US 20090318382A1

(19) **United States**

(12) **Patent Application Publication**

(10) **Pub. No.: US 2009/0318382 A1**

(43) **Pub. Date: Dec. 24, 2009**

Ghigo et al.

(54) **USE OF BACTERIAL POLYSACCHARIDES FOR BIOFILM INHIBITION**

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(21) Appl. No.: **12/307,045**

(22) PCT Filed: **Jun. 25, 2007**

(86) PCT No.: **PCT/IB07/02875**

§ 371 (c)(1),
(2), (4) Date: **Apr. 17, 2009**

(30) **Foreign Application Priority Data**

Jun. 30, 2006 (EP) 06291080.7

Publication Classification

(51) **Int. Cl.**

A01N 43/04	(2006.01)
C08B 37/00	(2006.01)
C07H 1/00	(2006.01)
A61L 29/16	(2006.01)

(52) **U.S. Cl.** **514/54**; 435/274; 536/123.1; 604/265

(57) **ABSTRACT**

A method comprises preventing or inhibiting bacterial adhesion and/or bacterial biofilm development by treating a substrate with a composition of a soluble group II capsular polysaccharide obtained from a bacterial strain.

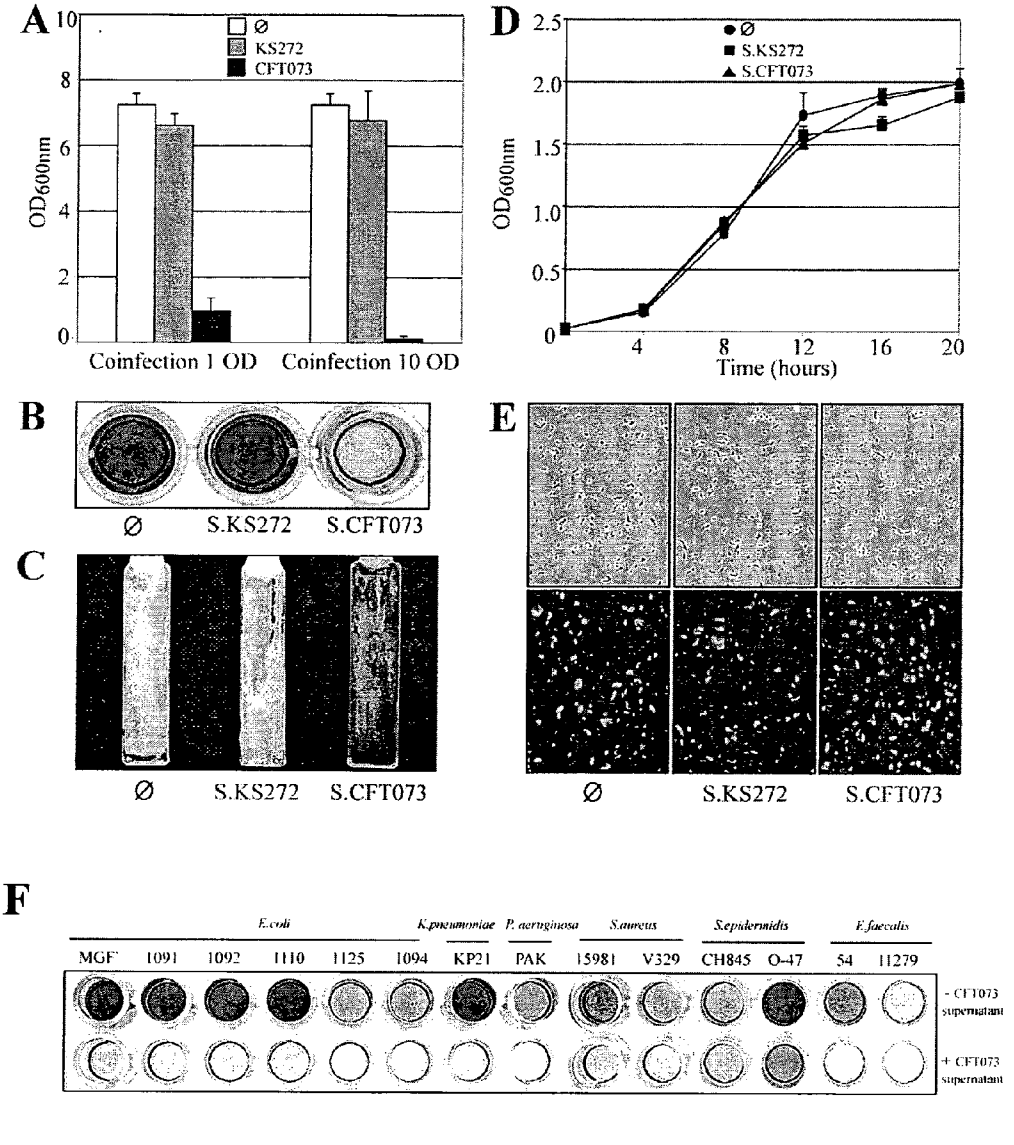
A
Ø
KS272
CFT073
OD600nm
Coinfection 1 OD
Coinfection 10 OD
D
Ø
S.KS272
S.CFT073
OD600nm
Time (hours)
B
Ø
S.KS272
S.CFT073
C
Ø
S.KS272
S.CFT073
E
Ø
S.KS272
S.CFT073
F
E.coli
K.pneumoniae
P. aeruginosa
S.aureus
S.epidermidis
E.faecalis
MGF
1091
1092
1110
1125
1094
KP21
PAK
15981
V329
CH845
O-47
54
11279
+ CFT073 supernatant
+ CFT073 supernatant

Figure 1

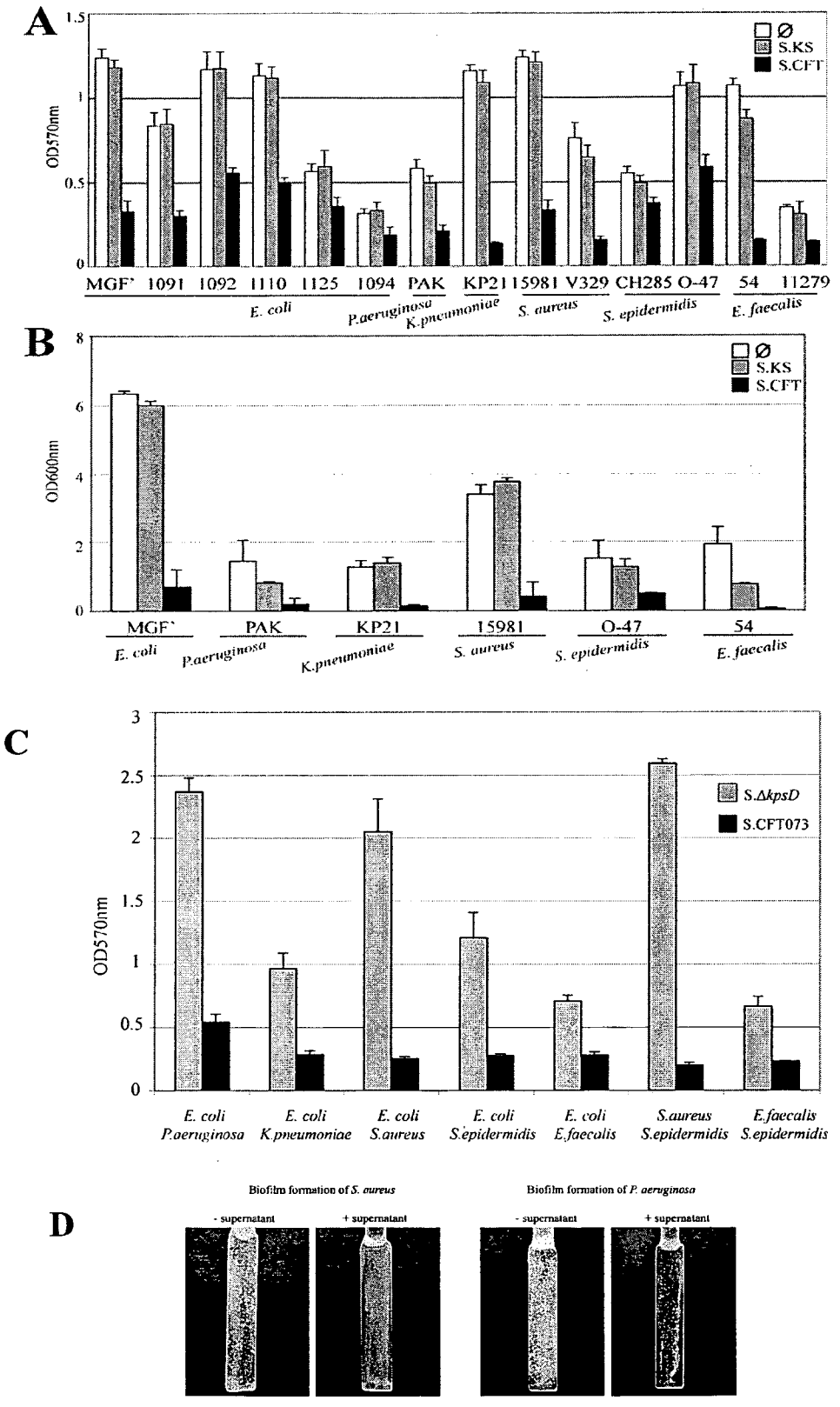
A
OD570nm
1.5
1
0.5
0
Ø
S.KS
S.CFT
MGF' 1091 1092 1110 1125 1094 PAK KP21 15981 V329 CH285 O-47 54 11279
E. coli
P.aeruginosa
K.pneumoniae
S. aureus
S. epidermidis
E. faecalis
B
OD600nm
8
6
4
2
0
Ø
S.KS
S.CFT
MGF'
PAK
KP21
15981
O-47
54
E. coli
P.aeruginosa
K.pneumoniae
S. aureus
S. epidermidis
E. faecalis
C
OD570nm
3
2.5
2
1.5
1
0.5
0
S.ΔkpsD
S.CFT073
E. coli P.aeruginosa
E. coli K.pneumoniae
E. coli S.aureus
E. coli S.epidermidis
E. coli E.faecalis
S.aureus S.epidermidis
E.faecalis S.epidermidis
D
Biofilm formation of S. aureus
- supernatant
+ supernatant
Biofilm formation of P. aeruginosa
- supernatant
+ supernatant

