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High throughput exopolysaccharide screening platform: From strain cultivation to monosaccharide composition and carbohydrate fingerprinting in one day

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ABSTRACT

Microbial exopolysaccharides (EPS) are multifunctional biogenic polymers, which exist in highly diverse chemical structures. To facilitate a fast determination of the carbohydrate composition of novel isolated strains or modified EPS variants a fast screening and analytical method is required. The platform as realized and described in this article is based on the fast carbohydrate analysis via liquid chromatography coupled with ultra violet and electrospray ionization ion trap detection in 96-well format to detect different sugars, sugar derivatives and substituents such as pyruvate. Monosaccharide analysis from hydrolyzed polysaccharides was validated successfully by 16 commercially available polymers with known structure. The method is sensitive enough to distinguish various types of sphingans which solely differ in small alterations in the monomer composition. Even a quantitative detection of single monomers as present in complex plant polysaccharides like karaya gum, with the lowest recovery, was in accordance with literature. Furthermore, 94 bacterial strains for the validation of the screening platform were completely analyzed and 41 EPS producing strains were efficiently identified. Using the method a carbohydrate-fingerprint of the strains was obtained even allowing a very fast differentiation between strains belonging to the same species. This method can become a valuable tool not only in the fast analysis of strain isolates but also in the targeted screening for polysaccharides containing special rare sugars as well in the screening of strain libraries from genetic engineering for altered structures.

1. Introduction

Carbohydrate polymers play diverse, yet important roles in biological systems. One structurally very diverse class of such molecules is formed by the so called exopolysaccharides (EPS), which are produced by a wide range of bacteria, fungi, algae and archaea (Parolis et al., 1996). The natural function of these molecules typically lies in biofilm formation for the protection of the microorganisms, for the colonization of infected hosts (plants or animals) and also to mediate pathogenicity. Consequently, understanding these molecules and their production has been an important subject in medical science (Costerton, Stewart, & Greenberg, 1999), agriculture (Colegrove, 1983) and increasingly in chemical industry, where EPS as biopolymers can replace petro based polymers and such can contribute to a "greening" of the chemical industry (Baird, Sandford, & Cottrell, 1983) and (Stokes, Macakova, Chojnicka-Paszun, de Kruif, & de Jongh, 2011).

There are numerous different varieties of EPS that are distinguished by their complex structure, which is based on the different types of monosaccharides, the way these are connected and the substitutions that they are carrying. Depending on the different properties, which are conferred by the individual structures, EPS can be applied in various technical or medical fields. For example, they can be used as gelling agent, stabilizer, emulsifier, or for flocculation. Besides having unique properties they also have the advantage of being biobased with the potential to replace petro based polymers such as polyacrylates or polyvinyl alcohol (Tait & Sutherland, 2002) and (Rehm, 2010). In medical applications polysaccharides are also gaining importance e.g. as drug delivery vehicles or due to their antitumoral and antiviral activities (Sosnik, 2014) and (Zhang, Wardwell, & Bader, 2013). For the group of β-glucan polysaccharides, there is currently research taking place whether it has a positive influence on the immune system as well as anti-cancer effects (Chan, Chan, & Sze, 2009).

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It is now important to more quickly learn about the EPS offered by nature and to tap their full potential for the various fields as well as for an engineering for the formation of synthetic EPS. In order to do this, a fast and reliable method for the analysis of EPS producing strains including the identity of their polysaccharide is crucial. With this increasing importance of EPS on the one side and the ever growing collections of strains from all different habitats on the other side, such a method should be capable to quickly determine whether a certain microorganism is producing EPS in substantial amounts and to analyze the basic elements of their chemical structure.

Many different techniques have been used to verify microbial EPS production. Mucoid growth is often the method of choice for visual identification of EPS producing strains. However, often they do not show slimy growth leading to false negatives in screening approaches (Ruas-Madiedo & De los Reyes-Gavilan, 2005). Depending on the thickening characteristics of the polysaccharide, observation of viscosity in culture broth can also be an option to screen for EPS production (Garai-Ibabe et al., 2010). Precipitation with different alcohols represents a common detection, isolation and purification method for many EPS (Freitas, Alves, & Reis, 2011), however, not all EPS will easily precipitate. Taken together, these approaches have severe limitations: They are not very reliable; they do not provide quantitative data and actually do not give any information on the identity of the polymer. More thorough analyses require different purification steps for the isolation from the culture broth (e.g. cross flow filtration, protein removal, ion exchange purification) as well as extensive polymer identification (Cerning, 1990). All these steps are, however, tedious and have been performed only in low throughput.

Therefore, we intended to establish and validate a more informative high throughput EPS screening platform, for the detection and rough identification of polymers directly out of small volume cultivations. For this purpose, we combined our recently developed high throughput carbohydrate analysis method (Rühmann, Schmid, & Sieber, 2014) with micro-plate based cultivation and sample preparation to create a powerful and fast tool for screening the highly diverse field of microbial EPS (Fig. 1). The main focus of the screening is to identify novel bacterial producers of EPS in combination with the direct determination of their monomeric composition. Furthermore, the method gives a chance to get more information about already known EPS producers and the different polymers they can produce, additional to the ones already described in literature.

