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Clostridium cellulolyticum: model organism of mesophilic cellulolytic clostridia

Mickaël Desvaux *

Institute for Biomedical Research, The University of Birmingham – The Medical School, Vincent Drive, Edgbaston, Birmingham B15 2TT, UK

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Abstract

Clostridium cellulolyticum ATCC 35319 is a non-ruminal mesophilic cellulolytic bacterium originally isolated from decayed grass. As with most truly cellulolytic clostridia, *C. cellulolyticum* possesses an extracellular multi-enzymatic complex, the cellulosome. The catalytic components of the cellulosome release soluble cello-oligosaccharides from cellulose providing the primary carbon substrates to support bacterial growth. As most cellulolytic bacteria, *C. cellulolyticum* was initially characterised by limited carbon consumption and subsequent limited growth in comparison to other saccharolytic clostridia. The first metabolic studies performed in batch cultures suggested nutrient(s) limitation and/or by-product(s) inhibition as the reasons for this limited growth. In most recent investigations using chemostat cultures, metabolic flux analysis suggests a self-intoxication of bacterial metabolism resulting from an inefficiently regulated carbon flow. The investigation of *C. cellulolyticum* physiology with cellobiose, as a model of soluble cellodextrin, and with pure cellulose, as a carbon source more closely related to lignocellulosic compounds, strengthen the idea of a bacterium particularly well adapted, and even restricted, to a cellulolytic lifestyle. The metabolic flux analysis from continuous cultures revealed that (i) in comparison to cellobiose, the cellulose hydrolysis by the cellulosome introduces an extra regulation of entering carbon flow resulting in globally lower metabolic fluxes on cellulose than on cellobiose, (ii) the glucose 1-phosphate/glucose 6-phosphate branch point controls the carbon flow directed towards glycolysis and dissipates carbon excess towards the formation of cellodextrins, glycogen and exopolysaccharides, (iii) the pyruvate/acetyl-CoA metabolic node is essential to the regulation of electronic and energetic fluxes. This in-depth analysis of *C. cellulolyticum* metabolism has permitted the first attempt to engineer metabolically a cellulolytic microorganism.

Keywords: Bacterial metabolism; Cellulose degradation; Cellulosome; Metabolic flux analysis; Cellulolytic clostridia; Metabolic engineering

Contents

* Tel.: +44 121 414 4368; fax: +44 121 414 3599.
E-mail address: m.desvaux@bham.ac.uk.

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1. Introduction

Cellulose is a linear insoluble biopolymer composed of the repeated union of β-D-glucopyranose linked by β-1,4 glycosidic bonds (Fig. 1(a)). Consequently, and in contrast to other glucan polymers such as starch or callose, the repeating structural unit in cellulose is not glucose but the disaccharide cellobiose. With a degree of polymerisation (DP) ranging from 2 to 7, the β-1,4 glucose oligomers, also called cellodextrins or cello- oligosaccharides, are water soluble [1]. In cellulose, the glucan chain can reach a length of more than 25,000 glucose residues [2]. The association of cellulose macromolecules leads to the formation of a microfibril containing 15–45 chains in a regular crystalline arrangement (Fig. 1(b)). At the microscopic scale, the association of these microfibrils formed a cellulose fibril also called macrofibril or fibre at the macroscopic scale [3]. Native cellulose is paracrystalline since within the microfibril alternates amorphous and crystalline regions. Moreover, cellulose fibres contain various types of irregularities such as twists or voids, which increase their total surface area.

Despite its low density, cellulose is the most prominent, resistant and stable natural-organic compound known and, as a consequence, it tends to accumulate in the environment [4]. According to the most recent estimation, the net primary production of biomass in terrestrial ecosystems would be of 60 milliard tonnes of carbon per year and about half of this carbon would be fixed under the form of cellulose [5]. It is generally assumed that cellulose is synthesised by plants [6,7] but this polymer can also be produced by some animals, algae and bacteria [8,9]. In plant, cellulose is generally associated with other biopolymers, i.e. hemicelluloses, pectins, proteins and lignin, and it then designated lignocellulose [2,10]. Depending on the plant, tissue and stage of development considered, the structural organisation and proportion of the different polymers in the lignocellulose are highly variable; in grass for example, cellulose,

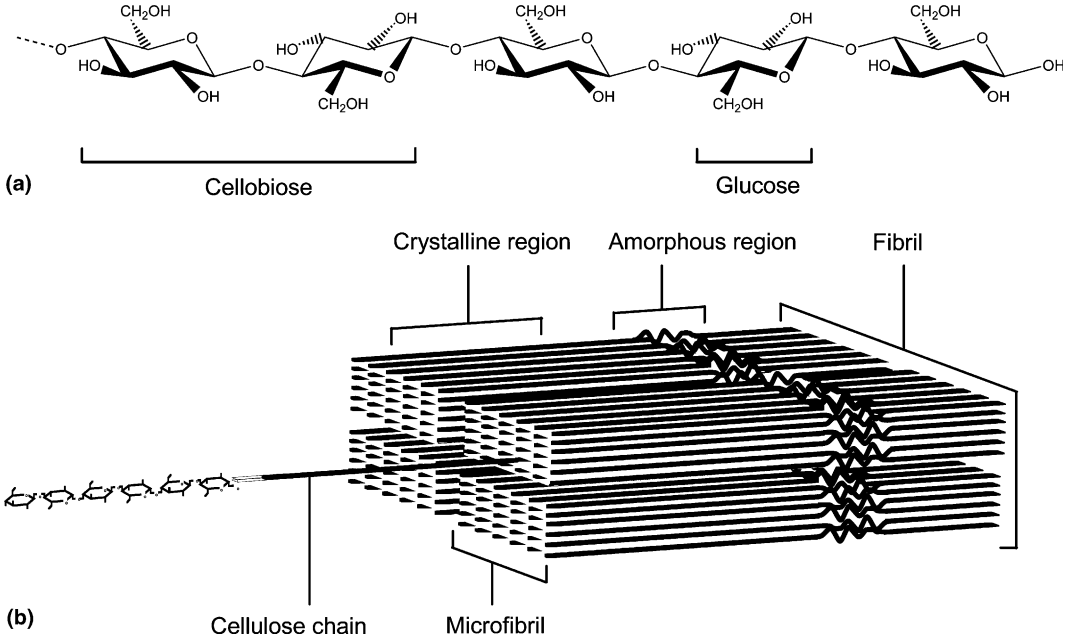

Fig. 1. (a) Scheme of the primary structure of cellulose. (b) Scheme of the structure of a cellulose fibril.