Figure 2

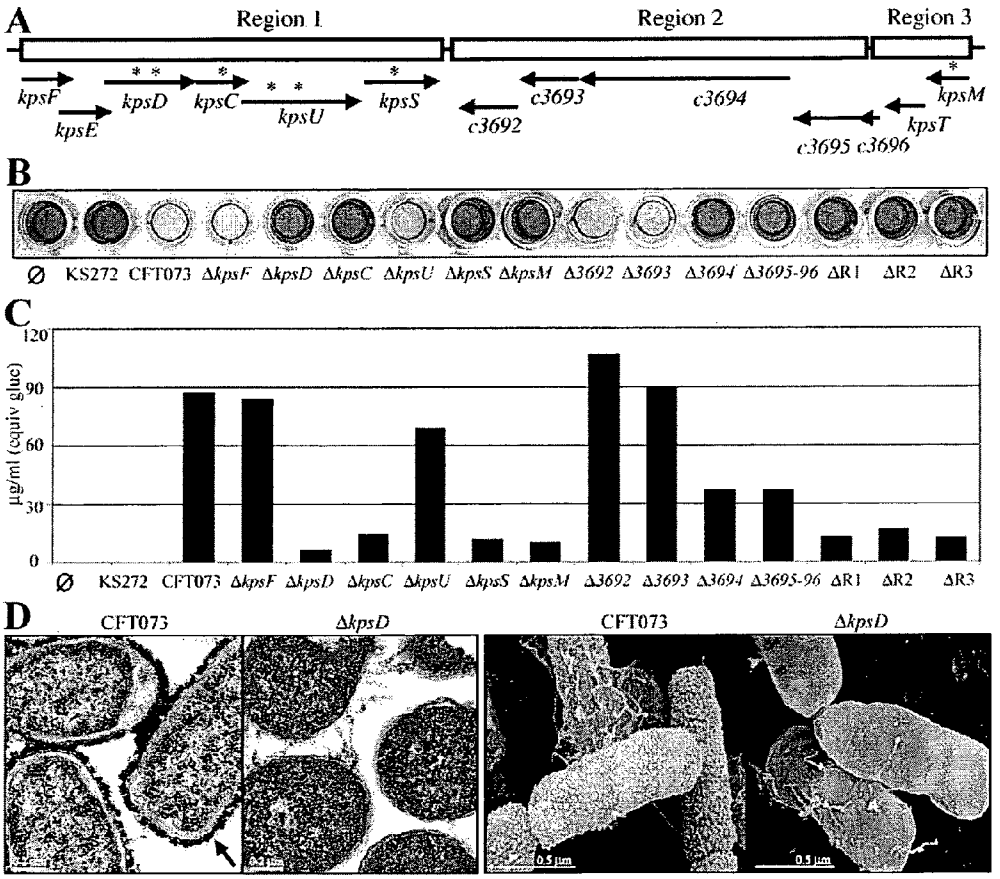
A
Region 1
Region 2
Region 3
kpsF
kpsE
kpsD
kpsC
kpsU
kpsS
c3692
c3693
c3694
c3695 c3696
kpsT
kpsM
B
∅ KS272 CFT073 ΔkpsF ΔkpsD ΔkpsC ΔkpsU ΔkpsS ΔkpsM Δ3692 Δ3693 Δ3694 Δ3695-96 ΔR1 ΔR2 ΔR3
C
µg/ml (equiv gluc)
120
90
60
30
0
∅ KS272 CFT073 ΔkpsF ΔkpsD ΔkpsC ΔkpsU ΔkpsS ΔkpsM Δ3692 Δ3693 Δ3694 Δ3695-96 ΔR1 ΔR2 ΔR3
D
CFT073
ΔkpsD
CFT073
ΔkpsD
0.5 µm
0.5 µm

Figure 3

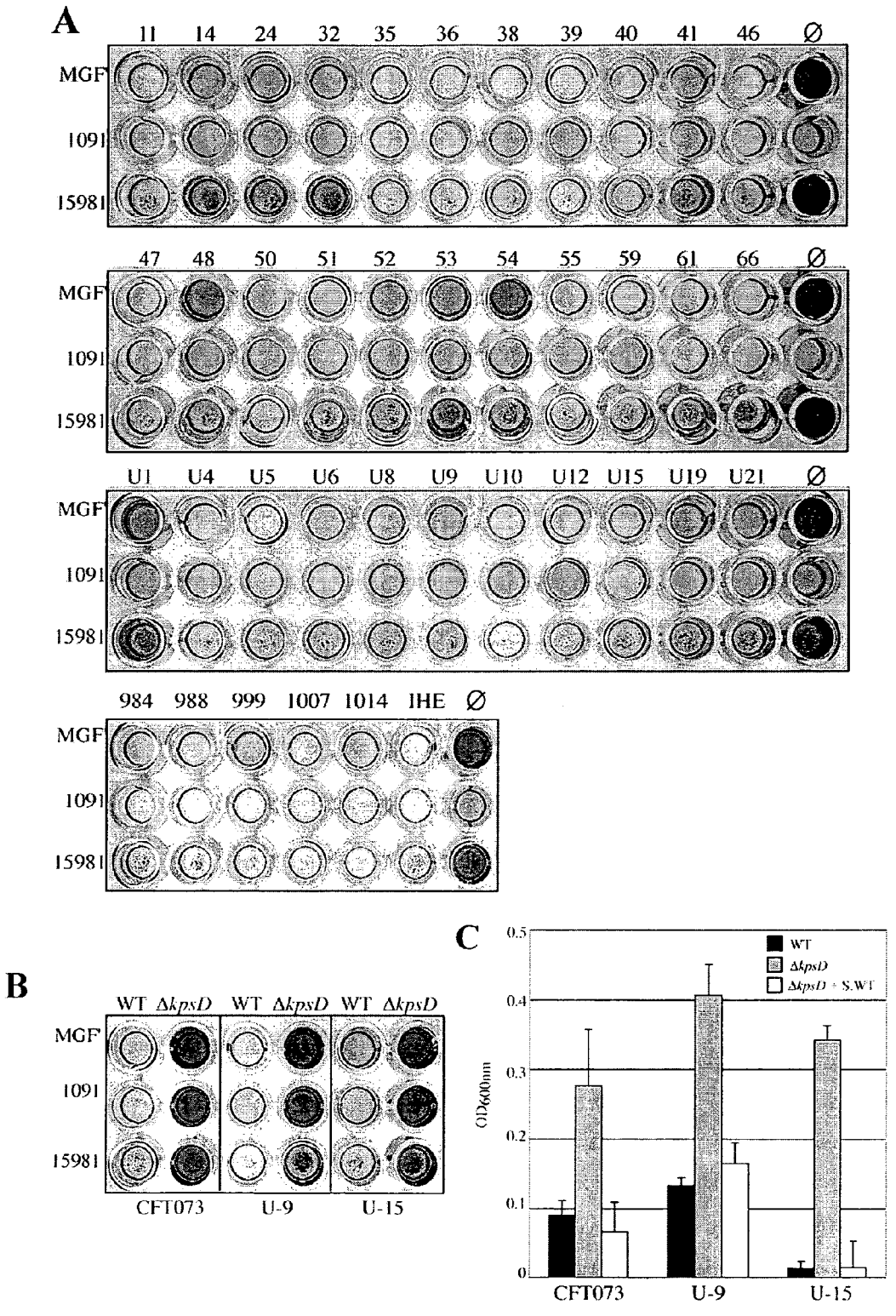
A
11 14 24 32 35 36 38 39 40 41 46 Ø
MGF
1091
15981
47 48 50 51 52 53 54 55 59 61 66 Ø
MGF
1091
15981
U1 U4 U5 U6 U8 U9 U10 U12 U15 U19 U21 Ø
MGF
1091
15981
984 988 999 1007 1014 IHE Ø
MGF
1091
15981
B
WT ΔkpsD WT ΔkpsD WT ΔkpsD
MGF
1091
15981
CFT073 U-9 U-15
C
WT
ΔkpsD
ΔkpsD + S.WT
OD600nm
0.5
0.4
0.3
0.2
0.1
0
CFT073 U-9 U-15

