of a double focussing mass spectrometer. The CID spectrum shows ions at m/z 666 (N-acetylhexosamine) and m/z, 594 (Muramic acid) and a fragment ion at m/z, 392. These observations point to the fact that the N-acetylhexosamine and muramic acid residues are linked independently to the molecule and are locked at the termini of the molecule. The fragment ions at m/z, 782 and 766 are due to the loss of serine and tetratol, respectively from the precursor ion. The remain-
ing part of the molecule was intuitively guessed as hexuronic acid. Based on the above mass data, the structure of the (M+Na)+ is depicted as 13.

Serine

\[ \text{NH} \]
\[ 666 \quad | \quad 782 \]
\[ \beta-	ext{GlpNAc-(1-4)-a-GalA-(1-2)-tetritol} \]
\[ 594 \quad | \quad 764 \]
\[ \alpha-	ext{MurNAc(1-3)} \]

Example: The characterization of the oligosaccharide (derived from lipopolysaccharide of *Salmonella enteritidis*) has been achieved, in part, by taking recourse to MALDI-TOF-MS.

Similarly, the structure determination of monophosphoryl lipid A (prepared from lipopolysaccharide of *Chlamydia trachomatis*) involving the use of MALDI-MS and liquid secondary ion mass spectrometry, has also been reported.

Electrospray Ionization Mass Spectrometry (ESI-MS)

In this technique, the highly charged droplets, dispersed from a capillary, and in an electric field, are evaporated, in a region maintained at high vacuum. This increases the charge on the droplets and the multiple charged ions are drawn into a mass spectrometer. The outstanding feature of ESI spectrum is that the ions carry multiple charges which reduce their m/z ratio compared to a singly charged species. This allows mass spectra to be obtained for large molecules like, for example, the oligosaccharide fraction derived from *Coxiella burnetii* strain Nine Mile. ESI-MS is also reported to give an in intense molecular ion at m/z, 1176 [Pyr Hex HexA Hex-ol + Na]+. MS-MS of the parent ion, m/z, 1176 shows a linear arrangement of the molecule and is sequenced as follows.

PyrHex-HexA-Hex-Hexol
Pyr = Pyruvate residue

Example: ESI-MS has also been employed in arriving at the structure of repeating unit of the nodular polysaccharide, NPS. The major fraction produced by Smith degradation, followed by BioGel P-2 chromatography of *Bradyrhizobium japonicum*. NPS, on ESI-MS exhibited two major mass peaks, m/z, 562 and 584. These mass fragment ions have been assigned to [M+1]+ and [M+Na]+ ions of the oligomer containing one galactosyl, two rhamnosyl residues, plus an additional component (90 amu)-deoxytetritol.

