Emerging Topics in Life Sciences (2018) **2** 337–348
https://doi.org/10.1042/ETLS20180003

Review Article

Metabolic engineering of capsular polysaccharides

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With rising concerns about sustainable practices, environmental complications, and declining resources, metabolic engineers are transforming microorganisms into cellular factories for producing capsular polysaccharides (CPSs). This review provides an overview of strategies employed for the metabolic engineering of heparosan, chondroitin, hyaluronan, and polysialic acid — four CPSs that are of interest for manufacturing a variety of biomedical applications. Methods described include the exploitation of wild-type and engineered native CPS producers, as well as genetically engineered heterologous hosts developed through the improvement of naturally existing pathways or newly (*de novo*) designed ones. The implementation of methodologies like gene knockout, promoter engineering, and gene expression level control has resulted in multiple-fold improvements in CPS fermentation titers compared with wild-type strains, and substantial increases in productivity, reaching as high as 100% in some cases. Optimization of these biotechnological processes can permit the adoption of industrially competitive engineered microorganisms to replace traditional sources that are generally toxic, unreliable, and inconsistent in product quality.

Introduction

Capsules of gram-negative bacteria are made up of long polysaccharide chains known as capsular polysaccharides (CPSs) [1]. These extracellular structures are important virulence factors involved in the host defense against pathogenic bacteria [2] and can generate a highly hydrated capsular layer to minimize dehydration in the face of harsh environmental conditions and block infection by most bacteriophages [3,4].

Recently, the production of CPSs using recombinant systems has received increasing interest due to the inherent benefit of circumventing potential contamination risks and other obstacles associated with natural producers and animal sources [5]. The bacterial polysaccharides discussed in this review possess a range of valuable properties, including their high M_W (molecular weight), biocompatibility, and unique mechanical and viscoelastic properties [6], making them attractive targets for production in genetically engineered microorganisms [7]. Polysialic acid (PSA) and hyaluronan (HA) are widely used as hydrogels and biological scaffolds [8–11], while heparosan and unsulfated chondroitin are important precursors that can be chemically or enzymatically modified to heparin (HP)/heparan sulfate (HS) and chondroitin sulfate (CS), for use in anticoagulation drugs and osteoarthritis treatment [12–14].

Metabolic engineering is targeted toward overexpressing specific gene pathways while suppressing competing pathways, to increase the biosynthesis of a desired product and allow the full potential of cell metabolism to be exploited [15–17]. Target products can be efficiently manufactured by transferring product-specific enzymes or complete metabolic pathways from an often unmanageable host organism into a more easily manipulated and readily available engineered microorganism [15]. Some of the native microbial sources of CPSs of interest in this review are listed in Table 1. The expression of natural biosynthetic pathways in heterologous hosts may be preferred for several reasons, including restricted availability of genetic engineering approaches or poor growth properties of the natural host [18]. The use of 'platform cell factories' such as *Escherichia coli*, *Bacillus subtilis*, and *Corynebacterium*

Received: 10 April 2018
Revised: 11 June 2018
Accepted: 27 June 2018

Version of Record published:
11 September 2018

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Emerging Topics in Life Sciences (2018) **2** 337–348
https://doi.org/10.1042/ETLS20180003

Table 1 Occurrence and structure of CPSs from native microbial/bacterial sources

CPS	Repeat unit	Bacterium	References
Chondroitin with fructose of C3 of GlcUA	-4)-β-D-GlcA-(1 → 3)-β-D-GlcNAc-(1→ β-Fru-(1 → 3	*E. coli* K4	[93–95]
Chondroitin	[→4) β-D-(GlcA) (1 → 3) *N*-acetyl-β-D-(GalNAc) (1→]$_n$	*P. multocida* Type F	[96]
Heparosan	[→4) β-D-GlcA (1 → 4) α-D-GlcNAc (1→]$_n$	*E. coli* K5; *P. multocida* Type D	[97,98]
Hyaluronan	[→4) β-D-GlcA (1 → 3) β-D-GlcNAc (1→]$_n$	*Streptococcus Group A or C; P. multocida* Type A	[99,100]
α(2 → 8) Polysialic acid	*[→8)Neu5Acα(2 → 8)Neu5Ac(2→]$_n$*	*E. coli* K1; *N. meningitidis* B	[101–104]
α(2 → 8)/α(2 → 9) Polysialic acid	*[→9)Neu5Acα(2 → 8)Neu5Ac(2→]$_n$*	*E. coli* K92 (Bos 12)	[105,106]
α(2 → 9) Polysialic acid	*[→9)Neu5Acα(2 → 9)Neu5Ac(2→]$_n$*	*N. meningitidis* C	[104]

glutamicum [17] has the associated advantages of their well-characterized genetic and physiological properties, and a myriad of available gene expression and genome editing tools (plasmids, promoters, and terminators) [18,19].