Figure 4

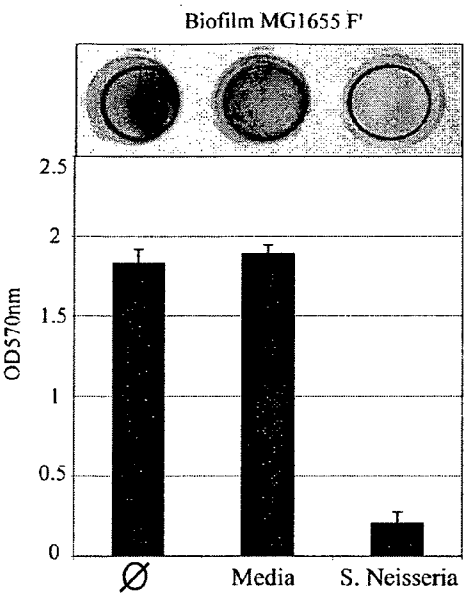

Figure 5

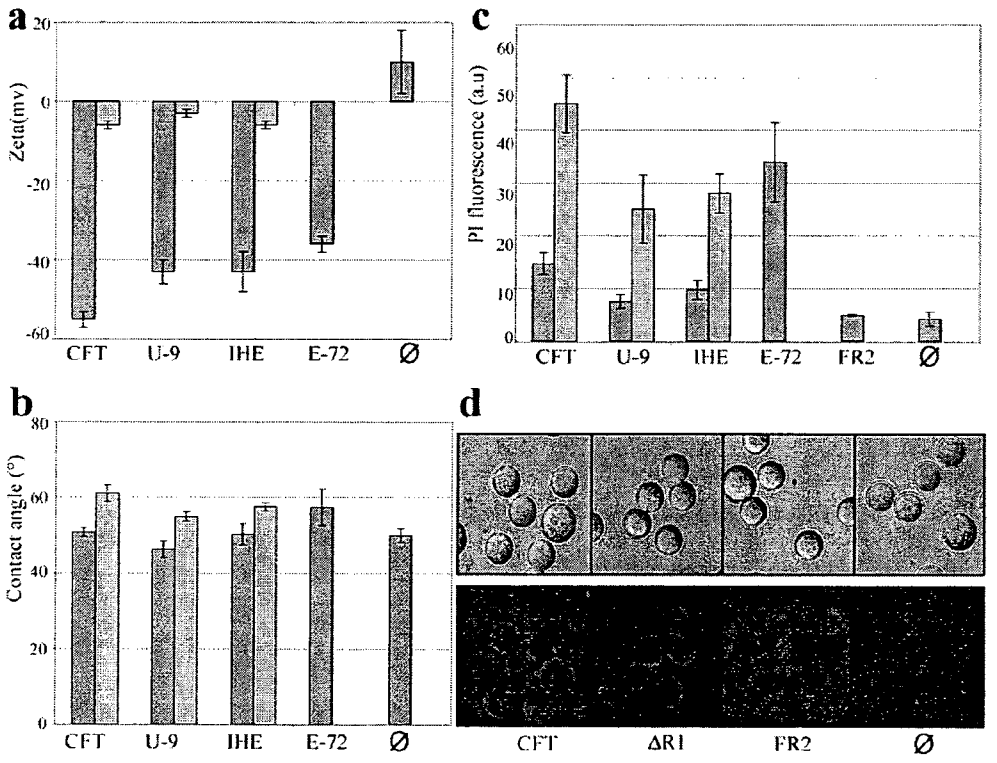

Figure 6

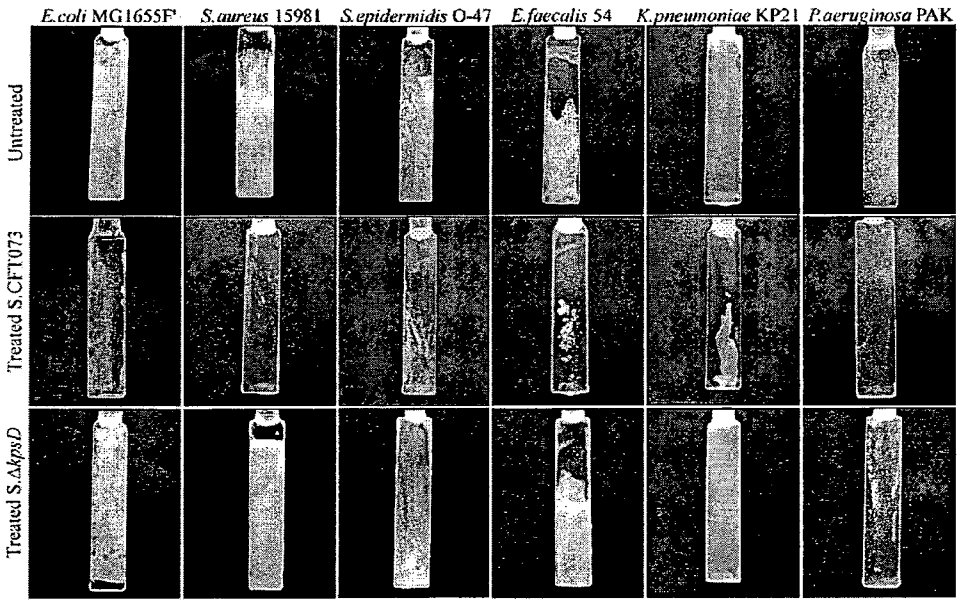

Figure 7

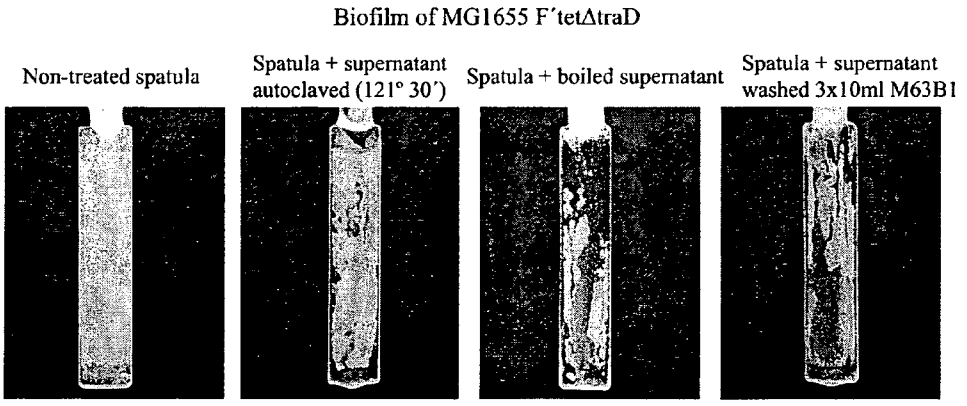

Figure 8

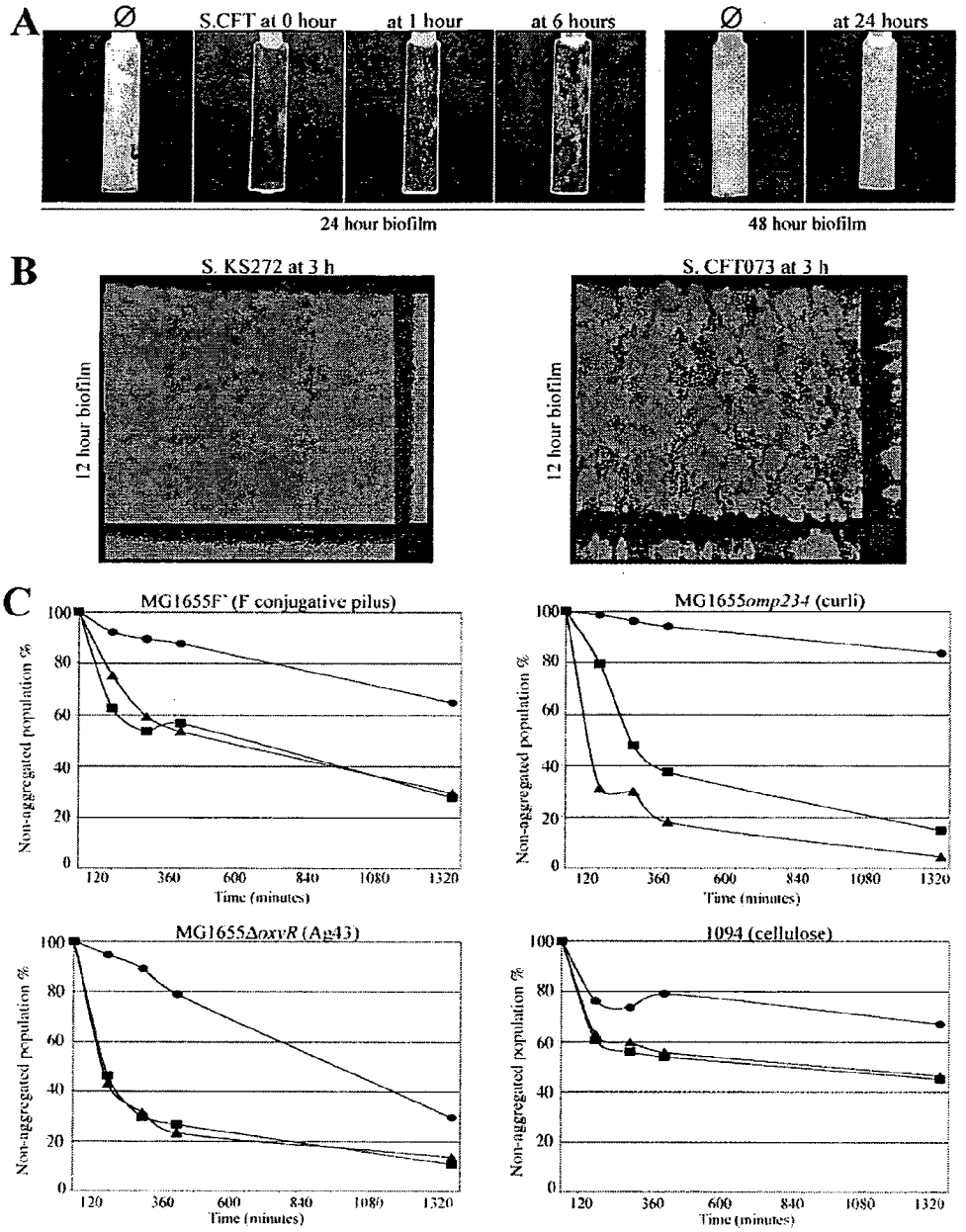
A
Ø
S.CFT at 0 hour
at 1 hour
at 6 hours
Ø
at 24 hours
24 hour biofilm
48 hour biofilm
B
S. KS272 at 3 h
S. CFT073 at 3 h
12 hour biofilm
12 hour biofilm
C
MG1655F' (F conjugative pilus)
MG1655omp234 (curli)
MG1655ΔoxyR (Ag43)
1094 (cellulose)
Non-aggregated population %
Time (minutes)
0
20
40
60
80
100
120
360
600
840
1080
1320

