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Aerobic and anaerobic growth modes and expression of type 1 fimbriae in *Salmonella*

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Abstract

The aim of this study was to clarify the growth rates of facultatively anaerobic *Salmonella enterica* serovar Enteritidis strain in aerobic and anaerobic conditions and the expression of type 1 fimbriae in relation to the growth phases. The cultivation was carried out in a Portable Microbe Enrichment Unit (PMEU) where in same conditions one can grow the cells in parallel by modifying, e.g. aerobiosis only. The results obtained show that although the anaerobic metabolism is generally believed to be a slower producer of biomass or metabolites, in these circumstances *S. enterica* serovar Enteritidis strain gave comparable growth rates in anaerobiosis with nitrogenation as in aerobic cultures with constant aeration. Fimbrial antigens were produced in the beginning of logarithmic phase of the growth cycle both in the aerobic and anaerobic conditions. The fimbria remained in the presence of oxygen. This capability is possibly used for the intrusion of oxygen containing tissues of host body by the invading pathogens. In conclusion *S. enterica* serovar Enteritidis strain suspensions grow equally well in constant nitrogenation and aeration, and fimbria were produced in both conditions, during the early logarithmic phase but they prevailed in the presence of aeration.

Keywords: *Salmonella*; Fimbriae; Enrichment; Growth; Aerobiosis; Anaerobiosis; Facultative anaerobes; Antibodies; Pathogenesis; Gastrointestinal tract

1. Introduction

In microbial physiology and human pathophysiology especially interesting groups of prokaryotes are facultatively anaerobic bacteria [1]. They include enteric pathogens, which can use both aerobic and anaerobic modes of growth. The members of the genus *Salmonellae* exhibit a vast amount of different immunological reactivities, forming a multitude of serovars [2]. It has been generally accepted that the aerobic mode of growth and metabolism is much more effective than the anaerobic one [1]. This may not necessarily be true when the same conditions are used as the value of the key metabolite ATP is the same not depending how it is made if enough energy substrates are availabe and harmful metabolites are removed with proper gas flows, which is made possible, e.g. in the Portable Microbe Enrichment Unit (PMEU) [3].

The members of the genus *Salmonella* have genes coding at least 12 different fimbrial types, including in the *S. enterica* serovar Enteritidis SEF 14, 17, 18, and 21, long polar fimbriae (lpf) and plasmid-encoded fimbriae (pef), many of which have been associated to the bacterial virulence [4]. The SEF 21 fimbriae is corresponding to the type 1 fimbriae of *S. enterica* serovar Typhimurium [5,6]. Usually it is assumed that fimbrial expression can be provoked during the anaerobic growth mode, but this is possibly relevant only for the first phases of the growth.

When moving ahead in the host gastrointestinal tract, the invading salmonellas face after acidic stomach favourable pH and nutritional conditions in the small intestine [7,8] and form colonies, eventually biofilms on host cells in mucosal membranes [9]. Salmonellas rapidly invade the gut epithelium in

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humans. They possess several surface structures, fimbriae, for attachment. The most intensively studied fimbrial structures are the enterobacterial type 1 fimbriae, which mediate the mannose-sensitive binding of the bacterial cells to the target cells [6]. Type 1 fimbriae are straigth rod-like structures of about 1 μm in length and 7 nm thick. The fimbrins are synthesized at the cytoplasmic membrane and directed to periplasmic space, where chaperones readily bind to them [10]. The chaperones liberate the fimbrin molecules and add them to growing fimbrial filament. The fimbrial assembly occurs about 3 min after the subunit synthesis. The reservoir of the synthesized fimbrins is limited in number [11]. The fimbrial assembly is suggested to occur without protein synthesis. In static cultures the fimbriation has been suggested to offer a selective advantage for these cells by allowing them to a better access of oxygen due to cell mesh on the broth surface [12]. *Salmonella* strains in the intestine, after initial attachment, invade the body by breaking through the host membrane structures [13]. This invasion is genetically activated by the *hilA*, which is independently regulated by multiple factors [14].

