

# Probiotic *Escherichia coli* strain Nissle 1917 outcompetes intestinal pathogens during biofilm formation

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Many bacterial infections are associated with biofilm formation. Bacterial biofilms can develop on essentially all kinds of surfaces, producing chronic and often intractable infections. *Escherichia coli* is an important pathogen causing a wide range of gastrointestinal infections. *E. coli* strain Nissle 1917 has been used for many decades as a probiotic against a variety of intestinal disorders and is probably the best field-tested *E. coli* strain in the world. Here we have investigated the biofilm-forming capacity of Nissle 1917. We found that the strain was a good biofilm former. Not only was it significantly better at biofilm formation than enteropathogenic, enterotoxigenic and enterohaemorrhagic *E. coli* strains, it was also able to outcompete such strains during biofilm formation. The results support the notion of bacterial prophylaxis employing Nissle 1917 and may partially explain why the strain has a beneficial effect on many intestinal disorders.

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## INTRODUCTION

Many bacteria live as sessile communities adhered to surfaces, rather than as planktonic isolated cells. These compact microbial consortia, referred to as biofilms, are commonly associated with many economic and health problems (Costerton *et al.*, 1999). In medicine, biofilm-associated infections have a major impact on permanent and temporary artificial implants placed in the human body, often with devastating consequences. Many persistent and chronic bacterial infections are now believed to be linked to the formation of biofilms (Costerton *et al.*, 1995, 1999). Bacterial biofilms can develop on many living surfaces and virtually all artificial implants, producing chronic and often intractable infections. Notable biofilm-associated infections include chronic cystitis, endocarditis, otitis media, periodontitis, prostatitis, wound infections and catheter- and stent-associated infections (Warren, 2001; Costerton *et al.*, 2003; Parsek & Singh, 2003; Brady *et al.*, 2008). Bacterial biofilms have been reported to affect 90% of indwelling stents in patients (Reid *et al.*, 1992). Biofilm-forming *Escherichia coli* strains are responsible for most infections in patients with indwelling bladder catheters (Warren, 2001). Biofilm-associated bacteria are often hard to eradicate by antibiotics and can tolerate hundred- or thousandfold higher doses than the corresponding planktonic bacteria (Costerton *et al.*, 1999). *E. coli* has also been reported to be able to form intracellular biofilm-like aggregates inside

bladder cells, making them hard to reach by both host defence mechanisms and antibiotics (Anderson *et al.*, 2003).

*E. coli* strain Nissle 1917, of serotype O6:K5:H1, is an excellent colonizer of the human gut and has been reported to be able to colonize and establish itself in the human intestine even in the presence of a natural resident bacterial flora (Lodinova-Zadnikova *et al.*, 1992; Schulze & Sonnenborn, 1995; Lodinova-Zadnikova & Sonnenborn, 1997). The strain was originally isolated during World War I from a soldier who escaped a severe outbreak of diarrhoea affecting his regiment. Nissle 1917 seems to have a beneficial effect on several types of intestinal disorders and appears to be well tolerated by humans. Nissle 1917 has been marketed as a probiotic remedy against intestinal disorders in several European countries since the 1920s (Schulze & Sonnenborn, 1995). During this period of time, it has been ingested by an appreciable number of people and is probably the best 'field-tested' *E. coli* strain in humans in the world.

The large intestine and colon are the sites most heavily colonized by micro-organisms in the gastrointestinal tract. There is mounting evidence that bacterial biofilm formation plays an important role in intestinal colonization. Notably there is growing interest in biofilms in the colon with respect to their role in bowel disease (reviewed by Macfarlane & Dillon, 2007). In this background, we have studied the biofilm-forming capacity on abiotic surfaces of Nissle 1917 and compared it with a range of other intestinal strains. Notably, we have probed the ability of

Abbreviations: EPEC, enteropathogenic *E. coli*; ETEC, enterotoxigenic *E. coli*.

Nissle 1917 to compete with pathogenic strains during biofilm formation.