References

### Table 1 – Some typical pathogenic and non-pathogenic bacteria

<table>
<thead>
<tr>
<th>Name of the organism</th>
<th>Remarks</th>
<th>Ref No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bradyrhizobium aspalati</td>
<td>Nitrogen-fixing symbionts (South African legumes)</td>
<td>72</td>
</tr>
<tr>
<td>Bradyrhizobium japonicum and B. elkanii</td>
<td>Gram negative bacteria, nitrogen fixation in soya bean</td>
<td>50</td>
</tr>
<tr>
<td>Burkholderia capsaci</td>
<td>Mucoid strain</td>
<td></td>
</tr>
<tr>
<td>Campylobacter lari strain PC 637</td>
<td>Human pathogen responsible for enteritis</td>
<td>15</td>
</tr>
<tr>
<td>Campylobacter lari type strain ATCC 35221</td>
<td>Human pathogen</td>
<td>51</td>
</tr>
<tr>
<td>Chlamydia trachomatis</td>
<td>Human pathogen</td>
<td>23</td>
</tr>
<tr>
<td>Chlamydia trachomatis serotype L2</td>
<td>Human pathogen</td>
<td>73</td>
</tr>
<tr>
<td>Coxiella burnetii Strain Nine Mile</td>
<td>Etiological agent for Q-fever (Pneumonitis, hepatitis and neurologic complications)</td>
<td>24</td>
</tr>
<tr>
<td>Cryptococcus neoformans Sero type-C</td>
<td>Infection associated with AIDS, pulmonary pathogen</td>
<td>53</td>
</tr>
<tr>
<td>Erwinia amylovora</td>
<td>Plant pathogenic bacteria, causes fire blight on apple and pear trees</td>
<td>25</td>
</tr>
<tr>
<td>Erwinia chrysanthemi CU643</td>
<td>Gram negative phytopathogen responsible for soft rot in a number of plants</td>
<td>74</td>
</tr>
<tr>
<td>Erwinia stewartii</td>
<td>Corn pathogen</td>
<td>7</td>
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<tr>
<td>Escherichia coli</td>
<td>It is a complex group of bacteria and many of its sero-types are important human pathogens causing extra-intestinal infections</td>
<td>54</td>
</tr>
<tr>
<td>Escherichia coli 0173</td>
<td>Entetro-invasive organism</td>
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<td>Eubacterium seburreum</td>
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<td>12</td>
</tr>
<tr>
<td>Haemophilus influenza strain Rd</td>
<td>(Gram-negative bacteria)</td>
<td>76</td>
</tr>
<tr>
<td>Haemophilus influenza mutant strain RM 118-26</td>
<td>A genus of bacteria (Gram-negative)</td>
<td>77</td>
</tr>
<tr>
<td>Hafnia alvei, strain-32</td>
<td>Enterobacteriaceae – pathogens found in some nosocomial infections and septicaemia</td>
<td>9</td>
</tr>
<tr>
<td>Hafnia alvei, Strain 744 and PCM strain 1194 and 1210</td>
<td>-</td>
<td>52</td>
</tr>
<tr>
<td>Hafnia alvei Strain 1216</td>
<td></td>
<td>10</td>
</tr>
<tr>
<td>Hafnia alvei Strain 13337 &amp; 1187</td>
<td></td>
<td>55</td>
</tr>
<tr>
<td>Klebsiella &amp; Rhizobium species</td>
<td>Bacteria</td>
<td>16</td>
</tr>
<tr>
<td>Legionella pneumophila</td>
<td>Pathogen causing severe respiratory infection in humans</td>
<td>11</td>
</tr>
<tr>
<td>Sero group I</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mycobacterium bovis, BCG</td>
<td>Tubercol bacillus</td>
<td>43</td>
</tr>
<tr>
<td>Name of the organism</td>
<td>Remarks</td>
<td></td>
</tr>
<tr>
<td>--------------------------------------------------</td>
<td>--------------------------------</td>
<td></td>
</tr>
<tr>
<td><em>Mycobacterium bovis, BCG</em></td>
<td>Tubercle bacillus</td>
<td></td>
</tr>
<tr>
<td><em>Mycobacterium xenopi</em></td>
<td>-</td>
<td></td>
</tr>
<tr>
<td><em>Proteus vulgaris OX 19</em></td>
<td>Human pathogen</td>
<td></td>
</tr>
<tr>
<td>(Sero group 01)</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Pseudomonas sp</em> strain 1.15</td>
<td>Fresh water biofilm</td>
<td></td>
</tr>
<tr>
<td><em>Rhizobium etli</em> Strain CE 358 and CE 359</td>
<td>Nitrogen fixing bacteria</td>
<td></td>
</tr>
<tr>
<td><em>Rhizobium fredii</em> HH 103</td>
<td>Nitrogen fixing bacteria</td>
<td></td>
</tr>
<tr>
<td><em>Rhizobium species NGR 234</em></td>
<td>Nitrogen fixing bacteria</td>
<td></td>
</tr>
<tr>
<td><em>Rhodopseudomonas sphaeroides</em> ATCC 17023</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td><em>Salmonella enterica SV Arizonae 062</em></td>
<td>Human pathogen</td>
<td></td>
</tr>
<tr>
<td><em>Salmonella enteritidis</em></td>
<td>Human pathogen</td>
<td></td>
</tr>
<tr>
<td><em>Shigella dysenteriae</em> Type 7</td>
<td>Human pathogen</td>
<td></td>
</tr>
<tr>
<td><em>Sinorhizobium fredii</em> HH 103</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td><em>Sphingomonas paucimobilis Strain 1-886</em></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Streptococcus pyogenes</em></td>
<td>Human pathogen</td>
<td></td>
</tr>
<tr>
<td><em>Vibrio cholerae</em> 0139</td>
<td>Pathogen responsible for cholera</td>
<td></td>
</tr>
<tr>
<td><em>Vibrio cholerae</em> 0139</td>
<td>Pathogen responsible for cholera</td>
<td></td>
</tr>
<tr>
<td><em>Vibrio salmonicida</em></td>
<td>Gram-negative bacteria, highly pathogenic to Atlantic salmon</td>
<td></td>
</tr>
<tr>
<td><em>Strain NCMB 2262</em></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Vibrio vulnificus</em> ATCC 27562</td>
<td>Gram-negative bacterial pathogen (Septicemia)</td>
<td></td>
</tr>
<tr>
<td><em>Yokenella regenburgei</em> strain PCM 2476, 2477, 2478 and 2494</td>
<td>Bacteria</td>
<td></td>
</tr>
</tbody>
</table>
Table 2 — Structures of oligo/polysaccharides (bacterial)