Figure 9

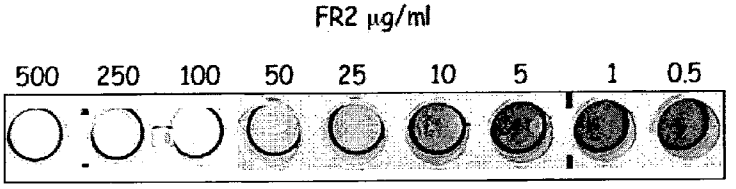

Figure 10

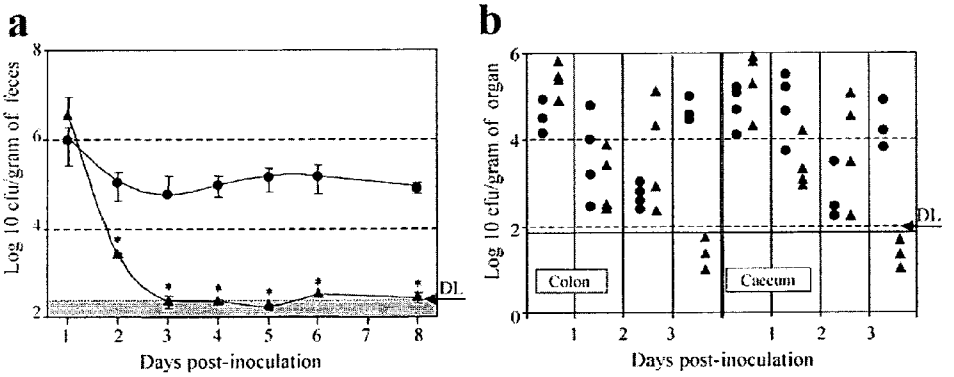

Figure 11

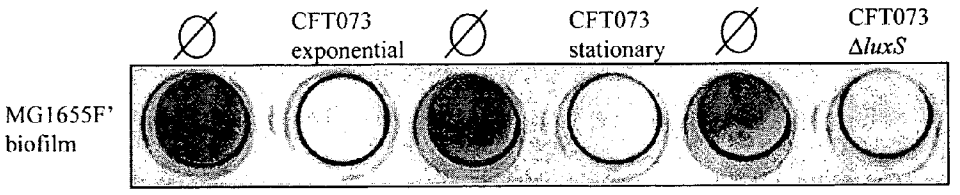

Figure 12

USE OF BACTERIAL POLYSACCHARIDES FOR BIOFILM INHIBITION

[0001] The present invention pertains to the field of biofilm prevention. More particularly, the invention provides novel components which can prevent and/or inhibit bacterial biofilm formation on various surfaces.

[0002] A biofilm is an accumulation of microorganisms embedded in a polysaccharide matrix and adherent to a biological or a non-biotic surface. Diverse microorganisms (bacteria, fungi, and/or protozoa, with associated bacteriophages and other viruses) can be found in these biofilms. Biofilms are ubiquitous in nature and are commonly found in a wide range of environments, including domestic and industrial water systems.

[0003] Biofilms are also etiologic agents for a number of disease states in mammals. Examples include infections of the oral soft tissues, teeth, middle ear, gastrointestinal tract, urogenital tract, airway/lung tissue, peritoneal membrane and eye. Biofilms also develop on medical indwelling devices, such as dental implants, urinary tract prostheses, peritoneal dialysis catheters, indwelling catheters for hemodialysis and for chronic administration of chemotherapeutic agents (Hickman catheters), cardiac implants such as pacemakers, prosthetic heart valves, ventricular assist devices (VAD), synthetic vascular grafts and stents, prostheses, internal fixation devices, percutaneous sutures, and tracheal and ventilator tubing.

[0004] Biofilm development in industrial devices such as water systems or agri-food plants also raises safety problems.

[0005] Planktonic bacteria (i.e., single-celled bacteria suspended in liquid media) are usually used as models for research and antibiotics design. However, bacteria in biofilms are far more resistant to antibiotics than their planktonic counterparts, and less accessible to the immune system. Moreover, conjugation occurs at a greater rate between cells in biofilms than between planktonic cells. This increased opportunity for gene transfer among bacteria is important, since bacteria resistant to antimicrobials or chemical biocides can transfer the genes for resistance to neighboring susceptible bacteria. Gene transfer can also convert a previous avirulent commensal organism into a highly virulent pathogen.

[0006] Biofilm formation is not limited to the attachment of bacteria to a surface. Indeed, when growing in depth, biofilm bacteria interact more between each other than with the actual physical substratum on which the biofilm initially developed. In a biofilm, bacteria can communicate through chemical signalling mechanisms, so that the community undergoes phenotypic changes when a minimum density (the quorum) is reached in the biofilm. This phenomenon, called "quorum sensing", can be responsible for the expression of virulence factors.

[0007] Besides *E. coli* biofilm-related polysaccharides such as colanic acid polymer, cellulose and (1-6) β-N-acetyl-glucosamine, *E. coli* isolates also produce two serotype-specific surface polysaccharides: the lipopolysaccharide (LPS) O antigen and capsular polysaceharide K antigen. These two classes of surface exposed polysaccharidic polymers have been shown to play indirect roles in biofilms by shielding of bacterial surface adhesin (Schembri et al., 2004).

[0008] The strategies described to date for preventing and/or disrupting biofilms are mainly based on quorum sensing inhibitors (Schachter, 2003).

[0009] The present invention provides a novel strategy for inhibiting biofilm formation, since the inventors have demonstrated, using in vitro mixed-species bacterial biofilm, that some bacteria release in the culture supernatant a soluble group II capsular polysaccharide that prevents biofilm formation by a wide range of Gram-negative and Gram-positive bacteria. As described in the experimental part below, these capsule components induce physico-chemical alterations of surface, leading to a reduction of cell-surface and cell-cell contacts that limits both initial adhesion and bacterial biofilm development.

[0010] A first object of the present invention is hence the use of a soluble group II-like capsular polysaccharide from a bacterial strain, for the preparation of a composition which prevents or inhibits adhesion of micro-organisms and/or biofilm development, in particular bacterial adhesion and/or bacterial biofilm development. In what follows, the term "polysaccharide", although used in the singular, can designate a mixture of different polysaccharides. The capsular polysaccharides produced by the bacteria are indeed of various sizes. In fact, *E. coli* capsules, which constitute the outermost protective layer of the cell surface, are classified into four groups based on genetic and biosynthetic criteria. Group II capsule is one of the 4 capsular types described in *E. coli*, and is constituted of high molecular weight and charged polysaccharidic polymers produced by most uropathogenic *Escherichia coli* (UPEC) and other extra-intestinal *E. coli*. Group II capsule displays a conserved modular genetic organization characterized by 3 functional regions. Region 1 (kpsFEDCUS) and region 3 (kpsMT) are conserved in all group II capsulated bacteria and encode proteins required for ABC-dependent export. Region 2 encodes a diversity of polysaccharidic structural components such as K1, K2 (CFT073), K5 and K96 capsular serotypes (Whitfield, 2006; Whitfield and Roberts, 1999). Group II-like capsules have also been described in *Hemophilus influenzae* and in *Neisseria meningitides* (Roberts, 1996).

[0011] In a preferred embodiment of the invention, a soluble group II-like capsular polysaccharide is obtained in the supernatant of a culture of bacteria selected amongst *Escherichia coli, Hemophilus influenzae* and *Neisseria meningitidis*. However, in the present text, the phrase "group II-like capsular polysaccharides" can designate capsular polysaccharides which are produced by other bacteria, provided they retain the anti-biofilm properties observed for the capsular polysaccharides produced by the above-mentioned strains. For example, capsular polysaccharides produced by the strain 47 of the ECOR collection (Ochman and Selander, 1984) are herein considered as a "group II-like capsular polysaccharide", although this strain apparently produces a hybrid group II/group III capsule.

[0012] The present invention can be performed with polysaccharides having different purification levels. For example, the crude supernatant of a bacterial culture (separated from the bacteria by filter-sterilizing or centrifugation) can be used according to the invention as a composition comprising soluble group II-like capsular polysaccharides. However, in order to increase the anti-biofilm activity of the composition, as well as its safety, the soluble group II-like capsular polysaccharide can be obtained as a purified fraction. Three levels of purification are described in the experimental part below, as non-limitative examples. Alternatively,

a composition according to the invention can be obtained directly from the bacterial culture, for example after lysis of the bacteria.

[0013] Another object of the present invention is a composition for inhibiting bacterial adhesion and/or bacterial biofilm development, which comprises a soluble group II-like capsular polysaccharide from a bacterial strain. Such a composition can comprise polysaccharides having different purification levels. In a preferred embodiment, such a composition comprises a purified fraction of the supernatant of a culture of bacteria selected amongst *E. coli, H. influenzae* and *N. meningitidis*, comprising soluble group II-like capsular polysaccharides.

[0014] The present invention also relates to a process for purifying an anti-biofilm group II-like capsular polysaccharide from a bacterial strain, comprising the following steps:

[0015] (i) separating the supernatant of a culture of a bacterial strain expressing a group II-like capsule from the bacterial cells,

[0016] (ii) precipitating the polysaccharides present in the obtained supernatant, and

[0017] (iii) optionally, resuspending the precipitate.