## METHODS

**Bacterial strains and growth media.** The strains used in this study are listed in Table 1. A streptomycin-resistant variant of Nissle 1917 (Nissle 1917*str*) was made by P1 transduction from strain BJ4*str* (Krogfelt *et al.*, 1993). Nissle 1917 and Nissle 1917*str* behaved identically under all growth conditions. All cultivations were performed in LB or AB minimal medium (Clark & Maaloe, 1967) containing 2.5 mg thiamine ml<sup>-1</sup> and 0.5% glucose (ABTG) complemented with 0.02% Casamino acids. When appropriate, antibiotics were added to the medium at the following concentrations: kanamycin, 50 µg ml<sup>-1</sup>; and streptomycin, 100 µg ml<sup>-1</sup>.

**Biofilm formation in microtitre plates.** Cells were grown over day in LB or ABTG and 10 µl was used to inoculate 1 ml medium in 24-well flat-bottom microplates (Iwaki). The microplates were incubated statically at 37 °C overnight. Unbound cells were removed by inversion of the microplate and tapping on absorbent paper; adhered cells were then stained with 0.1% crystal violet for 30 min. Excess stain was removed by washing with PBS. Crystal violet was then solubilized by the addition of ethanol and the A<sub>595</sub> was measured. Each strain was assayed in three to four wells on each plate and all experiments were repeated at least three times.

**Growth rate and carrying capacity determination in microplates.** The strains were inoculated from freshly grown overnight cultures to equal cell densities in minimal laboratory medium (ABTG supplemented with Casamino acids) and grown statically at 37 °C in microtitre plates (200 µl per well). The plate was scanned every 45 min at A<sub>595</sub>. Each strain was grown in eight wells. Growth curves were calculated from means of seven or eight wells per strain and growth rate constants (µ) were calculated from the four time points (R<sup>2</sup> > 0.995) resulting in the steepest straight slope near the start of the cultivation. The carrying capacity (maximum cell density reached at the end of the cultivation) was determined for each strain from three experiments and compared with that of Nissle 1917.

**Competition during biofilm formation in microtitre plates.** The wells of a 24-well flat-bottom microtitre plate (Iwaki) were filled with

1 ml ABTG (supplemented with 0.02% Casamino acids) and inoculated with an equal amount of cells of the two strains in competition to reach a final OD<sub>600</sub> of 0.01. After incubation at 37 °C overnight, the planktonic cells were removed and the wells were washed three times with 1 ml PBS. The adhered cells were resuspended in 1 ml PBS and the mixture was vortexed vigorously for 30 s to destroy cell aggregates. The absence of bacterial aggregates was confirmed by examination of the cell suspensions using light microscopy. The number of c.f.u. of each strain was determined both in the inoculum (to confirm the initial 1:1 ratio) and in the final biofilm population by plating serial dilutions of the cell suspension onto plain LB-agar plates and LB plates supplemented with the appropriate antibiotic. In the competition between Nissle 1917 and Eco1036, kanamycin was used, whereas in all other competitions, streptomycin was used; in competitions with strains MG1655, 1016, 1020, 1000 and H10407, the streptomycin-resistant Nissle 1917*str* was used, and in competition with F-18, the wild-type version of Nissle 1917 was used since F-18 is streptomycin resistant (see Table 1 for antibiotic resistance patterns). All competition experiments were performed in duplicate in parallel and repeated at least twice independently.

**Competition during planktonic growth.** A subset of strains were competed against Nissle 1917 during planktonic growth in shake flasks. Twenty millilitres of ABTG (supplemented with 0.02% Casamino acids) was inoculated with an equal amount of cells of the two strains in competition to reach a final OD<sub>600</sub> of 0.01. After incubation at 37 °C and 130 r.p.m. overnight, 100 µl liquid was removed and vortexed vigorously for 30 s, diluted and plated onto plain LB-agar plates and LB plates supplemented with the appropriate antibiotic. The number of c.f.u. of each strain was determined in the inocula as well to confirm the initial 1:1 ratio. All competition experiments were performed in duplicate in parallel and repeated at least twice independently.

