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FEMS Microbiology Reviews 29 (2005) 49–64

FEMS MICROBIOLOGY Reviews

www.fems-microbiology.org

"*Microthrix parvicella*", a filamentous bacterium causing bulking and foaming in activated sludge systems: a review of current knowledge

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Received 12 November 2003; received in revised form 5 July 2004; accepted 13 September 2004

First published online 19 October 2004

Abstract

This review summarizes the microbiology and physiology of "*Microthrix parvicella*" and the methods of its growth control in activated sludge wastewater treatment plants. This filamentous bacterium is of high interest because of its worldwide involvement in severe bulking and foaming at wastewater treatment plants. We present a critical analysis of physiological and kinetic data on "*M. parvicella*" and discuss its growth and storage abilities in various environments with the aim of understanding the strategies of this organism to successfully compete with other bacteria in activated sludge. Additionally, this review elaborates on research needs for defining reliable control strategies of bulking and foaming based on key features of "*M. parvicella*".
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Keywords: "*Microthrix parvicella*"; Activated sludge; Bulking; Foaming; Filamentous bacteria; Control strategies

Contents

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doi:10.1016/j.femsre.2004.09.005

1. The "*Microthrix parvicella*" puzzle

"*Microthrix parvicella*" is a filamentous bacterium commonly occurring in activated sludge wastewater treatment plants (WWTPs) with different operating conditions and aeration basin configurations. Several global surveys have shown that this organism is most frequently responsible for the problems of solid–liquid separation in bulking and foaming [1–3] and substantial efforts have been directed towards solving the operational and performance problems it causes in WWTPs. Activated sludge represents a unique ecosystem and this bacterium has been detected only there. Sequences of 16S rRNA that appear in clone libraries from several other environments including marine sediments [4], soil [5,6], and marine environments [5] are only distantly related to *Candidatus* Microthrix parvicella (<92% 16S rRNA sequence similarity).

"*Microthrix parvicella*" is a long, thin (diameter of 0.6–0.8 μm), non-branched and unsheathed filamentous bacterium. Its coiled appearance and characteristic gram-positive reaction make it easy to recognize it by microscopy in activated sludge samples (Fig. 1). The organism was originally described by Pasveer [7] and while several isolates have been cultured [8–14], only a few of them are maintained in pure culture [11–13].

1.1. Isolation

Many attempts have been made to isolate "*M. parvicella*" from WWTPs and twenty years elapsed since the first isolates were obtained in the 1970s, before a taxonomically identified reference strain of "*M. parvicella*" became available [11]. The first pure culture was obtained by van Veen [8], although several earlier studies mention attempts to isolate this organism [7,15,16]. The descriptions of the filaments studied by Pasveer [7] are identical to current descriptions of "*M. parvicella*". Pure cultures exhibited filamentous morphology only in an atmosphere of 90% CO_2 + 10% air, at a pH below 6 and when glucose was the carbon source. These isolates were then "identified" as *Escherichia coli* [7] without giving any details of the identification methods used. Farquhar and Boyle [15,16] stated that their filaments resembled "filament-forming lactic acid bacteria" and they biased their isolation conditions towards favouring this group of microorganisms. None of these first reports described any difficulties in filament isolation, so it is probable that the microorganism isolated was not "*M. parvicella*". The description of "*M. parvicella*" filaments and their isolation as given by van Veen [8] is detailed, and suggest that isolation is complex and time consuming. Later Eikelboom [9] utilized a medium comprising sludge hydrolysate and a complex vitamin mixture to obtain several isolates of "*M. parvicella*". No indication of any possible difficulties in obtaining these cultures was given. The organisms grew to 1 mm colonies on the sludge agar after 10 d subculturing. The same isolation medium was used by Slijkhuis and Deinema [17] but they reported that the sludge hydrolysate contained colloidal particles, which negatively affected cell yield. Slijkhuis [10] subsequently developed a chemically defined medium for studying the pattern of carbon utilization by "*M. parvicella*". This axenic culture study generated the first physiological data on the presumptive "*M. parvicella*". In contrast to previous work [8], the isolates obtained by Slijkhuis had an unusual metabolism: they did not grow on simple substrates such as sugars and organic acids, and required oleic acid or its polyoxyethylene sorbitan ester (Tween 80) as carbon and energy source. The 'Slijkhuis isolates' were characterized only morphologically [10] and detailed biochemical properties or 16S rRNA analyses are not available to compare them to the recently isolated strains. In the next decade no other pure cultures were obtained. Slijkhuis' results had led to a widespread opinion that Tween 80 was essential substrate for growth of "*M. parvicella*", although this substrate did not support the growth of subsequent isolates.

Our comprehension of this filamentous bacterium was further complicated by description of an isolate of "*M. parvicella*" obtained by micromanipulation on Tween 80-based growth medium by Forster and coworkers [18–20]. Their isolate showed morphology that was quite different to that published for any pure culture of "*M. parvicella*". Their description of this isolate included an ability to undergo a rod-filament transition, to form spores and possess motility and to sometimes be present as gram-negative rods and then convert to gram-positive extended filaments. Most likely their report describes the wrong organism and their results have never been confirmed by any available phylogenetic analysis of the isolate nor by later work. Blackall et al. [11] suggested that this isolate was a *Bacillus* sp., and filamentous members of this genus are commonly present in activated sludge.

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