[0018] The above process is preferably performed with a bacterial strain selected amongst *E. coli, H. influenzae* and *N. meningitidis*, more preferably with an uropathogenic *E. coli.*

[0019] In this process, step (i) can be carried out by centrifuging and/or filter-sterilizing the bacterial culture, in order to eliminate the bacterial cells. For example, in industrial processes, tangential filtration can be performed without any preliminary centrifugation. Tangential filtration can be performed continuously.

[0020] The skilled artisan can use any precipitation process known in the art to perform the second step of the above-described process. For example, the precipitation in step (ii) can be performed with three volumes of ethanol for one volume of supernatant.

[0021] In an advantageous variant of the process according to the invention, the precipitate obtained in step (ii) is first resuspended in water, dialyzed against deionised water, and then lyophilized before step (iii).

[0022] The resuspension in step (iii) can be done in water or in any buffer suitable for the intended use. An example of buffer which can be used is TrisHCl 20 mM, pH 7.5, with 25% propanol-1.

[0023] At the end of step (iii), the anti-biofilm polysaccharides are obtained as a semi-purified product, which can be used as such according to the invention, especially in applications which do not need medical-grade products.

[0024] In order to further purify the polysaccharides, the purification process can comprise an additional step (iv) of purification by chromatography, especially ion exchange chromatography, for example using a DEAE-Sepharose column. In this embodiment of the invention, an optional centrifugation step can be performed between step (iii) and step (iv), to discard the insoluble fraction.

[0025] The skilled artisan can choose any appropriate buffer for performing step (iv). An example of buffer which can be used is TrisHCl 20 mM, pH 7.5, with 25% propanol-1. According to an advantageous embodiment of the process, the precipitate is resuspended in TrisHCl 20 mM, pH 7.5, with 25% propanol-1 in step (iii), and the column used in step (iv) is equilibrated with the same buffer.

[0026] When performing a step of purification by ion-exchange chromatography, the group II-like capsular polysaccharides can be eluted using a salt gradient, for example a NaCl gradient. In an efficient embodiment of the process, described in the experimental part, the group II-like capsular polysaccharides are eluted with 300 mM NaCl in TrisHCl 20 mM, pH 7.5, 25% propanol-1.

[0027] Of course, the soluble group II-like capsular polysaccharides obtained through a process as above-described can be used, according to the invention, for the preparation of a composition which prevents or inhibits bacterial adhesion and/or bacterial biofilm development. An anti-biofilm composition comprising such purified polysaccharides is also part of the present invention.

[0028] In a particular embodiment, the composition of the present invention is formulated for preventive or therapeutic administration to a subject in need thereof. Non-limitative examples of compositions according to this aspect of the invention are oral solutions, solutions for infusion into the ear, collyrium, toothpaste or therapeutic dentifrice, etc. These compositions can be used, for example, to prevent the (re)-colonization of the gut, the lung, the ear, the sinus or any other organ or cavity, by pathogenic bacteria.

[0029] In another embodiment, the composition according to the invention is a liquid or a paste, for example a paint, which can be applied on any kind surfaces in order to prevent biofilm formation on these surfaces.

[0030] Another aspect of the present invention is an anti-biofilm coating, comprising a group II-like capsular polysaccharide from a bacterial strain. In such a coating, the group II-like capsular polysaccharide can have different purification levels, as described above. In a preferred embodiment of the coating according to the invention, the group II-like capsular polysaccharide is from a bacterial strain selected amongst *Escherichia coli, Hemophilus influenzae* and *Neisseria meningitidis*. This coating can be obtained, for example, by application of a composition as above-described. It can also be in the form of sheets which can be applied on any kind of device on which biofilm formation must be avoided.

[0031] Accordingly, a medical or industrial device, which is at least partly coated with an anti-biofilm coating comprising a group II-like capsular polysaccharide from a bacterial strain, is also part of the present invention. Such an object can be obtained, for example, by dipping part of the device or the whole device, into a liquid composition as described above. The skilled artisan can choose the incubation duration, depending on the material, the concentration of the composition in group II-like capsular polysaccharide, the intended use, and the like. Typically, said incubation can last from 10 seconds to 30 minutes. Short incubations (≦1 to 5 minutes) are usually sufficient. If necessary, the coated device can then be sterilized by a variety of treatments, without damaging the coating. For example, it can be intensively washed and/or autoclaved. Any kind of device made of glass, pyrex, PVC, polycarbonate, polypropylene and the like, can advantageously be coated according to this aspect of the invention.

[0032] Non-limitative medical devices which can advantageously be coated according to this aspect of the invention are scalpels, burs and other non-disposable surgery and/or dentistry tools, and indwelling devices, such as dental implants, urinary tract prostheses, peritoneal dialysis catheters, indwelling catheters for hemodialysis and for chronic administration of chemotherapeutic agents (Hickman catheters), cardiac implants such as pacemakers, prosthetic heart valves, ventricular assist devices (VAD), synthetic vascular grafts

and stents, prostheses, internal fixation devices, percutaneous sutures, and tracheal and ventilator tubing.

[0033] Non-limitative examples of industrial devices which can advantageously be coated according to this aspect of the invention are plumbing materials, such as pipes, tubes, valves and the like, air-cooled towers, warm water systems, coolant circuits of nuclear power plant, especially secondary and tertiary circuits, agri-food materials, such as silos, fermenters, colanders, etc., furniture elements such as lab tables, counter tops and the like, especially for clean rooms, etc.

[0034] The invention is further illustrated by the following figures and examples.

FIGURE LEGENDS

[0035] FIG. **1**: Biofilm inhibitory effect of CFT073. A, Biofilm formation of MG1655 F' in microfermentors inoculated with 1 or 10 OD_{600nm}, equivalent of KS272 (grey) or CFT073 (black) cells. MG1655F' biofilm alone (Ø, white). Results are average of 6 replicates ±s.d. $P<0.001$ compared with MG1655F' biofilm. B, Microtiter plate MG1655F' biofilm alone (Ø), or in the presence of KS272 or CFT073 supernatant, (S.KS272 and S.CFT073, respectively). C, MG1655F' biofilm in microfermentors perfused with medium without supernatant (Ø) or with S.KS272 or S.CFT073. D, Growth curves of MG1655F' alone (Ø) or with S.KS272 or S.CFT073. E, MG1655F' cell viability alone (Ø) or with S.KS272 or S.CFT073 visualized with BacLight staining. F, Qualitative analysis of the biofilm formation in microtiter plate by different bacteria in the presence of CFT073 supernatant (S. CFT).

[0036] FIG. **2**: Effect of CFT073 supernatant on Gram-positive and Gram-negative bacterial biofilm formation. A, Quantification of the microtiter plate biofilm formation of different bacteria, alone (Ø), with KS272 (S.KS) or CFT073 (S.CFT) supernatant. Levels of crystal violet retained were measured spectrophotometrically (OD_{570nm}). B, Quantification of biofilm formed by several pathogenic bacteria in microfermentors using media not supplemented (Ø), or supplemented with S.CFT or S.KS. Error bars represent standard deviation of two independent experiments. C, Effect of CFT073 supernatant (S.CFT073) in mix biofilms of *E. coli* (MG1655F') with *P. aeruginosa* (PAK), *K. pneumoniae* (KP21), *S. epidermidis* (O-47), *S. aureus* (15981) and *S. epidermidis* (O-47) with *S. aureus* (15981) and *E. faecalis* (54). Supernatant of *E. coli* CFT073ΔkpsD strain (S. ΔkpsD) that do not secrete any group II capsule is used as negative control. D, Qualitative analysis of biofilm formation of *S. aureus* and *P. aeruginosa*, in a microfermentor using media not supplemented, or supplemented with CFT073 supernatant.

[0037] FIG. **3**: Relationship between capsule production and anti-biofilm activity of the CFT073 supernatant. A, Genetic organization of the CFT073 capsule R1, R2 and R3 regions. Genes with transposon insertions are marked with an asterisk. B, Biofilm formation of MG1655F' cultivated in the presence of the capsule mutant supernatants. C, Hexose levels in the supernatants. kpsF, kpsU, c3692 and c3693 correspond to mutants that do not impair capsule production. D, Stationary phase CFT073 or CFT073Δ bacterial cell capsules stained with ferritin and examined by transmission electron microscopy (X100000; bar=0.2 μm) (left panel); 125 and 105 cells were observed respectively. Stained CFT073 capsule is indicated by an arrow. On the right panel: scanning electron micrographs of stationary-phase CFT073 or CFT073ΔkpsD (X50,000; bar=0.5 μm); 45 and 37 cells were observed respectively.

[0038] FIG. **4**. Correlation between anti-biofilm activity and group II capsule. Biofilm formation of *E. coli* MG1655F' and 1091 strains, and of the *S. aureus* 15981 strain cultured with: (A) supernatants of *E. coli* exhibiting anti-biofilm activity (see Table 1) (beside strain 47, all the strains tested produce group II capsule) (B) supernatants of CFT073, U-9, U-15 strains and their respective kpsD mutants. (C) Biofilm formation in microfermentor of UPEC strains CFT073, U-9, U-15 (black) and their respective kpsD mutants (grey) grown in M63B1glu, and kpsD mutants grown in media supplemented with their corresponding wild-type supernatant (white). Biofilms were grown for 36 h at 37° C. Error bars represent standard deviation of the mean. Strains identified by simple numbers correspond to those of the EcoR collection (Ochman and Selander, 1984).