**Biofilm formation in flow-cell chambers.** Flow-cell experiments on Nissle 1917 were performed at 37 °C in ABTG, essentially as described previously (Christensen *et al.*, 1999; Ferrières *et al.*, 2007a), with an additional overnight wash of the flow-cell system with sterile MilliQ water. Each channel was inoculated with 250 µl (final OD<sub>600</sub>=0.05) of the strain grown overnight. Biofilm formation was monitored at 24 and 48 h post-inoculation using a ×40/1.3 Plan-Neofluar oil objective and was visualized by fluorescence staining with SYTO9. Acquired pictures (at least six pictures taken randomly along

**Table 1.** Strains used in this study

<i>E. coli</i> strain	Relevant characteristics	Antibiotic resistance*	Reference
Nissle 1917	Non-pathogenic probiotic isolate (O6:K5:H1)	–	Grozdanov <i>et al.</i> (2004)
Nissle 1917 <i>str</i>	Streptomycin-resistant Nissle 1917	Strep	This study
413/89-1	Stx-producing bovine isolate (O26:H <sup>-</sup> )	Strep†	Wieler <i>et al.</i> (1996)
83972	Asymptomatic bacteriuria isolate (O?:K?:H <sup>-</sup> )	–	Hancock <i>et al.</i> (2008b)
Eco1036	Faecal commensal isolate	Strep†, Kan, Cam, Amp	
EPEC 1016/CB207	Bovine A/E isolate (O55)	–	Ebel <i>et al.</i> (1996)
EPEC 1020	Enteropathogenic isolate (O103:H2)	–	
EPEC 1000	Enterotoxigenic isolate (O78:H11)	–	
F-18	Faecal commensal isolate	Strep	Wadolowski <i>et al.</i> (1988)
BJ4 <i>str</i>	Commensal isolate		Krogfelt <i>et al.</i> (1993)
H10407	Enterotoxigenic isolate (O78:K80:H11)	Amp	Evans <i>et al.</i> (1975)
MG1655	K-12 reference strain		

\*Strep, streptomycin; Kan, kanamycin; Cam, chloramphenicol; Amp, ampicillin.

†Low resistance, i.e. produces a few colonies on LB-agar plates containing streptomycin (8–32 g l<sup>-1</sup>).

each channel) were processed for display using the IMARIS software (Bitplane). The flow-cell experiment was performed in six parallel channels and was repeated twice.

**Inhibition assay.** The ability to form colicin was tested on overnight cultures grown in LB. One hundred microlitres of overnight culture of the indicator strain was spread on minimal medium plates (ABTG supplemented with 0.02 % Casamino acids) and allowed to dry. An aliquot of 2  $\mu$ l overnight culture of the test strain was then spot-inoculated onto the plate. After overnight incubation at 37 °C, the plates were inspected for a clear zone of growth inhibition against the indicator lawn. All seven strains used for competition against Nissle 1917, i.e. MG1655, Eco1036, F-18, EPEC (enteropathogenic *E. coli*) 1016, EPEC 1020, ETEC (enterotoxigenic *E. coli*) 1000 and H10407, were spread as indicator strains with Nissle 1917 as test strain, and Nissle 1917 was spread as indicator strain with the seven strains as test strains. Each strain was tested independently twice.

## RESULTS

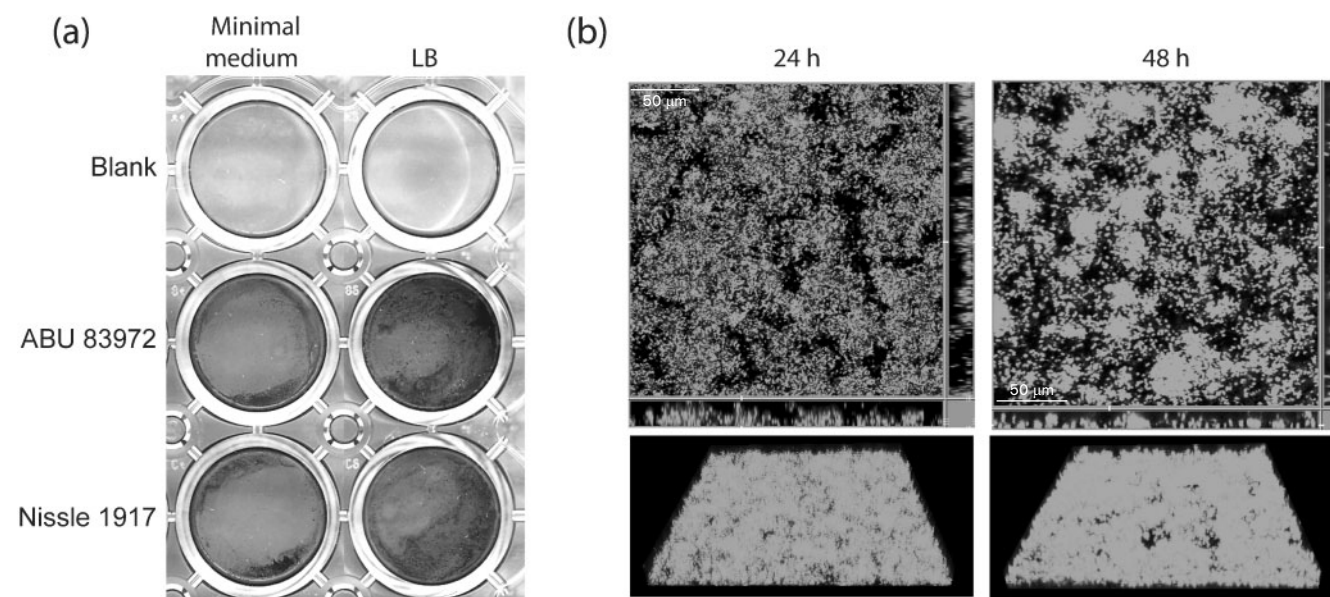
### Biofilm-forming capacity of Nissle 1917