<table>
<thead>
<tr>
<th>S.No.</th>
<th>Oligo/ polysaccharide</th>
<th>Structure/ Information</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Lipo-chitin oligosaccharide from <em>Bradyrhizobium aspalati</em></td>
<td>The lipo-chitin oligosaccharides are highly substituted on the non-reducing-terminal residue but unsubstituted on the reducing terminal residue. The molecular backbone consists of 3→5 β-(1→4)-linked N-acetyl-D-glucosamine residues substituted on the non-reducing terminus with a C16:0, C16:1, C18:0, C18:1, C19:1 cy, or C20:1 fatty acyl chain and are both N-methylated and 4, 6- dicarbamoylated.</td>
<td>73</td>
</tr>
<tr>
<td>2.</td>
<td>nodule polysaccharide derived from <em>Bradyrhizobium</em> species</td>
<td>→4)-α-L-Rhap-(1→3)-β-L-Rhap-(1→4)-β-L-Rhap-(1→4)-D-Me-β-D-Glcp(1→3)</td>
<td>50</td>
</tr>
<tr>
<td>3.</td>
<td>repeating unit of the EPS of <em>Burkholderia cepacia</em></td>
<td>→3)-β-D-Glcp-(1→3)-α-D-Galp-(1→6)-β-D-Glcp(1→4)-β-D-Glcp(1→3)</td>
<td>49</td>
</tr>
</tbody>
</table>

— Contd
extracellular polysaccharide obtained from *Campylobacter lari* strain PC 637

\[
\text{LOS:} \quad \alpha-D-\text{GlcP}-(1\rightarrow3)-\beta-D-\text{GalNAc}-(1\rightarrow3)-\beta-D-\text{GalNAc}-(1\rightarrow3)-\beta-D-\text{GalP}(1\rightarrow3)-2 \quad 1 \quad \alpha-D-\text{GalP} \\
\beta-D-\text{GlcP} \quad 4 \quad 1 \quad \alpha-D-\text{GalP} \\
L-\alpha-D-\text{Man-Hepp}-(1\rightarrow3)-L-\alpha-D-\text{man-Hepp}-(1\rightarrow5)-\text{Kdo} \quad 2 \quad 2 \quad 1 \quad \alpha-D-\text{GalP} \quad \alpha-D-\text{GalP} \\
\]
highly branched region of lipooligosaccharide of the *Campylobacter lari* type strain; ATCC 35221.

Abbreviation: AEP = 2-aminoethyl phosphate and for the tetraglycosyl phosphate repeating unit of the extracellular polysaccharide. 

\[
\text{EPS:} \\
\text{[(PO}_3\text{)}\text{→4]}-\beta\text{-D-Glc}p\text{-}(1\text{→5})6\text{-d-α-L-Gal-Hepp}(1\text{→})_n
\]

\[
-6\text{-d-α-L-gal-Hepp} \\
\text{2} \\
\text{|} \\
\text{1} \\
\text{6-d-α-L-Gal-Hepp}
\]

\*

5  

\[
\text{β-D-Glc}p\text{-NAc(1→3)-α-D-GalpNAc(1→3)-α-D-Galp(1→2)-L-α-D-man-Hepp(1→5)-α-Kdo(1→5)-Kdo}
\]

\[
\text{2} \\
\text{|} \\
\text{1} \\
\text{α-D-Glc}p
\]

\[
\text{L-α-D-man-Hepp}(1\text{→5})\text{-α-Kdo}(1\text{→5})\text{-Kdo}
\]

\[
\text{2} \\
\text{|} \\
\text{1} \\
\text{α-D-Galp}
\]