[0039] FIG. **5**. Anti-biofilm effect of *Neisseria meningitides* supernatant. Quantification of the microtiter plate biofilm formation of MG1655F' in the presence of *S. Neisseria*. OD_{570nm} nm of the crystal violet dye was determined as described in (O'Toole and Kolter, 1998).

[0040] FIG. **6**: Physico-chemical properties of the CFT073 supernatant. a, ζ potential of cationic colloids incubated with the dialyzed supernatants from: CFT073 (CFT), U-9, IHE3034 (IHE), EcoR72 (E-72) (dark grey) and their respective capsule mutants (light grey). (Ø) correspond to M63B1glu treatment. b, Water droplet contact angle on surface incubated with CFT, U-9, IHE, E-72 (dark grey) and the capsule mutants (light grey). c, Propidium iodide adsorption onto cationic particles incubated with CFT, U-9, IHE, E-72, FR2 (CFT073 supernatant purified fraction), (dark grey) and their respective capsule mutants (light grey). The extent of the adsorption is given by the fluorescent intensity (>670 nm). d, Fluorescence microscopy of cationic particles incubated with CFT, S.CFT073 μl (ΔR1), FR2 and not incubated (Ø). Error bars represent the standard deviation of the mean.

[0041] FIG. **7**: Biofilm inhibition effect of CFT073 supernatant on coated surfaces. Biofilm formation in microfermentors by several bacteria using: untreated glass slides (upper panel), glass slides treated with CFT073 supernatant (middle panel) and glass slides treated with CFT073ΔkpsD supernatant (lower panel).

[0042] FIG. **8**. Impact of the treatment of spatula coated with S.CFT073 supernatant (S.CFT). Biofilm formation in microfermentors by MG1655F' using untreated glass slides and glass slides treated with S.CFT or with boiled S.CFT, and then autoclaved or submitted to intensive wash.

[0043] FIG. **9**: CFT073 supernatant affects cell-cell interaction. A, MG1655F' biofilm formation in microfermentors with media supplemented with CFT073 supernatant (S.CFT) at times 0 h, 1 h, 6 h (24 h of culture) and 24 h (48 h of culture). Ø: no addition of S.CFT. B, GFP-tagged MG1655F' inoculated in a flow-cell and monitored by confocal microscopy. CFT073 or KS272 supernatants were supplemented after 3 h of culture and biofilms were grown for 12 h total. C, Autoaggregation assay with strains that aggregate via different mechanisms: MG1655F' (F conjugative pilus expression); MG1655ompR234 (curli overexpression); MG1655ΔoxyR (Ag43 autotransporter adhesin overexpression); 1094 (cellulose production). Cells were diluted to OD_{600} of 2 in 3 ml of M63B1 (triangle), CFT073 supernatant (circle) and ΔkpsD supernatant (rectangle).

[0044] FIG. **10**. Anti-biofilm activity of the FR2 fraction. CFT073 supernatant purified fraction (FR2) was added to the MG 1655F' culture in concentrations ranging from 0.5 to 500 μg/ml. Biofilm formation of MG1655F' was visualized after 24 h. Concentration of 50-100 μg/ml inhibited MG1655 F' biofilm.

[0045] FIG. **11**. Intestinal colonization by CFT073 and CFT073ΔR1. a, Bars represent the standard error of the log 10 mean number of CFU per gram of feces; a Mann-Whitney test was used for statistical analysis, the level of statistical significance (*) was set at P values of <0.016. b, Colon and caecium colonization by CFT073 (circles) and CFT073ΔR1 (triangles). DL: Detection limit.

[0046] FIG. **12**. Effect of growth phase and quorum-sensing in the anti-biofilm properties of CFT073 supernatant. Biofilm formation of MG1655F' in microtiter plate in presence of supernatants purified from cells in exponential phase, stationary phase and ΔluxS mutant. 10^{10} cells in exponential phase (OD_{600nm}=0.4) and in stationary phase (OD_{600nm}=2) were centrifuged and supernatants were precipitated with 3 volumes of ethanol. The supernatant of ΔluxS mutant was purified from an overnight culture.

EXAMPLES

Example 1

Methods

[0047] Bacterial Strains, Growth Conditions and Microscopy Analysis

[0048] Bacterial strains are listed in Table 1 below. Gram-negative bacteria were grown at 37° C. in M63B1 minimal medium with 0.4% glucose (M63B1glu) or in LB rich medium. Gram-positive bacteria were grown in TSB with 0.25% glucose (TSBglu) at 37° C. The effect of CFT073 supernatant on bacterial growth and viability rate was evaluated using growth curve determination, colony forming unit count on LB plate and BacLight Live/Dead viability stain (Molecular Probes). Ferritin-staining and Scanning Electronic Microscopy was performed as described in (Bahrani-Mougeot et al., 2002). Epifluorescence and transmitted light microscopy were acquired using a Nikon E400 microscope. Autoaggregation assays were performed as described in (Beloin et al., 2006).

TABLE 1

Strains used in this study

Strains	Relevant characteristics	References
E. coli strains		
CFT073	UPEC group II capsule (K2)	(Mobley et al., 1990)
MG1655F'	MG1655 F' tet-ΔtraD plasmid	(Ghigo, 2001)
KS272	Commensal *E. coli* K-12	(Strauch and Beckwith, 1988)
1091	Commensal *E. coli*	C. Le Bouguenec
1092	Commensal *E. coli*	C. Le Bouguenec
1094	Commensal *E. coli*	(Da Re and Ghigo, 2006)
1096	Commensal *E. coli*	C. Le Bouguenec
1097	Commensal *E. coli*	C. Le Bouguenec
1102	Commensal *E. coli*	C. Le Bouguenec
1103	Commensal *E. coli*	C. Le Bouguenec
1110	Commensal *E. coli*	C. Le Bouguenec
1125	Commensal *E. coli*	C. Le Bouguenec
1127	Commensal *E. coli*	C. Le Bouguenec
U-1	UPEC group II capsule	C. Forestier
U-2	UPEC group II capsule (K2)	C. Forestier
U-3	UPEC non-group II capsule	C. Forestier
U-4	UPEC group II capsule	C. Forestier
U-5	UPEC group II capsule	C. Forestier
U-6	UPEC group II capsule (K2)	C. Forestier
U-7	UPEC non-group II capsule	C. Forestier
U-8	UPEC group II capsule	C. Forestier
U-9	UPEC group II capsule	C. Forestier
U-10	UPEC group II capsule	C. Forestier
U-11	UPEC non-group II capsule	C. Forestier
U-12	UPEC group II capsule	C. Forestier
U-13	UPEC group II capsule	C. Forestier
U-14	UPEC non-group II capsule	C. Forestier
U-15	UPEC group II capsule	C. Forestier
U-16	UPEC group II capsule	C. Forestier
U-17	UPEC non-group II capsule	C. Forestier
U-18	UPEC non-group II capsule	C. Forestier
U-19	UPEC group II capsule	C. Forestier
U-20	UPEC group II capsule	C. Forestier
U-21	UPEC group II capsule (K2)	C. Forestier
984	Commensal *E. coli* group II capsule (K1)	M. C. Ploy
988	Commensal *E. coli* group II capsule (K1)	M. C. Ploy
999	Commensal *E. coli* group II capsule (K1)	M. C. Ploy
1007	Commensal *E. coli* group II capsule (K1)	M. C. Ploy
1014	Commensal *E. coli* group II capsule (K1)	M. C. Ploy

TABLE 1-continued

Strains used in this study

Strains	Relevant characteristics	References
IHE3034	*E. coli* causing meningitis group II capsule (K1)	(Meier et al., 1996)
EcoR strains	*E. coli* Reference Collection (72 strains)	(Ochman and Selander, 1984)
Other bacteria		
15981	*S. aureus* clinical strain	(Valle et al., 2003)
V329	*S. aureus* bovine mastitis subclinical isolate	(Cucarella et al., 2001)
O-47	*S. epidermidis* clinical strain	(Heilmann et al., 1996)
CH845	*S. epidermidis* clinical strain BM94314	(Galdbart et al., 2000)
54	*E. faecalis* clinical strain	(Toledo-Arana et al., 2001)
11279	*E. faecalis* clinical strain	(Toledo-Arana et al., 2001)
KP21	*Klebsiella pneumoniae* strain	C. Forestier
PAK	*Pseudomonas aeruginosa*	(Vasseur et al., 2005)
8013	*Neisseria meningitidis* strain, serogroup C, class 1	(Deghmane et al., 2002)
Mutants		
44H3	CFT073 kpsD::TnSC189	This study
25F11	CFT073 kpsD::TnSC189	This study
23D5	CFT073 kpsU::TnSC189	This study
16B9	CFT073 kpsU::TnSC189	This study
14E12	CFT073 kpsC::TnSC189	This study
76H11	CFT073 kpsS::TnSC189	This study
30H8	CFT073 kpsM::TnSC189	This study
ΔkpsD	CFT073 kpsD::km	This study
ΔkpsC	CFT073 kpsC::km	This study
ΔkpsU	CFT073 kpsU::km	This study
ΔkpsS	CFT073 kpsD::km	This study
ΔkpsM	CFT073 kpsM::km	This study
Δ3692	CFT073 Δ3692::km	This study
Δ3693	CFT073 Δ3693::km	This study
Δ3694	CFT073 Δ3694::km	This study
Δ3695-96	CFT073 Δ3695Δ3696::km	This study
ΔR1	CFT073 with a deletion from kpsD to kpsS	This study
ΔR2	CFT073 with a deletion from c3692 to c3696	This study
ΔR3	CFT073 with a deletion from kpsT to kpsM	This study
U-9 ΔkpsD	U-9 kpsD::km	This study
U-15 ΔkpsD	U-15 kpsD::km	This study
IHE3034 ΔkpsD	IHE3034 kpsD::km	This study
ΔluxS	CFT073 ΔluxS	This study
CFT073gfp	CFT073λATTgfp	This study
ΔR1gfp	ΔR1λATTgfp	This study
ΔoxyR	MG1655 oxyR::km	(Beloin et al., 2006)
ompR234	MG1655 ompR234 malA::km	(Vidal et al., 1998)