The biofilm-forming capacity of Nissle 1917 was assessed in microtitre plates by quantitative crystal violet staining. A range of pathogenic and commensal *E. coli* strains (randomly chosen from our laboratory strain collection) were included for comparison. It transpired that the non-pathogenic probiotic isolate *E. coli* Nissle 1917 formed a biofilm comparable with that formed by another probiotic isolate – the good biofilm-forming strain 83972 (Hancock

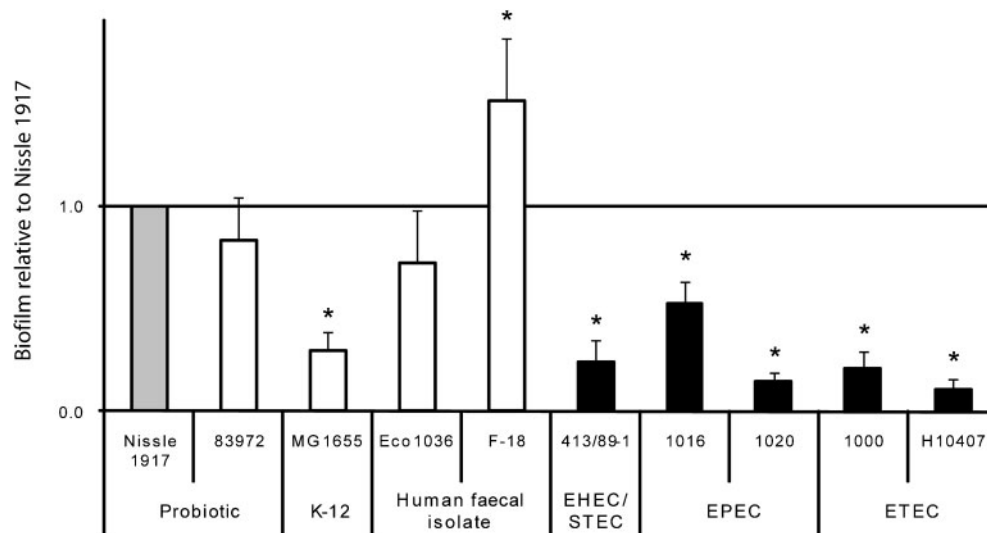
*et al.*, 2007); in minimal laboratory medium there was no difference in the biofilm-forming capacity between the two strains whereas in LB the 83972 strain performed somewhat better (Fig. 1a). The biofilm formation of Nissle 1917 in minimal media was significantly higher than that of most of the *E. coli* strains investigated; the pathogenic *E. coli* strains 413/89-1, 1016, 1020, 1000 and H10407 all formed significantly less biofilm corresponding to 11–52 % of that of Nissle 1917. The only strain outperforming Nissle 1917 was the commensal isolate F-18, which formed 51 % more biofilm than the Nissle isolate, whereas the other commensal isolate, Eco1036, showed no difference to Nissle 1917 (Fig. 2).

