

Table of Contents

1 Introduction	15
1.1 Classification of Microbial Polysaccharides.....	15
1.1.1 Extracellular polysaccharides (EPS).....	15
1.1.2 Capsular polysaccharides (CPS).....	16
1.2 Classification of Microbial Polysaccharides Based on Monosaccharide Composition and Chemical Structure	17
1.2.1 Monosaccharides, a highly diverse class of polysaccharide building blocks	17
1.2.2 Structure of bacterial exopolysaccharides	18
1.3 Physiological Role of Extracellular Polysaccharides.....	20
1.4 Biosynthesis of Extracellular Polysaccharides in Bacteria	21
1.5 Production of Bacterial Exopolysaccharides	23
1.6 Applications of Bacterial Exopolysaccharides	25
1.7 Methods to Identify the Unexplored Diversity of Microbial Exopolysaccharides.....	28
1.8 Sugar Labeling with PMP Derivatization	38
1.9 Scope of Work.....	39
2 Material and Methods.....	41
2.1 Material	41
2.1.1 Equipment.....	41
2.1.2 Software and databases.....	42
2.1.3 Special consumables.....	43
2.1.4 Enzymes and reagents	43
2.1.5 Sugar standards.....	44
2.1.6 Polymer standards.....	46
2.1.7 Primer for 16S and 5.8S rRNA gene amplification	46
2.2 Media and Buffer.....	46
2.2.1 Screening media	47
2.2.2 Trace element solution.....	47
2.2.3 Ammonium-acetate buffer pH 5.6.....	47
2.2.4 Eluent A.....	47
2.2.5 500 mM potassium phosphate buffer (pH 5.7).....	47
2.3 Microbiological and Molecular Biological Methods.....	47
2.3.1 Bacterial strains and strain storage	47
2.3.2 Strain cultivation.....	47
2.3.3 Phylogenetic analysis	48
2.3.3.1 16S and 5.8S rRNA gene amplification	48
2.3.3.2 Agarose gel electrophoresis	48
2.4 General Screening Protocol.....	48
2.4.1 General workflow of the screening platform.....	48
2.4.2 General workflow of the automated modular high throughput screening platform	49
2.5 Screening Modules.....	49
2.5.1 Visual observation of viscosity.....	49
2.5.2 Precipitation.....	49

2.5.3	Glucose-assay	49
2.5.4	Pyruvate-assay	50
2.5.5	Phenol-sulfuric-acid method.....	50
2.6	HT-PMP-method	50
2.6.1	HT-PMP-derivatization	50
2.6.2	HPLC analysis of monosaccharide-PMP-derivatives.....	50
2.6.3	ESI-ion-trap parameter	51
2.7	List of putative EPS Producers	51
3	Results.....	53
3.1	Fast Carbohydrate Analysis via Liquid Chromatography Coupled with Ultra Violet and Electro spray Ionization Ion Trap Detection in 96-well Format.....	53
3.2	High Throughput Exopolysaccharide Screening Platform: From Strain Cultivation to Monosaccharide Composition and Carbohydrate Fingerprinting in One Day.....	62
3.3	Automated Modular High Throughput Exopolysaccharide Screening Platform Coupled with Highly Sensitive Carbohydrate Fingerprint Analysis	75
4	Discussion	101
4.1	Validation of the Screening Platform by the Use of Different Bacterial Strains	101
4.2	Detailed Discussion of the Carbohydrate Fingerprints of Exemplary Strains	104
4.2.1	Proposed identification of a polymer produced from a <i>Gluconacetobacter</i> sp. strain.....	104
4.2.2	Proposed identification of the polymers produced from three <i>Rhizobia</i> strains.....	105
4.2.3	Proposed identification of different capsular polysaccharides from <i>Klebsiella</i>	107
4.3	Perspectives	109
4.3.1	Unknown sugar identification, for example for different deoxy glucoses.....	109
4.3.2	Linkage analysis and sequencing of polysaccharides.....	110
4.3.3	Further improvement of the screening platform	111
5	References	113
6	Appendix	119
7	Abbreviations.....	133
8	List of Figures	137
9	List of Tables.....	139
	Curriculum vitae	141