[0049] Biofilm Formation Procedures

[0050] Microfermentors experiments: Biofilm was performed as described previously (Ghigo, 2001). Mixed biofilm cultures: an 8 hours MG1655F' biofilm formed in the internal microfermentors glass slide was infected with 1 OD_{600nm} equivalent of CFT073-gfp overnight culture. After 24 hours of continuous culture in M63B1glu, pictures of the glass slides were taken. Biofilm biomass was estimated by determining the OD_{600nm} of the resuspension of the biofilm formed on the internal glass slide (Ghigo, 2001). Biofilm inhibition assays: the incoming medium was mixed in a 1:1 ratio with filtered supernatants and brought into the microferrnentors at different time after bacteria inoculation (0, 1, 6 or 24 hours). The biofilm was further cultivated for an additional 24 hours before biomass determination. Analysis of bacterial interaction with treated surfaces: the glass slides were incubated 1 min with filtered CFT073 supernatant and rinsed once in deionised water prior to inoculation in microfermentors. Biofilm formation on the slide was determined after 24 hours.

[0051] Microtiter plate experiments. Static biofilm formation assay were performed in 96-well PVC microtiter plates (Falcon) as described in (O'Toole and Kolter, 1998). Biofilm inhibition assays: overnight cultures were adjusted to OD_{600}=0.04 before inoculating 100 μl in 96-well plates in the presence or absence of 50 μl of supernatant. Flow-chamber experiments. Biofilms were performed in M63B1glu at 37° C. in 3× channels flow-cells (1×4×40 mm). The flow system was assembled and prepared as described in (Christensen et al., 1999). Inocula were prepared as follows: 16-20 hours old overnight cultures in M63B1glu were harvested and resuspended as normalized dilutions (OD_{600}=0.005). 300 μl were injected into each flow channel. Input medium was mixed in a 1:1 ratio with filtered supernatant. Flow was started 1 h after inoculation at a constant rate of 3 ml h^{-1} using a Watson Marlow 205S peristaltic pump. All Assays were at least performed in triplicate.

[0052] Purification of CFT073 or Other Group II Capsulated Strain Supernatants Displaying Anti-Biofilm Activity

[0053] Three levels of purification have been tested:

[0054] (i) Filtration (sterilization) of the active supernatants (S.CFT, used in all the experiments on microtiter plates or in microfermentors)

[0055] Overnight cultures in M63B1glucose 0.4% were centrifuged for 30 min at 5000 rpm at 4° C. and filtered through 0.25 μm filter to eliminate bacteria.

[0056] (ii) Precipitation of polysaccharides contained in active supernatants

[0057] The polysaccharides contained in the filtered supernatant were precipitated with 3 volumes of ethanol, resuspended in deionized water and dialyzed against deionized water in 10 kDa cut-off dialysis cassettes (Pierce biochemical).

[0058] (iii) purification of the capsular polysaccharides active fraction (capsular active fraction FR2)

[0059] the partially purified supernatant active fraction obtained in step (ii) was lyophilized and resuspended in 80 ml of buffer Tris HCl 20 mM pH 7.5 containing 25% de propanol-1.

[0060] This resuspension was centrifuged for 10 minutes at 3000 rpm to eliminate the insoluble particles.

[0061] the soluble supernatant was loaded on a DEAE-Sepharose column (30 ml, 2.6×6 cm, Amersham) and equilibrated with Tris HCl 20 mM pH 7.5, 25% de propanol-1 buffer.

[0062] the column was washed with Tris HCl 20 mM pH 7.5, 25% propanol-1 buffer at the rate of 20 ml/h.

[0063] After the wash, the column was eluted with a NaCl gradient (0 to 1 M in 400 ml) and the polysaccharide concentration of each eluted fractions (4.5 ml) was tested by the Dubois method (Dubois et al., 1956): 100 μl of phenol at 5% and 500 μl of concentrated sulfuric acid followed by vortex agitation and read at 492 nm)

[0064] The positive fractions (about 10 fractions of 4.5 ml) were pooled together and dialyzed against deionized water and lyophilized

[0065] 1 mg of the lyophilysate was resuspended in 1 ml of deionized water

[0066] Handling of Culture Supernatants and Polysaccharide Analysis

[0067] Overnight cultures in M63B1glu at 37° C. were centrifuged 30 minutes at 5000 rpm at 4° C. After filtration of the supernatant with a 0.2 μm filter, macromolecules were precipitated with 3 volumes of ethanol and dialysed against deionised water using 10 kDa cassettes (Pierce). Total amounts of phosphate and neutral sugars were determined by ammonium molybdate/ascorbic acid and phenol/sulfuric acid methods, respectively. Polysaccharide composition was determined by HPLC (ion-exclusion column) and by gas liquid chromatography as in (d'Enfert and Fontaine, 1997; Fontaine et al., 2000). CFT073 supernatant active fraction, FR2, was purified using a DEAE-Sepharose column (Amersham) and eluted with 300 mM NaCl in 25% propanol-1, 20 mM TrisHCl pH7.5. Molecular weight of the polymer was estimated by gel filtration chromatography on Superdex-200 (Amersham) using dextran as standard. Polysaccharide degradations were done by total acid hydrolysis (trifluoroacetic acid, 4N, 4H, 100° C.) or by aqueous hydrofluoric acid (48% aq. HF, 2 days on water-ice).

[0068] Mutagenesis and Molecular Techniques

[0069] Mariner transposon mutagenesis of *E. coli* CFT073 was performed as described in (Da Re and Ghigo, 2006). The supernatants of 10,000 transposon mutants incubated 24 h, in LB at 37° C. in 96-well microtiter plates were extracted after centrifugation of the plates 15 min at 10000 rpm and their effect on MG1655F' biofilm formation was analysed. Transposon insertion sites were determined as described in (Da Re and Ghigo, 2006). Homology searches were performed using Blast 2.0. Deletion mutants were generated as detailed at http://www.pasteur.fr/recherche/unites/Ggb/3SPCRprotocol.html, using primers presented in Table 2.

TABLE 2

Primers used in this study.

Target gene	Primer name	Sequence	SEQ ID No:
Primers used to generate deletion mutants			
kpsD	KpsD.500-5	gaccagcttgcctttgcagaaacg	1
	KpsD.500-3	ctttttcagcattacgcggatagg	2
	KpsD.GB.L-5	TGCTCGATGAGTTTTTCTAAGGAGTTGAAatgagcaa	3
	KpsD.GB.L-3	gattttgagacacaacgtggctttCATcacAAACTCATTCAGCGACA	4
	KpsD.ext-5	ttgcgcttaagtttaaccaaaccg	5
	KpsD.ext-3	gctctggcatggactccggtaact	6
kpsU	KpsU.500-5	atgaacgcagttcagctttatcgcc	7
	KpsU.500-3	ccaaatttcggcttgaggattttc	8
	KpsU.GB.L-5	TGCTCGATGAGTTTTTCTAAcaggaactggctgaaaacgcatga	9
	KpsU.GB.L-3	gattttgagacacaacgtggctttCATTTCAACTCCttacaaagacaga	10
	KpsU.ext-5	tgcagaacggcgataccttaatcg	11
	KpsU.ext-3	ctcggcaatcaaacgtactcgttg	12
kpsC	KpsC.500-5	gaggcagatatcaacattaacc	13
	KpsC.500-3	gttgaaggttttaagttctcaac	14
	KpsC.GB.L-5	TGCTCGATGAGTTTTTCTAAACAATTTCATAGTTGACTATTAC	15
	KpsC.GB.L-3	gattttgagacacaacgtggctttgagtaaatgccaatcatgcgttttc	16
	KpsC.ext-5	cgactcacattacgattatgcg	17
	KpsC.ext-3	gaaaatgatttgtggtggcggtagc	18
kpsS	KpsS.500-5	agagcaaccttgagttattacg	19
	KpsS.500-3	aaagacaagggatagctttagg	20
	KpsS.GB.L-5	TGCTCGATGAGTTTTTCTAATTTATTCTAAATTATCAACG	21
	KpsS.GB.L-3	gattttgagacacaacgtggctttCATAAATAATCTGTGTAATAGTCAA	22
	KpsS.ext-5	agcgactggttgaaagcaaactg	23
	KpsS.ext-3	ttcgatgagtcaagactattgg	24

TABLE 2-continued

Primers used in this study.