The ability to form a biofilm is often considered to be a virulence-associated trait. However, the biofilm results for our varied collection of commensal and pathogenic isolates did not support this. On the contrary, the four isolates forming most biofilm were all non-pathogenic (two probiotic and two commensal strains), while the five pathogenic (enterohaemorrhagic, enteropathogenic and enterotoxigenic) strains all formed significantly less biofilm than Nissle 1917 (Fig. 2).

In its natural environment, Nissle 1917 is exposed to hydrodynamic shear forces, likely to affect the development of the biofilm. Therefore, in order to mimic more realistic conditions, we investigated the ability of Nissle 1917 to form biofilm in a continuous flow-cell chamber system. The biofilm was allowed to develop on glass slides for 48 h



**Fig. 1.** (a) Biofilm formation in minimal laboratory medium (ABTG supplemented with Casamino acids) and LB of the two probiotic *E. coli* isolates ABU 83972 and Nissle 1917. Biofilms were grown in microtitre plates for 16 h, stained with crystal violet and subsequently washed. The microtitre plate was then scanned using an HP Scanjet 4800. (b) Biofilm growth of Nissle 1917 in flow-cell chambers in ABTG minimal medium monitored 24 and 48 h post-inoculum by confocal laser scanning microscopy. Orthogonal cross-sections through the biofilm were generated and simulated *xz*- and *yz*-vertical cross-sections are presented (upper panels), as well as 3D projections (lower panels). Scale bars, 50  $\mu$ m.



**Fig. 2.** Biofilm formation in minimal laboratory medium (ABTG supplemented with Casamino acids) of nine pathogenic (black bars) and non-pathogenic (white bars) *E. coli* strains compared with that of Nissle 1917 (grey bar). Biofilms were grown in microtitre plates and quantified by crystal violet staining. The  $A_{590}$  value for each strain is reported following standardization by the  $A_{590}$  value of the probiotic Nissle 1917 isolate. Asterisks indicate strains that formed significantly more or less biofilm than Nissle 1917 (paired two-tailed  $t$  test,  $P < 0.05$ ). The data shown are means of at least three independent experiments; the error bars indicate standard deviation.

and was monitored by confocal microscopy. It turned out that Nissle was a good biofilm former under these conditions also (Fig. 1b); the biofilm structure produced 24 h after inoculation was highly comparable to that of the commensal-like *E. coli* strains 83972 and VR50 observed earlier (Hancock *et al.*, 2007).

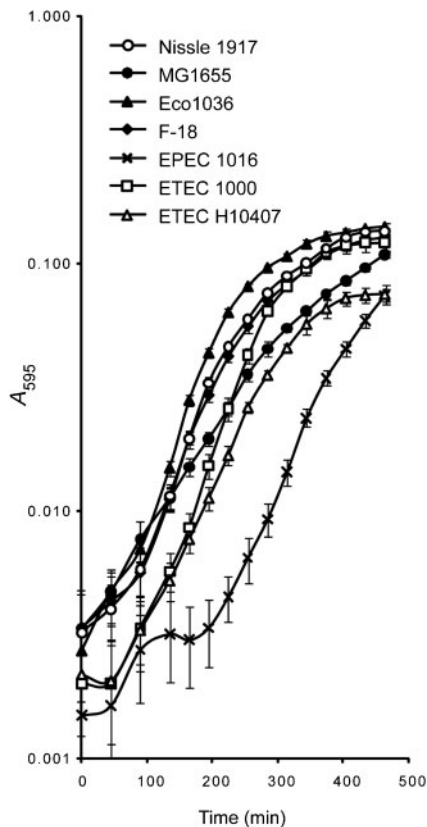
We also compared the growth of seven of the *E. coli* strains in minimal laboratory medium in microtitre plates (Fig. 3). It appeared that Nissle 1917 was a comparably fast grower in this medium and had a high growth rate compared with many of the other *E. coli* strains; Nissle 1917 displayed a growth rate that was 48% higher than that of the K-12 strain MG1655. However, two strains, Eco1036 and the ETEC isolate 1000, had growth rates that were 17% higher than that of Nissle 1917. Interestingly, despite the different growth rates of the seven *E. coli* strains, there was only one strain that had a carrying capacity (i.e. maximum cell density) that was significantly different from that of Nissle 1917; the ETEC strain H10407 reached final cell optical densities that were only 62% of that of Nissle 1917. Comparing the biofilm-forming capacity of the strains (Fig. 2) with their growth rates (Fig. 3) suggests no correlation between the ability to form a good biofilm and fast growth, measured under the same experimental conditions (i.e. statically in minimal laboratory medium in microtitre plates). It should be noted that the growth rates were determined *in situ* in microtitre plates under the same conditions as used for the biofilm competition experiments, i.e. static conditions at 37 °C. Consequently, some cells will be adherent to the walls of the well as