An overview of several aspects of the metabolic engineering optimization process is shown in Figure 1. The final yield of the target product can be augmented by metabolic pathway balancing in the chosen host organism using a mix of traditional approaches like up- and down-regulation of genes and promoter engineering, as well as more contemporary methods like dynamic regulation and compartmentalization [19–21]. Synthetic biology techniques, such as a genomics, pathway prediction, and combinatorial directed evolution to improve both regulatory and pathway elements, are also becoming increasingly valuable tools for pathway optimization and metabolic engineering applications [22–25].

Furthermore, process optimization has also been extremely effective in improving CPS yields, since bacterial CPS production can vary depending on temperature, pH value, composition of the culture medium, and the control or feeding strategy for fermentation cultivations [26–29].

Capsular polysaccharides in *E. coli*

CPS transport pathways

The four groups of CPS structures in *E. coli* can be classified based on their production mechanism. Groups 1 and 4 polysaccharides are heteropolymers having highly diverse sugar patterns, and are assembled through what is known as the Wzx/Wzy-dependent pathway [3,4,6], while group 2 and 3 CPSs display wide-ranging structural variation and are assembled through an ATP-binding cassette (ABC) transporter-dependent pathway [2,30–32]. The CPSs of groups 2 and 3 are fully polymerized in the cytoplasm before being transported out of the cell. These polysaccharides are elongated through the processive action of proteins like glycosyltransferases [3,7], a mechanism that is characteristic of K antigens [31–33].

Biosynthesis and assembly

The genes responsible for bacterial CPS synthesis are often organized within capsular gene clusters in the genome of the particular production organism [7]. A comprehensive understanding of the biosynthesis processes involved is essential for developing CPS production metabolic engineering approaches.

The capsule genes in *E. coli* group 2K antigens are organized into three regions, where the central region 2 is unique to the particular CPS and directs its polymerization [4]. Region 2 is bordered by genes coding for proteins that serve as diagnostic markers for their biosynthesis systems and are preserved in different serotypes, as shown in Figure 2. The conserved genes in regions 1 and 3 are transferrable and can be switched between different *E. coli* strains with group 2 capsules. These genes function independently of the structure of the CPS and are involved in a range of activities encompassing the export and assembly of the capsule on the cell surface [3,31]. The well-maintained biosynthetic steps for *E. coli* CPSs are representative of many bacteria, and their

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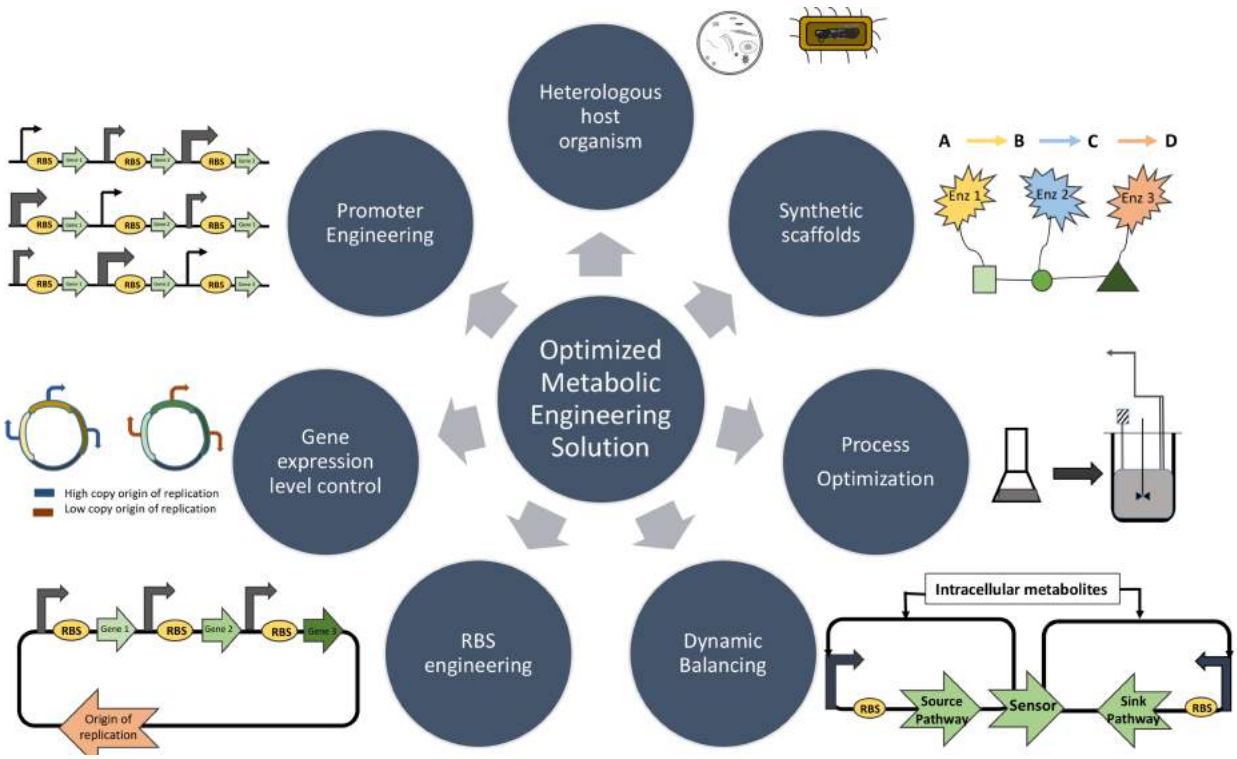