\*

6  

Kdo α2→8 Kdo α2→4 Kdo α2→6D-GlcNβ1→6D-GlcP N α1,4′-bispophosphate
7 major lipid A component of Chlamydial lipopolysaccharide

8 Lipopolysaccharide from Coxella burnetti strain Nine Mile in avirulent phase II

This is reported to be due to a glucosamine disaccharide that contains five fatty acids and a phosphate in the distal segment. Three fatty acyl groups are in the distal segment, and two are in the reducing end segment. The acyloxyacyl group is located in the distal segment in amide linkage.

\[ \alpha-D-Manp \end{array}\]

\[
\begin{array}{c}
1 \\
4 \\
\Leftrightarrow \\
2 \\
\end{array}
\]

\[
\alpha-Kdo \end{array}\]

\[
\begin{array}{c}
\downarrow \\
2 \\
\end{array}
\]

\[ \alpha-Kdo-(4\rightarrow 2\rightarrow)\alpha-Kdo \end{array}\]

9 core repeating unit of the polysaccharide of Cryptococcus neoformans serotype C

\[ \beta-D-GlcPA \end{array}\]

\[
\begin{array}{c}
1 \\
\downarrow \\
2 \\
\end{array}
\]

\[ \alpha-D-Manp(1\rightarrow 3)-\alpha-D-Manp(1\rightarrow 3)\rightleftharpoons \alpha-D-Manp(1\rightarrow 1) \]

10 Erwinia amylovora

\[ \alpha-D-Pyr4,6Ac,\alpha-Galp \end{array}\]

\[
\begin{array}{c}
\downarrow \\
4 \\
\beta-D-GlcPA \\
1 \\
\downarrow \\
4 \\
\end{array}
\]

\[ \alpha-D-Manp \end{array}\]

\[
\begin{array}{c}
\downarrow \\
6 \\
\Leftrightarrow \\
1 \\
\end{array}
\]

\[ \beta-D-GlcP \end{array}\]

(\[ \beta-D-GlcP \end{array}\)ₙ)
<table>
<thead>
<tr>
<th>11</th>
<th>Branched repeating unit with seven monosaccharide residues, all in their pyranose forms of the oligosaccharide derived from the <em>Erwinia stewartii</em> Extracellular polysaccharide of <em>Erwinia chrysanthemi</em> CU 643.</th>
</tr>
</thead>
</table>
|    | β-D-Glc p 1  
|    | ↓ 6  
|    | D-Gal p 1  
|    | ↓  
|    | β-D-Glc p 1  
|    | ↓ 4  
|    | [→3]-α-D-Gal p-(1→6)-β-D-Glc p-(1→3)-β-D-Gal p-(1→)ₙ  
|    | ↓ 6  
|    | ↑  
|    | β-D-Glc p 1  
| 12 | Extracellular polysaccharide of *Erwinia chrysanthemi* CU 643. |
|    | ...→3)-β-D-Gal p-(1→2)-α-L-Rhap-(1→4)-β-D-Glc p-(1→2)-α-L-Rhap-(1→2)-α-L-Rhap-(1→2)-α-L-Rhap-(1→2)-α-L-Rhap-(1→2) 
|    | ...→-(1)  
|    | **Contd** |
13. The novel sugar constituent of the O-specific polysaccharides of *Escherichia coli* 0114 has been reported to have the following structure:

![Chemical structure](image)

3-((N-acetyl-L-seryl)amino)-3,6-dideoxy-D-glucose

14. The repeating units of the polysaccharide obtained from *Escherichia coli* 0:25:

The repeating unit of the polysaccharide is the pentasaccharide. Its cleavage leads to the formation of a tetrasaccharide with the structure as shown:

\[
\alpha-L-Rhap \\
\downarrow \\
3 \\
\rightarrow 4(\alpha-L-FucNAc(1\rightarrow3))-\beta-D-GlcNAc(1\rightarrow4)-\alpha-D-Glc-(1\rightarrow6) \\
\uparrow \\
1 \\
\beta-D-GlcP
\]

15. O-Antigen polysaccharide from *Escherichia coli* 0173:

The repeating unit of the polysaccharide is the pentasaccharide. Its cleavage leads to the formation of a tetrasaccharide with the structure as shown:

\[
\alpha-D-GlcP-(1\rightarrow2)-\beta-D-GlcP-(1\rightarrow3)-\beta-D-GlcP \text{NAc}-(1\rightarrow3)-D-Glc
\]