For the validation of this novel high throughput EPS screening platform 16 commercially available polymers with known chemical structure and over 90 putatively EPS producing strains were used and successfully analyzed.

2. Materials and methods

2.1. Chemicals and reagents

All chemicals and enzymes were, unless otherwise stated, purchased in analytical grade from Sigma-Aldrich, Carl Roth GmbH (Germany) and Merck KGaA (Germany). The commercial polysaccharides were purchased from: Jungbunzlauer (xanthan gum food grade), Colltec (welan gum Collstab W200), Kelco (gellan gum Kelco®F, diutan gum Kelco-Create®DG, Harke (succinoglycan Rheozan®, konjac gum NI-F4A0), Sigma (hyaluronic acid), Roeper (karaya gum Ceroka®), Cargill (guar gum Viscogum™MP, locust bean gum Viscogum®FA, scleroglucan Actigum®CS 6), Molekular (gum arabic), Lonza (larch gum ResistAid®), IMCD (tara gum KUW, tragacanth KUW) and Roth (xylan).

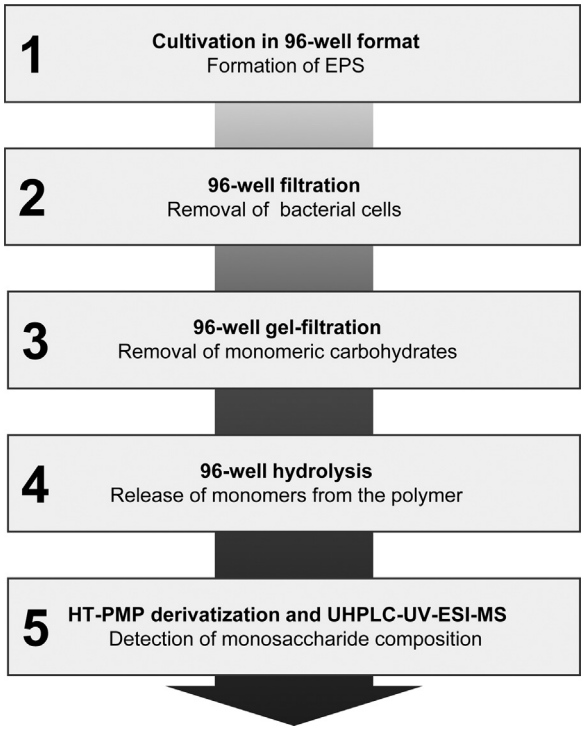

Fig. 1. Overview of the five main screening steps (arrow) of the high throughput exopolysaccharide (EPS) screening platform. After bacteria are grown in 96-well plates, cells are removed by centrifugation (step 1) and a 96-well filtration (step 2) before monomeric sugars from the growth media are removed by a 96-well gel filtration (step 3). Following the hydrolysis of the polymers (step 4), their monomeric composition can be analyzed via HT-PMP method (high throughput 1-phenyl-3-methyl-5-pyrazolone, step 5). (UHPLC–UV–ESI–MS, Ultra high performance liquid chromatography with ultra violet and electrospray ionization ion trap mass spectrometer).

2.2. Culture conditions (step 1)

The strains were stored at −80 °C in a 96-well micro titer plate with screening media containing 20% of glycerol. Precultures were grown in a 96-deep-well plate containing 1 mL screening media with 1.5 g/L casein peptone, 1.33 g/L magnesium sulfate heptahydrate, 10 g/L 3-(*N*-morpholino)-propanesulfonic acid (MOPS), 0.05 g/L calcium chloride dihydrate, 11 g/L glucose monohydrate, 1.67 g/L potassium dihydrogenphosphate, 2 mL/L RPMI 1640 vitamins solution (Sigma) and 1 mL/L trace element solution (2.5 g/L iron-(II)-sulfate heptahydrate, 2.1 g/L sodium tartrate dihydrate, 1.8 g/L manganese-(II)-chloride tetrahydrate, 0.075 g/L cobalt-(II)-chloride hexahydrate, 0.031 g/L copper-(II)-sulfate heptahydrate, 0.258 g/L boric acid, 0.023 g/L sodium molybdate and 0.021 g/L zinc chloride) in each well. Inoculation was performed with a 96-pin replicator. After incubation of the preculture for 48 h at 30 °C in a microplate shaker (1000 rpm) equipped with an incubator hood (TiMix 5 control and TH 15, Edmund Bühler GmbH). The main culture was inoculated with 10 μL of the preculture in 990 μL of fresh screening media and incubated in the same way as described above.

2.3. General workflow of the screening (step 1–4)

Removal of the cells from the main culture occurred via centrifugation at 3710 × *g* for 30 min at 20 °C. An aliquot (200 μL) of the supernatant was transferred to a 96-well filtration plate (1 μm A/B Glass, Pall Corporation) and filtered by centrifugation (3000 × *g* for 10 min at 20 °C). In the next step 30 μL of filtrated supernatant were placed in the center of each well of a gel-filtration plate (96-well SpinColumn G-25, Harvard Apparatus), that had been equilibrated by washing three times with 150 μL ammonium acetate buffer (pH