biofilm wherefore the growth rates determined may not represent absolute growth rates.

### Nissle 1917 outcompetes a range of pathogenic and commensal *E. coli* isolates during biofilm formation

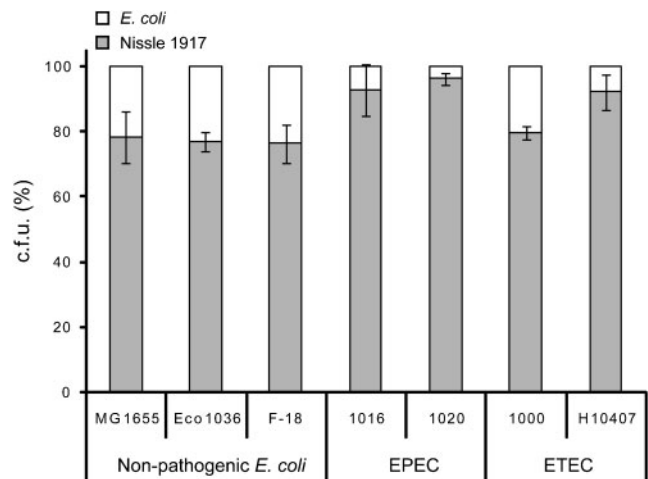
Having analysed the biofilm-forming capacity of a range of different *E. coli* isolates, we proceeded with investigating whether Nissle 1917 could outperform the other *E. coli* strains during biofilm formation. Consequently, we pitted Nissle 1917 against seven of the nine *E. coli* strains tested for biofilm formation (Fig. 2) in a 1:1 starting ratio (all gut isolates were included, with the exception of 413/89-1, which could not be distinguished from Nissle 1917 due to the resistance pattern). It transpired that, in all cases, Nissle 1917 contributed to more than 76% of the final biofilm population, consisting of up to 96% of the final population (Fig. 4). Interestingly, Nissle 1917 proved to be most efficient against the pathogenic isolates, i.e. ETEC and EPEC strains, where the final biofilm populations consisted of 79–96% Nissle 1917; while Nissle 1917 was somewhat less efficient against the three non-pathogenic isolates, i.e. MG1655, Eco1036 and F-18, where Nissle 1917 corresponded to 76–78% of the population at the end of the experiment.

Interestingly, Nissle 1917 was able to outcompete the only strain that was a significantly better biofilm former than Nissle 1917 when grown alone, i.e. F-18; after 16 h of competition the final biofilm population consisted of 76%



**Fig. 3.** Growth curves of Nissle 1917 and six of the *E. coli* isolates that were competed against Nissle 1917. The strains were grown statically in microtitre plates in minimal laboratory medium (ABTG supplemented with Casamino acids). Each curve represents the mean of seven or eight wells and error bars indicate standard deviation. Growth rates were determined and compared with that of Nissle 1917; strains Eco1036 and ETEC 1000 displayed growth rates 17% higher than that of Nissle 1917, while strains MG1655 and H10407 had growth rates that were 67 and 76% of that of Nissle 1917 (unpaired two-tailed *t* test,  $P < 0.05$ ).