Figure 1. An overview of several tools and strategies for metabolic engineering.

These include: using heterologous host organisms which often provide many advantages over native CPS producers; dynamic metabolite monitoring and balancing through important intermediates; spatial organization of enzymes using synthetic scaffolds; RBS engineering for each pathway gene to optimize efficiency of translation; gene expression level control through promoter engineering, plasmid and DNA copy number or chromosomal integration; process optimization of fermentation conditions to maximize yield from engineered microorganisms.

proposed pathways are illustrated in Figure 2. Two activated UDP-sugar intermediates are required for biosynthesis of the GAG-like CPSs, while only one of them is needed for biosynthesis of PSA, a non-GAG CPS (Figure 2). In the CPS biosynthesis process, these UDP-sugar precursors are alternately added to the non-reducing end of the polysaccharide chain [33,34] (Figure 3).

Chondroitin

The CPSs produced by K4 *E. coli* and type F *Pasteurella multocida* are related to CS, a class of sulfated GAGs characterized by [→4) β-D-glucuronic acid (GlcA) (1 → 3) *N*-acetyl-β-D-galactosamine (GalNAc) (1→]$_n$ disaccharide repeat units [4,35,36] (Figure 3). *E. coli* K4 naturally produces a CPS possessing a disaccharide repeat unit corresponding to fructosylated chondroitin, providing a cheaper and safer alternative to animal-sourced chondroitin through microbial fermentation [37,38]. Several research groups have used metabolic engineering to increase chondroitin production by optimizing its biosynthesis process.

Native chondroitin CPS producer (*E. coli* K4)

Cimini and co-workers found that homologous overexpression of the *rfaH* gene (a positive regulator that controls K4 expression of the polysaccharide biosynthesis genes) in *E. coli* K4 leads to a substantial increase in CPS yield, through its impact on the intracellular concentration of UDP-sugar precursors [39]. Similarly, overexpressing SlyA, a transcriptional regulator, led to enhanced expression of the K4 capsule gene cluster and a higher level of fructosylated chondroitin production [40]. These methods of homologous overexpression of biosynthesis activators and regulators achieved yields of 5.3 and 2.6 g/l, respectively, representing substantial improvements on the wild-type K4 strain [36,37]. Researchers also found that homologous overexpression [41], or

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https://doi.org/10.1042/ETLS20180003

