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Interrogation of *Streptomyces avermitilis* for efficient production of avermectins

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ABSTRACT

The 2015 Nobel Prize in Physiology or Medicine has been awarded to avermectins and artemisinin, respectively. Avermectins produced by *Streptomyces avermitilis* are excellent anthelmintic and potential antibiotic agents. Because wild-type strains only produce low levels of avermectins, much research effort has focused on improvements in avermectin production to meet the ever increasing demand for such compounds. This review describes the strategies that have been widely employed and the future prospects of synthetic biology applications in avermectin yield improvement. With the help of genome sequencing of *S. avermitilis* and an understanding of the avermectin biosynthetic/regulatory pathways, synthetic and systems biotechnology approaches have been applied for precision engineering. We focus on the design and synthesis of biological chassis, parts, devices, and modules from diverse microbes to reconstruct and optimize their dynamic processes, as well as predict favorable effective overproduction of avermectins by a 4Ms strategy (Mine, Model, Manipulation, and Measurement).

Introduction

Microbial natural products are valuable compounds used in agricultural, pharmaceutical, and food industries. However, there has been an industrial challenge in that wild-type strains isolated from nature usually produce low levels of these compounds that can never meet commercial demands. Avermectin and its analogs, a series of eight major 16-membered macrocyclic polyketides produced by *Streptomyces avermitilis*, are widely used in the fields of animal health and agriculture, according to their activities against a variety of nematodes and arthropod parasites, with low levels of side effects on humans.[1] Because the derivatives of avermectins lowered the incidence of River Blindness and other parasitic diseases, half of the 2015 Nobel Prize in Physiology or Medicine was awarded to avermectin discoverer, William C. Campbell and Satoshi Ōmura.[2] Avermectins contain four major (80–90%) components A1a, A2a, B1a, and B2a in varying proportions and four minor (10–20%) components A1b, A2b, B1b, and B2b,[3] among which the B1a component

Abbreviations: MDR-TB, multidrug-resistant tuberculosis; XDR-TB, extensively drug-resistant tuberculosis; MRSA, methicillin-resistant *Staphylococcus aureus*; PBD, Plackett–Burman design; DO, dissolved oxygen; OUR, oxygen uptake rate; EER, ethanol evolution rate; RF, radio frequency; APGD, atmospheric pressure glow discharge; HMGE, high-magnet gravitational environment; UV, ultraviolet rays; MMS, methyl methanesulphonate; NTG, *N*-methyl-*N*-nitro-*N*-nitrosoguanidine; NA, nitrous acid; MTP, microtiter plates; MB-CoA, 2-methybutyryl-CoA; IB-CoA, isobutyryl-CoA; MM-CoA, methylmalonyl- CoA; BCDH, branched-chain alpha-keto acid dehydrogenase; SAM, *S*-adenosylmethionine; RRF, ribosome recycling factor; GBL, *gamma*-butyrolactone; STPK, serine-threonine protein kinases; ChIP, chromatin immunoprecipitation; TAR, transformation-assisted recombination.

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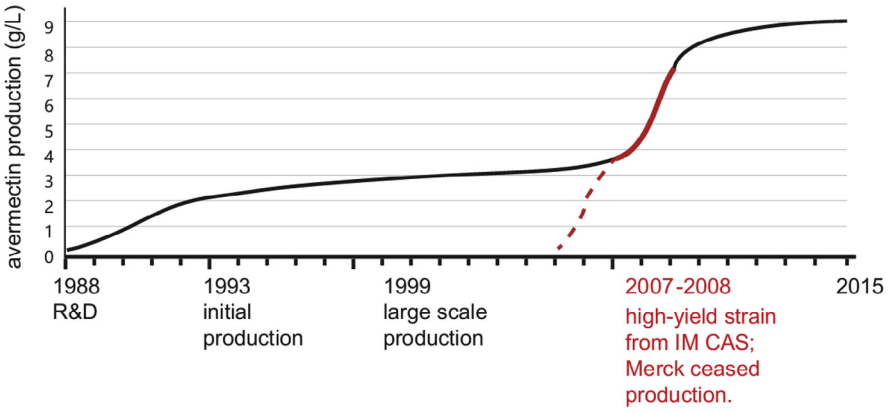

Fig. 1. Avermectin production improvement in China. The solid line indicates the avermectin production level in industry in China. The red line indicates contribution by the Institute of Microbiology, CAS. The red dashed line indicates starting from a wild type strain.