Nissle 1917 (compare Figs 2 and 4). This supports our previous observations that the ability to form a biofilm does not directly correlate with the ability to outcompete other strains in mixed populations (Ferrières *et al.*, 2007a, b). The data suggest that the ability of Nissle 1917 to outperform other *E. coli* strains during biofilm formation is not solely linked to the individual biofilm-forming capacity of the two strains. In agreement with the present study, we have in several other studies found no correlation between the ability to form a strong biofilm and the fitness characteristics, i.e. growth rate and carrying capacity, comparing several different *E. coli* and *Klebsiella* isolates; furthermore, no correlation between growth rate and the ability to compete in the biofilm growth mode was observed (Ferrières *et al.*, 2007a, b; Hancock *et al.*, 2007). In the present study, despite the fact that the two strains Eco1036 and ETEC 1000 both displayed growth rates 17% higher than that of Nissle 1917, Nissle 1917 was still able to



**Fig. 4.** Competition between the probiotic *E. coli* Nissle 1917 strain and seven pathogenic and non-pathogenic *E. coli* isolates during biofilm formation. An equal number of cells of Nissle 1917 and the competing strain were mixed in minimal laboratory medium (ABTG supplemented with Casamino acids) and incubated statically at 37 °C in microtitre plates. After 16 h, cells from the biofilm formed were harvested and plated out on plain LB-agar plates and on selective LB-agar plates to determine the percentage of c.f.u. of each strain in the mixed-biofilm population. The data shown are means of four values from two independent experiments, each in duplicate.

outcompete the two strains constituting 77 and 79%, respectively, of the final biofilm population (Figs 3 and 4). In our hands, the probiotic Nissle 1917 strain was able to outcompete a whole range of commensal and pathogenic *E. coli* isolates – proving most efficient against the pathogens – suggesting one of the possible mechanisms by which this strain is able to provide the beneficial effects observed when it is administered to patients with intestinal disorders.

#### The ability of Nissle 1917 to outcompete other strains does not depend on production of colicins

Nissle 1917 has been reported to produce colicin H and microcins H47 and M (Patzer *et al.*, 2003). To investigate whether these could be responsible for the ability of Nissle 1917 to outcompete other strains in this study, the seven outcompeted strains (Fig. 4) were used as indicator strains as confluent lawns on plates, in the exact same medium that was used in the biofilm competition experiments; Nissle 1917 was then spot-inoculated onto the plates. None of the indicator strains showed any inhibitory zones after incubation overnight at 37 °C. However, when the situation was reversed and Nissle 1917 was used as indicator strain and the other strains were spot-inoculated onto the plates, Nissle 1917 showed growth inhibition zones (2–3 mm) around the spots where strains F-18 and Eco1036 had been applied. Taken together, under the given growth

conditions Nissle 1917 does not seem to have an advantage over the seven strains tested by virtue of colicin or microcin production. Arguably, its ability to outcompete other strains during biofilm formation depends on other, presumably biofilm-specific, factors.

### Competition during biofilm growth showed no correlation to competition during the planktonic growth phase

Competitions in shaking cultures were performed to investigate whether Nissle 1917 is also able to outcompete strains in the planktonic phase of growth. Three of the strains performed similarly during planktonic competition compared with biofilm competition; in planktonic competitions with strains MG1655, EPEC 1020 and ETEC H10407, the cultures contained 78 %, 96 % and 88 % of Nissle 1917 after overnight growth (compare Fig. 4). One strain, i.e. ETEC 1000, was outcompeted to a higher degree in the planktonic growth mode than in biofilm growth; Nissle constituted 91 % of the population after overnight competition. Two of the strains that were competed against Nissle 1917, i.e. Eco1036 and F-18, were able to outcompete Nissle in planktonic growth although they were outcompeted by Nissle in the biofilm growth phase; the planktonic cultures contained only 13 % and 20 % Nissle 1917 after overnight competition (compare Fig. 4). Taken together, the results reveal that there is no correlation between competition during biofilm growth and competition during planktonic growth; Nissle 1917 was able to outcompete all strains in the biofilm growth phase, but failed to do so in the planktonic growth phase.