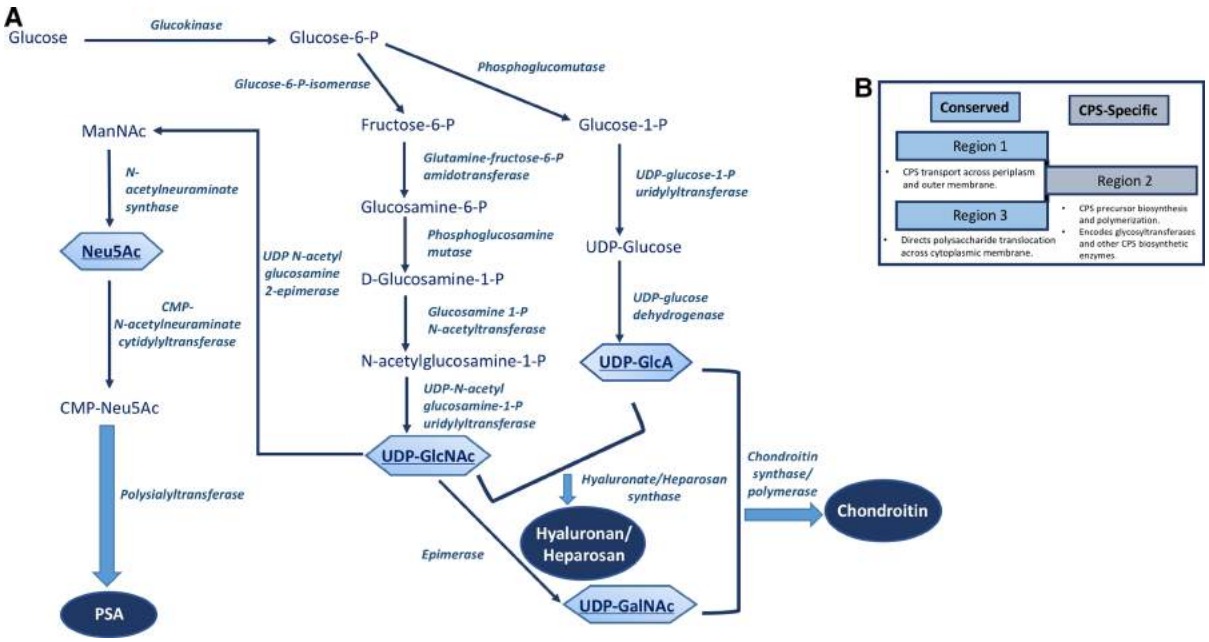

Figure 2. CPS metabolic pathways and locations of group 2 E. coli K antigen genes.

(**A**) Schematic presentation of metabolic pathways of the major CPSs discussed in the present paper and their precursors. Enzyme names are italicized, GAG precursors are underlined, and CPS products are enclosed in circles. NeuC, UDP-GlcNAc 2-epimerase; NeuB, Neu5Ac synthase; NeuA, CMP-Neu5Ac synthase; CMP-Neu5Ac, CMP-*N*-acetylneuraminic acid; NeuS, polysialyltransferase; Neu5Ac, *N*-acetylneuraminic acid; ManNAc, *N*-acetylmannosamine; GlcNAc, *N*-acetylglucosamine; GalNAc, *N*-acetylgalactosamine; GlcA, glucuronic acid; and PSA, polysialic acid. (**B**) Location of genes encoding enzymes and transport proteins for the assembly of group 2 *E. coli* K antigens for K1 (PSA), K4 (chondroitin) and K5 (heparosan). Genes encoded by regions 1 and 3 are well conserved within group 2 *E. coli* strains, while region 2 genes are unique to the specific CPS.

overexpression mediated by the IS2 transposon of *E. coli* K4 [42], of chondroitin polymerase (the *kfoC* gene), increased productivity by 100% and 2.5-fold in 22-l fed-batch, respectively, compared with wild-type *E. coli* K4. A mutation in this *kfoC* gene created a strain producing 80% more fructosylated chondroitin than the wild-type strain [43].

Introducing additional copies of *pgm* and *galU*, genes involved in biosynthesizing a biochemical precursor to UDP-GlcA, also increased polysaccharide concentrations in *E. coli* K4 [34]. Additionally, increasing UDP-GalNAc levels by using a glutamine-enriched medium correspondingly increased CPS yields [48]. Recent studies have identified an imbalanced ratio between UDP-GalNAc and UDP-GlcA in *E. coli* K4 during growth, with GlcA being present in lower amounts [45]. This challenge was circumvented by adding GlcA and GalNAc monosaccharides to the medium at the beginning of growth, leading to a 64% increase in K4 CPS production [46]. Flux balance was also successfully addressed via a *pfkA* knockout in *E. coli* K4, favoring the production of fructose-6-phosphate. This ultimately increased the ratio of intracellular UDP-GalNAc to UDP-GlcA from 0.17 in the wild-type strain to 1.05 in the engineered strain, and produced 8.43 g/l fructosylated chondroitin at a productivity of 227.84 mg/l/h [44].