has the most effective anthelmintic activity.[4] Recently, the pharmaceutical potential of avermectins has been extended against *Mycobacterium tuberculosis*, including multidrug-resistant tuberculosis (MDR-TB) and extensively drug-resistant tuberculosis (XDR-TB),[5] as well as the synergistic effect of avermectin B1a with methicillin (MET) against methicillin-resistant *Staphylococcus aureus* (MRSA).[6,7]

The efforts to produce improved avermectins have never stopped since the discovery of avermectin by Ōmura and co-workers in 1975.[1] Avermectins were first commercialized by Merck Sharp & Dohme Research Laboratories, Kitasato Institute, and Kitasato University in 1985. China joined this campaign in 1988 and succeeded in production in 1993. Four companies for avermectin production went public during 1988–2007. The Institute of Microbiology Chinese Academy of Sciences and other institutes, with strong support from those companies, significantly increased the production of avermectin B1a with a titer from 0.009 to 9 g/L (Fig. 1). Now, China is the only avermectin producing country in the world. Avermectin is the only bio-pesticide that has an annual sale above 3 billion RMB, creating great social and economic benefits. Thus, in this review, we summarize the various strategies used to improve production of avermectins.

Improving the production of avermectin by traditional mutagenesis methods

Microbial fermentation and random mutagenesis are conventionally applied industrially to produce natural products, displaying the advantages of production improvement of the natural product by strains with little genetic information. These approaches have been applied to improve avermectin production in the fermentation industry and increased the titer to 0.5 g/L by strain selection from ultraviolet (UV) light radiation, Methyl methanesulphonate (MMS) and N-methyl-N-nitro-N-nitrosoguanidine (NTG) treatment and media modifications.[8,9]

Optimization of media and the fermentation process

A low-cost medium was developed through optimization of nitrogen and carbon sources, as well as supplementation with 0.2 mM Co^{2+}.[10] The production of avermectin B1a has increased to a titer of 0.46 g/L, which is 48.8% higher than that of the production in the original medium. Then, statistical experimental designs were used in consideration of the interactions between different factors.[11] Out of nine components, corn starch and yeast extract were found to significantly affect the production of avermectin B1a by Plackett–Burman design (PBD). The optimum values of medium composition of 149.57 g/L corn starch and 8.92 g/L yeast extract were determined.[11]

Aside from the optimization of the fermentation medium, the addition of possible precursors or stimulators of avermectin also plays an important role during the fermentation process. The influence of the addition of the possible precursors of avermectin, acetate and propionate, were investigated on two different strains.[12] The addition of 0.8% (w/w) propionate at 24 h of cultivation resulted in a 12.8–13.8% improvement in the production of avermectin B1a after 5 days of incubation. However, there was no change when propionate was added at the beginning of cultivation. Additionally, the proportion of B1a is not affected by propionate supplementation. In the case of acetate, the avermectin yield improvement was not observed when the acetate was added either at the beginning of or 24 h into cultivation.[12]

The above evidence indicates that glucose metabolism affects avermectin biosynthesis.[10,12,13] Indeed, avermectin fermentation and 6-phosphoglu-conate dehydrogenase in the pentose phosphate pathway are significantly suppressed by the addition of glucose at the early stage of fermentation.[10] Even though the involvement of the pentose phosphate pathway in avermectin production is still unclear, it may help to supply NADPH in avermectin biosynthesis (data unpublished). Avermectin production can be further increased when glucose is fed at a late stage of fermentation in the flask, bench-top, and pilot-plant scales.[10,12,13] Moreover, a B1a ratio increase by glucose feeding was observed, and the stimulation is further enhanced by controlled glucose feeding.[7,9] It has been suggested that glucose affects avermectin formation by providing additional dTDP-oleandrose, an immediate precursor of avermectin.[14,15] The B1a ratio might be changed due to the feeding of glucose, which could regulate the activity of *aveD*, a crucial gene that is responsible for the conversion between avermectin B and A types.[16–18] However, the genetic mechanism of this phenomenon is still under investigation.

Some physiological parameters also affect avermectin production by influencing cell growth, such as dissolved oxygen (DO)[19] and the oxygen uptake rate (OUR).[20] Higher DO tension (usually >20% saturation) is beneficial for pellet formation and avermectin production during submerged cultivation.[19] By controlling OUR between 15 and 20 mmol/L/hour, the production of avermectin B1a reaches 5.568 ± 0.111 g/L, which is 21.8% higher than that of the control. This indicates that the stimulatory effects on avermectin B1a production might contribute to improve the precursor supply.[20] The OUR parameter is also used to determine the glucose feeding rate in avermectin production, as well as the ethanol evolution rate (EER).[21,22]