16. The O-antigen (*Escherichia coli* 0125 : K72 : H19) is branched and is built up of repeating hexasaccharide unit:

\[
\rightarrow 2(\alpha-D-Manp-(1\rightarrow3)\alpha-L-Fucp-(1\rightarrow3)\alpha-D-GalpNAc(1\rightarrow4)\alpha-D-GalpNAc-(1\rightarrow3)\alpha-L-Fucp-(1\rightarrow3)-\beta-D-GlcP-(1\rightarrow3)-\beta-D-Galp-(1\rightarrow3)
\]

---

Contd
17 O-antigen from enteropathogenic *Escherichia coli* 0126

The polysaccharide obtained from the above source is branched and built of tetrasaccharide repeating units

\[ \beta-L-\text{Fucp} \]

\[ \xrightarrow{2} \]

\[ \beta-L-\text{Fucp} \]

\[ \xrightarrow{1} \]

\[ \beta-L-\text{Fucp} \]

18 *Escherichia coli* K12 (S53)

\[ \xrightarrow{4-L-\text{Fucp}} \]

\[ \alpha \]

\[ 4 \text{Glc} \]

\[ \beta \]

\[ 3 \text{L-Fuc} \]

\[ \beta \]

\[ 4 \]

19 O-specific polysaccharide of *Haemophilus alvei* strain 1216 is reported to be built up of linear pentasaccharide units

\[ \xrightarrow{(R) \ CH_3CH(OH)CH_2CO \ OAC} \]

\[ 3 \]

\[ 6 \]

\[ \xrightarrow{4-\alpha-D-L-Rhap-(1\rightarrow4)\text{Galp}-(1\rightarrow3)\beta-D\text{-GalpNAc}-(1\rightarrow4)\alpha-D\text{-Glc}pNAc-(1\rightarrow} \]

20 Lipopolysaccharide of *Haemophilus influenzae* mutant strain RM 118-26

One of the major glycoforms (obtained by mild acid hydrolysis of lipopolysaccharide) is reported to contain three hexose moieties (Hex 3), in which a lactose unit, \( \beta-D\text{-Galp}-(1\rightarrow4)\beta-D\text{-GlcNac} \), is attached at the 0-2 position of the terminal heptose of the inner core element, \( L-\alpha-D\text{-Hepp}-(1\rightarrow3)\text{-GlcNac}-(1\rightarrow4)\text{-Galp}-(1\rightarrow6)\alpha-D\text{-Galp}-(1\rightarrow6)\alpha-Kdo \). The glycoform is 40% substituted by an O-acetyl group attached to the 6-position of the glucose moiety in the lactose unit.

21 Lipopolysaccharide from *Haemophilus influenzae*, strain Rd.

The major lipopolysaccharide glycoforms are reported to contain three (two Glc and one Gal), four (two Glc and two Gal) and five (two Glc, two Gal and one Gal NAc) hexoses-substituted by phosphocholine and phosphoethanol amine.
22 Klebsiella K1

\[\text{4-L-Fuc} \xrightarrow{\alpha} \text{3Glc} \xrightarrow{\beta} \text{4GlcA} \xrightarrow{\beta}\]

\(\text{H}_3\text{C} \xrightarrow{\text{COOH}}\)

23 Klebsiella K3

Contains Gal, Man, GalA and pyruvic acid (10%) but the structure has not been determined. 4,6-O-(1-carboxyethylidene) D-mannose is known to be present.

24 Klebsiella K30

\[\text{Glc} \xrightarrow{\alpha}\]

\[\text{4Glc} \xrightarrow{\beta} \text{4Man} \xrightarrow{\beta} \text{4Man} \xrightarrow{\beta}\]

\(\text{Me}, \beta / \text{Gal}, \text{HOOC}\)

25 Klebsiella K32

\[\text{3Gal} \xrightarrow{\alpha} \text{2-L-Rha} \xrightarrow{\alpha} \text{3-L-Rha} \xrightarrow{\beta} \text{4-L-Rha} \xrightarrow{\alpha}\]

\(\text{H}_3\text{C} \xrightarrow{\text{COOH}}\)