Improved fermentation strategies have also led to higher K4 CPS yields, reaching concentrations of 4.73 g/l in a microfiltration bioreactor with optimized feeding strategies and aeration conditions [28].

Non-native chondroitin producers

Metabolic engineering techniques have been employed to produce chondroitin using non-native *E. coli* strains and other bacteria. Unfructosylated chondroitin production was achieved by expressing *E. coli* K4 genes in the non-pathogenic *E. coli* BL21 Star(DE3) strain [47]. The ePathBrick system, a multigene pathway manipulation tool for efficient pathway configuration optimization [48], was used to manipulate the expression levels of three

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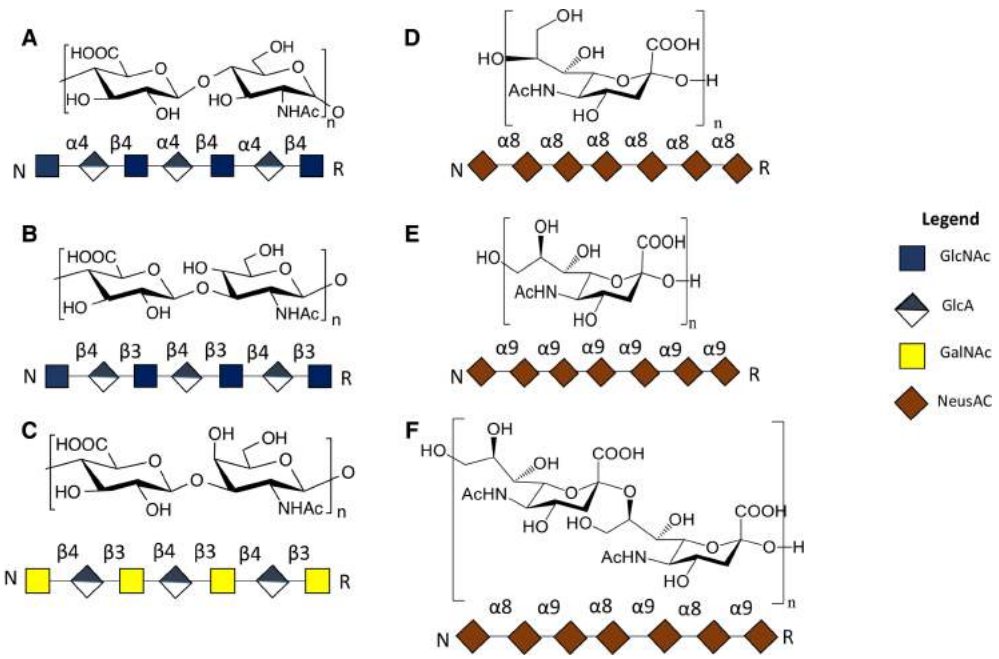

Figure 3. The chemical structures and symbolic representations of the disaccharide repeating units of CPSs in the present study.

(**A**) Heparosan: [(→4) β-D-glucuronic acid (GlcA) (1 → 4) *N*-acetyl-α-D-glucosamine (GlcNAc) (1→)]$_n$. (**B**) Hyaluronan: [→4) β-D-glucuronic acid (GlcA) (1→3) *N*-acetyl-β-D-glucosamine (GlcNAc) (1→]$_n$. (**C**) Chondroitin: [→4) β-D-glucuronic acid (GlcA) (1 → 3) *N*-acetyl-β-D-galactosamine (GalNAc) (1→]$_n$. (**D**) α(2 → 8) Polysialic acid: [→8)Neu5Acα (2 → 8)Neu5Ac(2→]$_n$. (**E**) α(2 → 9) Polysialic acid: [→9)Neu5Acα (2 → 9)Neu5Ac(2→]$_n$. (**F**) α(2 → 8)/α(2 → 9) Polysialic acid: [→9)Neu5Acα(2 → 8)Neu5Ac(2v]$_n$. N, non-reducing end; and R, reducing end. Figure adapted from Cress et al. [4].

essential chondroitin biosynthesis genes: *kfoA* [encoding the enzyme uridine diphosphate (UDP)-GlcNAc 4-epimerase, responsible for the epimerization of UDP-GlcNAc to UDP-GalNAc], *kfoC* (encoding a chondroitin polymerase that operates in a dual-action mode to transfer both GlcA and GalNAc residues to the non-reducing end of an oligosaccharide/polysaccharide acceptor), and *kfoF* [encoding the enzyme UDP-glucose dehydrogenase (UDPGDH), involved in the oxidation of UDP-glucose to UDP-GlcA] [47]. A maximum of 2.4 g/l chondroitin production was achieved using the high copy number vector, pETM6, with a pseudo-operon gene configuration, where *kfoF* was expressed most strongly. *kfoF* overexpression directed a higher flux towards UDP-GlcA synthesis, thereby limiting UDP-GalNAc production and down-regulating peptidoglycan biosynthesis — the major competing pathway for UDP-GlaNAc [47].