## DISCUSSION

Probiotic bacteria are usually defined as organisms that are able to confer health benefits to the host. In human health-care, the beneficial effects of various probiotic bacteria have been demonstrated over the years for the treatment of various disorders, for example the side effects of antibiotic treatment (Cremonini *et al.*, 2002) and urinary tract infections (Darouiche *et al.*, 2005; Sundén *et al.*, 2006). Bacterial interference is based on the concept that one bacterial strain can interfere with the ability of another to colonize and infect the host. The use of commensal-type bacteria to inhibit pathogens has a large potential because such bacteria are often natural competitors of pathogens, they are usually well tolerated by the host and they are easy to administer. Applications of commensal-type bacteria as probiotics have been shown to reduce the risk of infection in the gastrointestinal system and the urinary tract (Reid *et al.*, 2001). However, the mode of action employed by the commensals to outcompete the pathogens is often unknown. Nissle 1917 seems to have a positive effect on a number of intestinal disorders, such as colitis and diarrhoea in children, inflammatory bowel disease and ulcerative colitis (Lodinova-Zadnikova *et al.*, 1992; Schulze

& Sonnenborn, 1995; Sonnenborn & Schulze, 1997; Schultz *et al.*, 2004). Specifically, the strain has been reported to prevent colonization of the infant intestine by microbial pathogens (Lodinova-Zadnikova *et al.*, 1992; Lodinova-Zadnikova & Sonnenborn, 1997). Here we investigated the biofilm-forming properties of Nissle 1917 and its ability to compete with various intestinal *E. coli* strains of commensal and pathogenic origin during growth and biofilm formation.

The biofilm mode of bacterial growth generally confers a number of advantages such as improved niche colonization, resistance to hydrodynamic shear forces as well as tolerance to a range of antimicrobial agents. We have previously demonstrated that urinary tract infectious strains compete intensely for dominance in multi-strain biofilms (Ferrières *et al.*, 2007a, b). Nissle 1917 turned out to be an excellent biofilm former both on plastic (microtitre plates) and glass surfaces (flow-cell chambers); it also did so under both static and hydrodynamic flow conditions. These assays are generally accepted and widely used to monitor bacterial biofilm formation. In biofilm competition experiments, Nissle 1917 performed well against a range of other gut-colonizing *E. coli* strains including both commensals and pathogens. Interestingly, Nissle 1917 was able to outcompete a range of intestinal pathogenic *E. coli* strains of both ETEC and EPEC types. In some cases, Nissle 1917 completely dominated the biofilm and constituted up to 96 % of the biofilm-dwelling population at the end of the experiment; in competition against the enterotoxin-producing *E. coli* strain H10407, isolated from a case of cholera-like diarrhoea, Nissle constituted 92 % of the biofilm after 16 h of competition. Although Nissle 1917 is known to produce colicins (Patzner *et al.*, 2003), testing the seven outcompeted *E. coli* strains (Fig. 4) against Nissle 1917 for inhibition growth zones showed no evidence that Nissle 1917 possessed any advantage over the strains in this respect. On the contrary, two of the competing strains were able to inhibit growth of Nissle 1917 in this assay.

Nissle 1917 harbours a large number of genes encoding fitness/virulence factors. It can express several adhesins such as type 1 fimbriae, F1C fimbriae and Ag43, and it strongly produces curli and cellulose in a temperature-independent manner, several proteases and an impressive array of iron acquisition systems (Blum *et al.*, 1995; Grozdanov *et al.*, 2004). Several of these, i.e. type 1 and F1C fimbriae, Ag43, curli and cellulose, have been implicated in biofilm formation (Pratt & Kolter, 1998; Kjaergaard *et al.*, 2000; Prigent-Combaret *et al.*, 2000; Da Re & Ghigo, 2006; Lasaro *et al.*, 2009). Recently, we demonstrated that the ferric iron uptake system comprising yersiniabactin and its cognate uptake system, FyuA, are important during biofilm formation under iron-limiting conditions (Hancock *et al.*, 2008a). Nissle 1917 has the yersiniabactin system (Blum *et al.*, 1995; Grozdanov *et al.*, 2004). At present it is not possible to say which of these, or probably which combination of these, is responsible for the

good biofilm performance of Nissle 1917. However, its good performance with respect to biofilm formation and its ability to dominate during biofilm formation might account for its well-known ability to suppress intestinal disorders by virtue of its ability to outcompete pathogens. It is becoming clear that biofilm formation is not necessarily associated with virulence but rather seems to be a niche-fitness associated trait. Efficient biofilm formation can give commensal/probiotic-type strains an edge over pathogens as previously demonstrated in the case of the asymptomatic bacteriuria (ABU) strain 83972 (Ferrières *et al.*, 2007a) and in this study with Nissle 1917.

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