26 Klebsiella K58

\[\text{4-L-Fuc} \xrightarrow{\alpha} \text{3Glc} \xrightarrow{\alpha} \text{4GlcA} \xrightarrow{\beta}\]

\(\text{Gal} \xrightarrow{\alpha}\)

\(\text{H}_3\text{C} \xrightarrow{\text{COOH}}\)

— Contd
27 Klebsiella K70

\[-2\text{Glc-}\xrightarrow{\beta}3\text{Gal-}\xrightarrow{\beta}2\text{-L-Rha-}\xrightarrow{\alpha}4\text{GlcA-}\xrightarrow{\beta}4\text{-L-Rha-}\xrightarrow{\alpha}2\text{-L-Rha-}\xrightarrow{\alpha}\]

\[\text{H}_3\text{C- COOH}\]

Note: only 50% of the repeating units of this polysaccharide are pyruvated.

28 O-acetylated core of Legionella pneumophila serogroup I lipopolysaccharide

\[\alpha\text{-L-Rhap}^\dagger_{(1\rightarrow3)}\alpha\text{-L-Rhap}^\dagger_{(1\rightarrow3)}\beta\text{-D-QuipNAc-(1\rightarrow4)}\beta\text{-D-GlcNAc}-_{(1\rightarrow4)}\alpha\text{-D-Manp-(1\rightarrow5)}\text{-Kdo}\]

29 The 6-O-methylglucose lipopolysaccharide secured from Mycobacterium bovis BCG contain an unusual monosaccharide 2N-acetyl-2,6-dideoxy-\beta-glucopyranose in its molecular backbone.

30 Two polysaccharides characterised from Mycobacterium xenopi

The first one is composed of 16 D-glucopyranose residues, 11 of which are methylated. While the other contains 15 D-glucopyranose residues 10 of which are methylated.

31 O-specific polysaccharide chain of Proteus vulgaris OX19

lipopolysaccharide branched pentasaccharide repeating units containing D-galactose, 2-acetamido-2-deoxy-\alpha-D-glucose, 2-acetamido-2-deoxy-D-galactose and 2-acetamido-2,6-dideoxy-\alpha-D-glucose (QuiNAc, two residues) which are connected to each other via a phosphate group.
32 Acidic exopolysaccharide from *Pseudomonas* sp. Strain 1.15.

The parent repeating unit of the 1.15 exopolysaccharide is a branched hexasaccharide. The main chain is made up of a trisaccharide \(\alpha-L\text{-Fuc}p(1\rightarrow4)\alpha-L\text{-Fuc}p(1\rightarrow4)\beta-D\text{-Glc}p(1\rightarrow4)\) and the side chain consists of \(\alpha-D\text{-Gal}p(1\rightarrow4)\beta-D\text{-Glc}Ap(1\rightarrow4)\alpha-D\text{-Gal}p(1\rightarrow4)\) attached to O-3 of the first Fuc moiety. The terminal non-reducing Gal carries a 1-carboxy-ethylidene acetal in the R configuration at the position 4 and 6.

33 Intact lipopolysaccharide core region (obtained from *Rhizobium etli* strains CE 358 and CE 359)

It consists of trisaccharide (2) attached to O-4 of the Kdo residue in tetrasaccharide 1, and that an additional Kdo residue is attached to O-8 of the polymeric chain. It may be noted, however, that one mutant CE 358, completely lacks the O-chain polysaccharide, while the second mutant CE 359 contains a truncated portion of this polysaccharide.

\[
\text{Kdo-p-(2\rightarrow6)\text{-Gal}p(1\rightarrow6)-(\alpha-D\text{-Gal}p\text{-A-Gal}p\text{-A-(1\rightarrow4)\alpha-D-Man}p(1\rightarrow6)-}
\]

\[
\text{-Kdop-(2)}
\]

It consists of trisaccharide (2) attached to O-4 of the Kdo residue in tetrasaccharide 1, and that an additional Kdo residue is attached to O-8 of the polymeric chain. It may be noted, however, that one mutant CE 358, completely lacks the O-chain polysaccharide, while the second mutant CE 359 contains a truncated portion of this polysaccharide.

where Kdo = 3-deoxy-D-manno-2-oct-2 ulo-pyranosonic acid.