Unfructosylated chondroitin has also been produced in *B. subtilis*, a well-characterized Gram-positive bacterium, by integrating chondroitin synthase genes from *E. coli* K4 into the *B. subtilis* 168 chromosome. Fed-batch yields reached 5.22 g/l with the overexpression of *tauD*, a gene encoding the UDPGDH enzyme, which is strictly regulated for UDP-GlcA biosynthesis [49,50]. Together with the overexpression of *kfoA* (encoding UDP-*N*-acetylglucosamine 4-epimerase) and *glmM* (encoding phosphoglucosamine mutase), and up-regulation of biosynthesis genes using the strong P43 promoter, the fed-batch yield was further increased to 7.15 g/l [51].

Heparosan

Heparosan, an important precursor to bioengineered HP, consists of [→4) β-D-glucuronic acid (GlcA) (1 → 4) *N*-acetyl-α-D-glucosamine (GlcNAc) (1→)]$_n$ repeating disaccharide units [26] as shown in Figure 3. It is the CPS of *E. coli* K5 and *Pasteurella multocida* type D [52,53].

Native heparosan CPS producer (*E. coli* K5)

The product yield from the growth-associated capsule synthesis of heparosan in *E. coli* K5 reached as high as 15 g/l in process control optimized fed-batch fermentations [26,54,55]. The chain length of *E. coli* K5

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Emerging Topics in Life Sciences (2018) **2** 337–348
https://doi.org/10.1042/ETLS20180003

heparosan has been found to vary based on fermentation conditions [56] and can be controlled by the concentration of substrates like UDP-sugar, through type 3 synthase gene manipulations [49]. Transcription of *E. coli* K5 group 2 capsule gene clusters is regulated by nucleoid-associated proteins at 37°C and can be controlled to enhance production [54].

An analysis of *E. coli* K5 showed that UDP-GlcNAc and UDP-GlcA precursors were formed at different speeds during growth [45]. This precursor imbalance directly was found to directly affect the chain-elongation process in heparosan biosynthesis, with GlcA levels being the limiting factor [50,55,57]. Although the overexpression of genes involved in the synthesis of UDP-sugars increases the availability of precursors, higher CPS yields are not necessarily achieved [32,46]. Roman et al. demonstrated this in a study where enzymatic manipulations to increase UDP-GlcA availability resulted in lower CPS titers in *E. coli* K5 [57]. This suggests that intrinsic genetic configuration plays an important role in determining pathway output regardless of whether precursors are supplied [48].

E. coli strain Nissle 1917 has also been demonstrated to be an efficient producer of heparosan, after its genome was sequenced and shown to contain a cluster 2 that leads to heparosan biosynthesis [58].

Non-native heparosan producers

Since *E. coli* K5 is a pathogenic bacterium, non-pathogenic *E. coli* hosts have been exploited for heparosan production by exploring different combinations of overexpressed genes [26,57]. This has been accomplished by transforming a plasmid containing four *E. coli* K5 heparosan biosynthesis genes (*kfiA*, *kfiB*, *kfiC*, and *kfiD*) into *E. coli* BL21, producing 334 mg/l of heparosan [59]. An even higher yield of 1.5 g/l was achieved in BL21-derived strains when *kfiC* was fused with the *E. coli* Trigger Factor (*tig* gene) to stabilize *kfiC* [60,61]. Fed-batch cultivation of *E. coli* K-12 expressing selective K5 region 2 biosynthetic genes produced a titer of and co-expression of a K5 lyase enzymatically modified the heparosan to generate DP 2–10 oligosaccharides intracellularly [62].

Similar to chondroitin, heparosan production has been achieved in *B. subtilis* by introducing heparosan biosynthesis genes, *kfiA* and *kfiC*, into *B. subtilis* 168. Up-regulation of the *tauD* gene increased expression of UDPGDH, increasing fed-batch yields to 5.82 g/l [50]. Higher yields and M_W for heparosan from *E. coli* and *B. subtilis* were produced by using the bifunctional heparosan synthase 1 from *P. multocida* (pmHS1) instead of the *E. coli* K5 genes [63]. Through a modular approach for UDP-precursor production and pmHS1 heparosan synthesis, a titer of 237.6 mg/l heparosan was obtained using *B. subtilis* [63].