The type 1 fimbriae are also important mediators of bacterial adhesion and invasion of *Escherichia coli* and other enteric bacteria [15]. The role of type 1 fimbriae in the pathogenesis of *E. coli* has recently been summarised [16]. Similar structures have also been found in other bacteria, such as *Pseudomonas aeruginosa* [17]. Depending on physiological or environmental conditions bacteria can exist in fimbriated and nonfimbriated states. The phase variation is under transcriptional control [18,19]. In earlier studies we have examined the expression of type 1 fimbriae on *Salmonella* cells by using antibodies to the fimbrin protein with enzyme immuno assay and transmission electron microscopy [20,21]. Peak immunoreactivity occurred after 3 h cultivation at 42 °C in shaken cultures. In electron micrographs, the highest numbers of fimbriae occurred at the time of peak immunoreactivity, which suggests that fimbrins were assembled during a short period of the early exponential growth phase.

Antifimbrial antibodies can be produced both against the terminal and the middle part sequences of the fimbrial proteins [20]. Synthetic fibril-forming peptides derived from *E. coli* fimbriae have been described [22] as also for bacterial flagella [23]. These antibodies produced against the fimbrin peptides usually cross-react not only with different Salmonella strains but also with some related enteric species. This remarkable cross-reactivity of Salmonella type 1 fimbrial antibodies produced with synthetic peptides is an undesired property according to conventional ideas in diagnostics. However, it could be exploited in specifying antibodies with an adequately broad spectrum for the detection of different serotypes [20]. If the antibodies produced with the synthetic peptides were not adequately species-specific, selectivity in the immunoanalysis could be achieved by controlling the enrichment conditions prior to the analysis [24].

In *E. coli*, the synthesis of mRNA coding the type 1 fimbriae takes place in the lag and early logarithmic phases [11]. In *Salmonella* sp., the expression of type 1 fimbrial antigens occurs also in an early growth phase, which indicates that these appendages are assembled quickly after the arrival of the cells to the correct growth temperature and rich nutritional conditions [20]. This quick "mobilization" of fimbriae facilitates the attachment of enterobacteria to the small intestinal epithelia soon after the cells have passed the acidic conditions of the stomach. Therefore, the understanding of fimbrial kinetics is crucial also for the understanding the pathophysiology of bacterial invasions. Unfortunately present knowledge is still too limited.

The aims of the present study have been to clarify the anaerobic and aerobic growths and type 1 fimbrin antigen production during the growth by benefitting the conditions in the PMEU to increase understanding about the pathophysiological capacity of *Salmonellae* and other facultatively anaerobic pathogenic bacteria.

2. Materials and methods

2.1. Bacterial strains and culture conditions

The strain *Salmonella* serovar Enteriditis phagetype 4 (IHS 59813) and the *Salmonella* serovar Typhimurium phagetype 1 (IHS 59929) were stored at 37 °C in TYG medium (5% tryptone, 2.5% yeast extract, 1% glucose) and seeded every 2 weeks throughout the study. Cultivation for the EIA was started with 3–4 days old starter cultures by inoculating 5% of the cultures into fresh RVS medium (Rappaport–Vassiliadis soya peptone broth, Oxoid, UK). The cultures were shaken in Erlenmeyer flasks (100 ml each) at 20 and 42 °C or in peptone water at 37 °C for inoculating the PMEU for Transia experiments, see below. Samples were stored at 4 °C before coating to microtitration plates, up to 8 h after cultivation at 42 °C and up to 24 h after cultivation at 20 °C. For the plate cultivation samples were diluted to 10^{-2}–10^{-8} with 0.9% NaCl, added to XLD-plates (xylose–lysine–deoxycholate) and incubated at 37 °C for 24 h. The number of colonies were counted and colony forming units (cfu)/ml were calculated for every time point.

2.2. Enrichment under different gas flows in the PMEU

The cultivation syringes were positioned into a Portable Microbe Enrichment Unit (PMEU) (Finnoflag Oy, Kuopio and Siilinjärvi, Finland). This equipment has been designed for enhanced cultivation of microbes under aseptic gas flow in adjusted temperatures (Fig. 1) for use in monitoring in the hospitals [3]. It has also been applied in the screening of water hygiene [25]. In the present experimentation, the PMEU unit was set to the enrichment temperature of 35–40 °C. The air flow was adjusted to 0–100% of the pump capacity in the aerobic cultivations. During the anaerobic cultures, the gas stream was visually adjusted using the valves of the pressurized gas bottles.