34 *Rhizobium phaseoli* 127 K38

\[
\begin{align*}
\text{Me} & \quad \text{Glc} \quad \alpha \quad 4 \text{Glc} \quad \beta \quad 4 \text{Glc} \quad \alpha \\
\text{HOOC} & \quad \beta \quad 4 \text{Glc} \quad \beta \quad 4 \text{Glc} \quad \alpha
\end{align*}
\]

35 The repeating units of pyruvated polysaccharides *Rhizobium phaseoli* 127 K36

\[
\begin{align*}
\text{Me} & \quad \text{Gal} \quad \beta \quad 3 \text{Glc} \quad \beta \quad 4 \text{Glc} \quad \beta \\
\text{HOOC} & \quad \beta \quad 4 \text{Glc} \quad \beta \quad 4 \text{Glc} \quad \alpha
\end{align*}
\]

--- Contd
36. *Rhizobium phaseoli* 127
   
   \[ \text{K44} \]

   \[
   \begin{align*}
   4\text{Glc} & \rightarrow 4\text{Glc} \\
   4\text{Glc} & \rightarrow 4\text{Glc} \\
   \end{align*}
   \]

37. *Rhizobium phaseoli* 127
   
   \[ \text{K87} \]

   \[
   \begin{align*}
   4\text{Glc} & \rightarrow 4\text{Glc} \\
   4\text{Glc} & \rightarrow 4\text{Glc} \\
   \end{align*}
   \]

38. *Rhizobium fredii* HH103

\[
\begin{align*}
4\text{Glc} & \rightarrow 4\text{Glc} \\
4\text{Glc} & \rightarrow 4\text{Glc} \\
4\text{Glc} & \rightarrow 4\text{Glc} \\
4\text{Glc} & \rightarrow 4\text{Glc} \\
\end{align*}
\]

---

Nod NG R\(_B\)
(R=H, R\(^1\)=Acetate)

Nod NGRA (R=SO\(_3\)H, R\(^1\)=H)

X=H or Carbamoyl (0,1,or 2)

---

Contd
Nod factors from *Rhizobium fredii* HH103
<table>
<thead>
<tr>
<th>N</th>
<th>( R^1 ) (fatty acid)</th>
<th>( R^2 )</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>( \text{C}_{16}:1\Delta^9 )</td>
<td>( H )</td>
</tr>
<tr>
<td>2</td>
<td>( \text{C}_{16}:1\Delta^9 )</td>
<td>( \text{CH}_3 )</td>
</tr>
<tr>
<td>3</td>
<td>( \text{C}_{16}:1\Delta^9 )</td>
<td>( H )</td>
</tr>
<tr>
<td>3</td>
<td>( \text{C}_{16}:1\Delta^9 )</td>
<td>( \text{CH}_3 )</td>
</tr>
<tr>
<td>1</td>
<td>( \text{C}_{16}:1\Delta^9 )</td>
<td>( \text{CH}_3 )</td>
</tr>
<tr>
<td>3</td>
<td>( \text{C}_{16}:0 )</td>
<td>( H )</td>
</tr>
<tr>
<td>3</td>
<td>( \text{C}_{16}:1\Delta^9 )</td>
<td>( \text{CH}_3 )</td>
</tr>
<tr>
<td>2</td>
<td>( \text{C}_{16}:0 )</td>
<td>( \text{CH}_3 )</td>
</tr>
<tr>
<td>3</td>
<td>( \text{C}_{16}:1\Delta^9 )</td>
<td>( H )</td>
</tr>
<tr>
<td>2</td>
<td>( \text{C}_{16}:1\Delta^9 )</td>
<td>( \text{CH}_3 )</td>
</tr>
<tr>
<td>2</td>
<td>( \text{C}_{18}:1\Delta^9 )</td>
<td>( H )</td>
</tr>
<tr>
<td>2</td>
<td>( \text{C}_{18}:1\Delta^9 )</td>
<td>( \text{CH}_3 )</td>
</tr>
<tr>
<td>3</td>
<td>( \text{C}_{18}:1\Delta^9 )</td>
<td>( \text{CH}_3 )</td>
</tr>
<tr>
<td>1</td>
<td>( \text{C}_{18}:1\Delta^9 )</td>
<td>( \text{CH}_3 )</td>
</tr>
<tr>
<td>1</td>
<td>( \text{C}_{18}:0 )</td>
<td>( \text{CH}_3 )</td>
</tr>
<tr>
<td>2</td>
<td>( \text{C}_{18}:0 )</td>
<td>( \text{CH}_3 )</td>
</tr>
<tr>
<td>3</td>
<td>( \text{C}_{18}:0 )</td>
<td>( \text{CH}_3 )</td>
</tr>
</tbody>
</table>
polysaccharide from the *Shigella dysenteriae* Type 7

The high molecular weight lipopolysaccharide obtained from *Salmonella enteritidis* (virulent isolate SE6-E21 as well as avirulent isolate SE6-E5).