Hyaluronan

HA is composed of disaccharide repeats of D-glucuronic acid (GlcA) and *N*-acetyl-D-glucosamine (GlcNAc) joined alternately by β-1, 3 and β-1, 4 glycosidic bonds (Figure 3) [11]. The monosaccharide constituents of HA are identical with those of heparosan but their different polymerizing glycosyltransferases create distinct glycosidic linkages (β-1, 4 in heparosan) that differentiate their disaccharide structures. This unsulfated, linear, and high M_W GAG is the primary constituent of the CPS naturally produced by several microorganisms, including the well-studied bacteria *Streptococcus pyogenes* type A and C and *P. multocida* type A (Table 1) [4,64,65]. Since it does not undergo post-polymerization modifications like other GAGs, HA has been directly isolated and purified from *Streptococci* fermentation in a fully commercialized, high-titer process [14,66].

Several different microorganisms have also been engineered for HA production, including *E. coli*, *B. subtilis*, *Lactococcus lactis*, *Pichia pastoris*, and *C. glutamicum* [67–70]. Studies have shown that UDP-GlcA biosynthesis is a limiting factor for the production of extracellular polysaccharides like HA in *E. coli* [71] *and B. subtilis* [70], hence metabolic engineering approaches to enhance and tailor HA synthesis [65] have been developed by identifying a balanced availability of both precursor molecules to positively affect yield and M_W [6,60,61].

Strains of *E. coli*, *B. subtilis* and *Bacillus megaterium* have all been engineered for HA production by managing regulation through the use of altered and artificial promoters for pathway genes, such as Pgrac and T7 [21,22]. HA chain length can be modulated in *B. subtilis* by engineering and mutating regions in hyaluronan synthase (HAS) from *Streptococcus zooepidemicus* [72–74]. Furthermore, *B. subtilis* has been engineered to produce HA of controlled molecular weight during fermentation through a variety of strategies including controlling the expression level of hyaluronidase using ribosome-binding site (RBS) mutants of varying translation strength, up-regulating pathway genes but down-regulating competing glycolytic pathways, and functional leech hyaluronidase expression to resolve dissolved oxygen constraints [66,75,76].

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Engineered *C. glutamicum*, a GRAS (generally recognized as safe) organism free of exotoxins and endotoxins, was able to produce HA yields of 8.3 g/l through the artificial synthesis of HAS with codon preference of *C. glutamicum*, in conjunction with optimization of the process parameters and nutrient source [77]. HA was also produced in recombinant *E. coli* by expressing codon-optimized *Streptococcus gene* analogs coding for enzymes in the HA biosynthetic pathway, organized in an artificial operon that optimized yields [71].

Polysialic acid

PSA is a polymer of α-(2,8) and/or α-(2,9)-linked *N*-acetylneuraminic acid (Neu5Ac) and is produced as the capsules of several different bacteria [78,79]. Three PSAs have been identified in nature, differentiated based on their linkages (Figure 3) and specific sialyltransferase activities [10]. *E. coli* K1 produces an α-2,8-linked PSA that is identical with the CPS of *N. meningitidis B*. The *E. coli* K92 polysaccharide contains Neu5Ac in alternating α-2,8 and α-2,9 linkages. A CPS containing only α-2,9 linkages, such as that of *N. meningitidis C*, has not been found in *E. coli* [3].

Metabolic engineering strategies have been employed to improve bacterial PSA production. In a modified recombinant *E. coli* SA8 strain, PSA production was found to significantly improve when NeuD and NeuA, key enzymes of the PSA biosynthetic pathway, were overexpressed. By deleting *nanA*, the gene responsible for synthesizing Neu5Ac aldose, a lyase involved in Neu5Ac production, the competing catabolic pathway of Neu5Ac was blocked. This combinatorial approach led to an overall 85% PSA production increase and a final titer of 16.15 g/l in fed-batch cultivation [80].

Since the pH of the fermentation medium and its nutritional composition both significantly affect the activity of key enzymes regulating the biosynthesis of PSA in *E. coli* [81–83], several studies have focused on developing novel strategies to optimize these conditions for improved PSA production.

PSA is optimally produced by *E. coli* K92 when it is grown at 37°C [10]. This is the only wild-type strain with the ability to biosynthesize and regulate different CPSs (colonic acid and PSA) by growth temperature [84–86]. By optimizing physical and chemical aspects of the growth conditions of *E. coli* K235 and *E. coli* K1, their PSA production levels reached 1.35 and 1.5 g/l, respectively [83,87]. Additionally, the use of xylose as a substrate and casamino acids as a nitrogen source substantially increased the growth-associated production of PSA by *E. coli* K1 [27,29].

Novel strategies for fermentation process optimization like sorbitol supplementation and ammonia water feeding to control pH translated to elevated α-2,8-linked PSA yields of 5.5 g/l in recombinant *E. coli* CCTCC M208088 [8,82].

Precursor synthesis

UDP-glucose, UDP-GlCA and UDP-hexosamines are important building blocks for many CPSs (Figure 2), therefore metabolic engineering strategies for increasing the yield of these precursors are important steps toward microbial CPS production. In *E. coli* K4 and K5, CPS biosynthesis is performed by glycosyltransferase enzymes that extend the nascent chain by adding UDP-sugars to the non-reducing end. Similarly, Neu5Ac is sequentially added to the non-reducing end of growing PSA chains, with the first committed step in K1 PSA biosynthesis being catalyzed by UDP-GlcNAc 2-epimerase [4] (Figure 2).

The overexpression of pathway biosynthesis genes from *B. subtilis* and *Saccharomyces cerevisiae*, in combination with the deletion of extracellular GlcNAc importation and intracellular GlcNAc catabolism genes in *B. subtilis* yielded 5.19 g/l of GlcNAc in a fed-batch bioreactor [88]. GlcNAc titers in *B. subtilis* were similarly increased by using a DNA-guided scaffold system, which modulated the activity of key pathway enzymes for decreased cellular maintenance metabolism and high fed-batch yields of 20.6 g/l [24]. Additionally, analyses performed on *E. coli* K4 and K5 using a novel capillary electrophoresis method underlined the dependence of polymer synthesis initiation and CPS production on nucleotide sugar availability, and highlighted the key role of UDP-GlcA in regulating the polymerase enzyme [43,45].

Metabolic engineering has also been beneficial for enhancing the production of the Neu5Ac precursor for PSA biosynthesis — through various strategies including overexpression of biosynthesis pathway genes [89], heterologous pathway assembly [62,79], blocking competing pathways, and deletion/knockout of sialic acid catabolic genes [90,91].

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Emerging Topics in Life Sciences (2018) **2** 337–348
https://doi.org/10.1042/ETLS20180003

Conclusion

Heterologous hosts have been successfully harnessed for CPS production, achieving comparable, and in some cases, higher titers than native CPS producers. Though some techniques, particularly those related to oversupply of imbalanced precursors, have led to lowered CPS yields, most often metabolic engineering results in increased CPS production relative to wild-type strains, and improved production by heterologous hosts. The most promising strategies for increasing CPS production involve genetic manipulations that optimize precursor synthesis, precursor balance, and CPS polymerization, in combination with fermentation optimization. These successes suggest the need for continued and more extensive endeavors in the area of metabolic engineering for the development of economically viable CPS producers. Other tools, such as metabolic flux analysis and metabolic control analysis [92], can be employed for the future development of rational strategies for CPS yield and M_W improvement.

Summary

- Metabolic engineering strategies have enabled improvements in yield and titer for a variety of CPSs produced naturally in microorganisms.
- CPS production by native producers is shifting towards more effective heterologous host strains via heterologous pathways.
- Process optimization and precursor production significantly impact CPS yield from metabolically engineered microorganisms.

Abbreviations

ABC, ATP-binding cassette; CPS, capsular polysaccharide; CS, chondroitin sulfate; GAG, glycosaminoglycan; GalNAc, *N*-acetyl-D-galactosamine; GlcA, D-glucuronic acid; GlcNAc, *N*-acetyl-D-glucosamine; GRAS, generally recognized as safe; HA, hyaluronan; HAS, hyaluronan synthase; HP, heparin; HS, heparan sulfate; M_W, molecular weight; Neu5Ac, *N*-acetylneuraminic acid; PSA, polysialic acid; RBS, ribosome-binding site; UDP, uridine diphosphate; UDPGDH, UDP-glucose dehydrogenase.

Funding

This work was supported by the Rensselaer Polytechnic Institute Presidential Graduate Research Fellows Program.

Competing Interests

The Authors declare that there are no competing interests associated with the manuscript.

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