Polysaccharide from *Sinorhizobium fredii* HH103

It shows differences in respect of repeating unit of the polymer

Structure I:

- \( \text{[----}-2)-[\alpha-\text{TyVp}-(1\rightarrow3)]\beta-D-\text{Manp}-(1\rightarrow4)-\alpha-L-\text{Rhap}-(1\rightarrow3)-\alpha-D-\text{Galp}-(1\rightarrow) \)

Structure II:

- \( \text{[----}-2)-[\alpha-\text{TyVp}-(1\rightarrow3)]\beta-D-\text{Manp}-(1\rightarrow4)-\alpha-L-\text{Rhap}-(1\rightarrow3)-[\alpha-D-\text{GlcP}-(1\rightarrow4)]-\alpha \)

- \( -\text{D-Galp}-(1\rightarrow) \)

It consists of a homopolymer of 3:1 mixture of 5-acetamido-3,5,7,9-tetrae deoxy-7-\{(R)- and (S)-3-hydroxy butyramido\}-1-glycero-1-manno-nonulosonic acid. The monomers are linked via both glycosidic and amide linkages.
43 *Sphingomonas paucimobilis* Strain 1-886; EPS

44 *Streptococcus pneumoniae* type IV

45 Lipopolysaccharide of *Vibrio cholerae* 0139

**Abbreviations:** Fru = D-fructose; Kdo = 3-deoxy-D-manno-2-octulosonic acid, Gal = Galactose, GlcN = 2-amino-2-deoxy-D-glucose, Qui N = 2-amino-2,6-dideoxy-D-glucose. Glc = D-glucose; Hep = L-glycero-D-manno-heptose. Gal N = D-galacturonic acid; col = 3,6-dideoxy-L-xylo-hexose.
tetrasaccharide triphosphate isolated from the lipopolysaccharide of *Vibrio cholerae* 0139
oligosaccharide of *Vibrio salmonicida* (strain NCMB 2262)

\[
\begin{align*}
\alpha-D-Fucp \quad \text{(PEA)}_2 \\
\quad \downarrow \\
\alpha-D-Fucp \quad \text{NAc} \quad (1 \rightarrow 4) \quad \alpha-D-\text{Glc}p \quad (1 \rightarrow 6) \quad \beta-D-\text{Glc}p \quad (1 \rightarrow 4) \quad \alpha-D-\text{Hepp} \quad (1 \rightarrow 5) \quad \text{Kdo}
\end{align*}
\]

\[
\begin{align*}
\uparrow \\
\uparrow \\
\uparrow \\
\uparrow \\
1 \\
P
\end{align*}
\]

\[
\begin{align*}
\alpha-L-\text{Rhap} \quad (1 \rightarrow 4) \quad \alpha-D-\text{Glc}p \quad (1 \rightarrow 2) \quad \alpha-D-\text{Hepp}
\end{align*}
\]

CPS Serine NH-amide

\[
(\beta-D-\text{GlcNAc} \quad (1 \rightarrow 4) \quad \alpha-D-\text{GalA} \quad (1 \rightarrow 4) \quad \beta-L-\text{Rha} \quad (1 \rightarrow 4))
\]

\[
\alpha-\text{Mur NAc} \quad (1 \rightarrow 3)
\]

The aforesaid strains are reported to be composed of the same basic trisaccharide repeating unit of the following structure:

\[
\begin{align*}
\ldots \ldots \alpha-D-\text{Fucp} \quad \text{NAc} \quad (1 \rightarrow 2) \quad \alpha-D-\text{Hepp} \quad (1 \rightarrow 3) \quad \text{6-deoxy-\alpha-L-Talp} \quad (1 \rightarrow \ldots \ldots),
\end{align*}
\]

in which \(\alpha-D-\text{Hepp}\) is L-glycero-\(\alpha-D\)-manno-heptopyranose