Target gene	Primer name	Sequence	SEQ ID No:
kpsM	KpsM.500-5	TTACTACGCATAAAATTCATGG	25
	KpsM.500-3	aatgccatgcttaaaccaaagcc	26
	KpsM.GB.L-5	TGCTCGATGAGTTTTTCTAAcaatgctgacatcatgattaagattg	27
	KpsM.GB.L-3	gattttgagacacaacgtggctttcttgccatTTGGTGATGTGATCCT	28
	KpsM.ext-5	TCGCATGCGTTCTGGTTTGAG	29
	KpsM.ext-3	cacatcacaaaactctttcaatg	30
Kps	KpsD.500-5	gaccagcttgcctttgcagaaacg	31
	KpsS.500-3	aaagacaagggatagctttagg	32
	KpsD.GB.L-3	gattttgagacacaacgtggctttCATcacAAACTCATTCAGCGACA	33
	KpsS.GB.L-3	gattttgagacacaacgtggctttCATAAATAATCTGTGTAATAGTCAA	34
	KpsD.ext-5	ttgcgcttaagtttaaccaaaccg	35
	KpsS.ext-3	ttcgatgagtcaagactattgg	36
Kps	KpsR2.500-5	atataggagtatggagcgaaac	37
	KpsR2.500-3	ttgagtaaggaatatggcttag	38
	KpsR2.GB-L5	TGCTCGATGAGTTTTTCTAAGAAATCAGACGAGTTTTC	39
	KpsR2.GB-L3	gattttgagacacaacgtggctttcataacatACTATGTCCCCATGATTATT	40
	KpsR2.ext-5	catgtactcattttcacgtaaag	41
	KpsR2.ext-3	tgctaaaattgcattattaggtc	42
Kps	KpsM.500-5	TTACTACGCATAAAATTCATGG	43
	KpsR3.500-3	AATTAACCATATCTTTTGATTTGAG	44
	KpsR3.GB-L5	TGCTCGATGAGTTTTTCTAAatcagacttgtctttatcag	45
	KpsM.GB.L-3	gattttgagacacaacgtggctttcttgccatTTGGTGATGTGATCCT	46
	KpsM.ext-5	TCGCATGCGTTCTGGTTTGAG	47
	KpsR3.ext-3	cctagcaacaaaatatttagcgac	48
Kp95-	Kps95-96.500-	aaacaatatcatggccagtcgg	49
	Kps95-96.500-	aataacgttcaggtattgaagg	50
	Kps95-96.GB-	TGCTCGATGAGTTTTTCTAAccttgaGGTCTATATAACTGAA	51
	Kps95-96.GB-	gattttgagacacaacgtggctttcatcaaatgtaccaaaggtgataac	52
	Kps95-96.ext-	taaatcaacgttactgagaatg	53
	Kps95-96.ext-	gaatatccgagtgcataatacc	54
	Kps95-96.500-	aaacaatatcatggccagtcgg	55
C3694	c3694.500-5	aagcattagaattggaaccc	56
	c3694.500-3	ctttccatgtattcctctccaag	57
	c3694.GB.L-5	TGCTCGATGAGTTTTTCTAAgtgcaagtatttcttgtaaccc	58
	c3694.GB.L-3	GATTTTGAGACACAACGTGGCTTTCATatacgcatcaatagccttagccc	59
	c3694.ext-5	gcggagagctattttaaagcagg	60
	c3694.ext-3	cggaaaacgatatgacaatcctg	61
C3693	c3693.500-5	gtttattgttgcaggcatccaag	62
	c3693.500-3	atgccgttagatagttttattcc	63
	c3693.GB.L-5	TGCTCGATGAGTTTTTCTAAatggatgctcaaaaggaggtacg	64
	c3693.GB.L-3	GATTTTGAGACACAACGTGGCTTTCATcagcattggttggtaatgcatttg	65
	c3693.ext-5	acatattaacagtaatataacc	66
	c3693.ext-3	ctacaaatttggatactgcaaatc	67
C3692	c3692.500-5	ttatacttgcggtgatttgcag	68
	c3692.500-3	ATGACTCATAAAAATATATTCC	69
	c3692.GB.L-5	TGCTCGATGAGTTTTTCTAAtatttacagaataattattctgg	70
	c3692.GB.L-3	GATTTTGAGACACAAGGTGGCTTTCATtaagccaatagtcttgactcatcg	71
	c3692.ext-5	aattcatatgattgtagcaatg	72
	c3692.ext-3	CAACGTAGAATAAAAGCATTACC	73
luxS	LuxS.500-5	AAACTGCGCAGTTCCCGTTACC	74
	LuxS.500-3	CCTGATTTTGTTCCCTGGGAGG	75
	LuxS.GB-L5	TGCTCGATGAGTTTTTCTAATCAGTGGAACAAAAGAAG	76
	LuxS.GB-L3	gattttgagacacaacgtggctttcatTTAGCCACCTCCGGTAATTT	77
	LuxS.ext-5	CTGGAACCGGGTGATCCTCGAAG	78
	LuxS.ext-3	AGCAACAATGCTGGGGAAAAATGC	79
Primers used for			
	Kps95-F	aacgaaaattgcttgctctggc	80
	Kps94-R	cggtgccaagtttgaaataacg	81
	Kps94-F	gaaaatagtgtagacggtctcttc	82
	Kps92-R	tttggatactgcaaatcaccgc	83

TABLE 2-continued

Primers used in this study.

Target gene	Primer name	Sequence	SEQ ID No:
	KpsI1f	GCGCATTTGCTGATACTGTTG	84
	KpsK2r	AGGTAGTTCAGACTCACACCT	85
Primers used to check			
	KmGB.verif-5	TGGCTCCCTCACTTTCTGGC	86
	KmGB.verif-3	ATATGGCTCATAACACCCCTTG	87
Primers used for			
	ARB1	ggCCACgCgTCgACTAgTACNNNNNNNNNNgATAT	88
	ARB6	ggCCACgCgTCgACTAgTACNNNNNNNNNNACgCC	89
	ARB2	ggCCACgCgTCgACTAgTAC	90
	IR2	CTgACCgCTTCCTCgTgCTTTACgg	91
	IR2-60-5	TTCTGAgcgggactctggggtacg	92

[0070] Analysis of the Physico-Chemical Properties of the Active Fractions

[0071] Zeta potential was measured as in (Caruso et al., 1999) after 20 minutes of incubation of 10 μm in diameter cationic colloids latex particles with dialyzed precipitated supernatants (i.e., the level (ii) of purification indicated above). The latex particles bear permanent net positive charge due to their polyethylenimine (PEI) coating. The layer of PEI is a branched 6400 dalton molecular weight polymer bearing approximately 50% of methylated quaternary functions which confer a stable positive charge to the molecule. This polymer was deposited in aqueous phase on the initially carboxylated particles (Decher, 1997). Hydrophilic properties of the supernatants were investigated by determining the contact angle formed by a 2.5 μl ultrapure water droplet with a glass plane surface previously incubated in the supernatants for 20 minutes. Surface interactions were analyzed by monitoring the adsorption of propidium iodide on supernatant-treated cationic colloids. The affinity of the treated surfaces for the fluorescent probe was tested using flow cytometry (Leboeuf and Henry, 2006) and fluorescence microscopy. All incubations of particles with supernatant were performed at low particle/volume fraction (ca. 0.2%) likely leading to surface saturation by the active species.

[0072] In Vivo Mice Experiments

[0073] CFT073 and CFT073ΔR1 in vivo colonization were performed as described previously (Maroncle et al., 2006). Mice were intragastrically fed with 1010 CFU. Bacteria contained in fecal samples were numbered on agar plates. For examination of bacterial growth in the host, mice were sacrificed at various times after inoculation; colon and caecum were homogenized in physiological water, and plated to determine cfu per gram of tissue.

Example 2

Anti-Biofilm Activity of CFT073 Supernatant

[0074] In order to study UPEC interactions within multicellular biofilm (Hall-Stoodley et al., 2004) bacterial communities, an in vitro mixed bacterial biofilm model in microfermentors was developed (Ghigo, 2001). Using this model, a 8 hours biofilm formed by the commensal strain of *E. coli* K12 MG1655 F' was inoculated with different titers of the UPEC strain CFT073, and further cultivated for 24 hours. Upon increasing titers of CFT073, a strong reduction of the *E. coli* K12 MG1655 F' biofilm development was observed, which was not observed when the commensal *E. coli* strain KS272 was used (FIG. **1**A). This suggested that CFT073 could prevent MG1655 F' biofilm formation either by direct contact or by secretion of an inhibitory molecule. To distinguish between these two possibilities, the supernatant of CFT073 stationary phase culture was filter-sterilized and its effect on *E. coli* biofilm formation was tested. In the presence of CFT073 supernatant, MG1655 F' biofilm was severely affected (FIGS. **1**B,C). This biofilm inhibition did not result from a growth defect due to a bactericidal or bacteriostatic activity, since MG1655 F' growth rate and cell viability were not affected by the CFT073 supernatant (FIG. **1**D,E).

[0075] In order to determine the spectrum of the anti-biofilm activity of CFT073 supernatant, its effect was tested on several adherent bacteria (*E. coli, Klebsiella pneumoniae, Pseudomonas aeruginosa, Staphylococcus aureus, S. epidermidis* and *Enterococcus faecalis*). This analysis showed that CFT073 supernatant was active against a surprisingly wide range of bacteria, even in mixed cultures (FIG. **1**F and FIG. **2**).

Example 3

Correlation between Anti-Biofilm Activity and Type-II Capsule

[0076] To elucidate the genetic basis of the anti-biofilm effect, the supernatant activity of ca. 10,000 CFT073 random mariner transposon insertion mutants was tested. The inventors identified seven candidates impaired in their ability to inhibit MG1655 F' biofilm formation. All these mutants mapped in genes involved in the expression of the group II capsular polysaccharide, the outermost bacterial cell surface structure (Whitfield and Roberts, 1999). Group II capsule displays a conserved modular genetic organization characterized by 3 functional regions (Roberts, 1996) (FIG. **3**A). Region 1 (kpsFEDCUS) and region 3 (kpsMT) are conserved in all group II capsulated bacteria and encode proteins required for the ABC-dependent polysaccharide export. Region 2 is variable and encodes polysaccharide serotypes such as K1, K2 (CFT073), K5, K96 (Roberts, 1996). The R1,

R2 or R3 region, or each individual kps gene was deleted and it was observed that, except for kpsU, c3692 and c3693, all the mutants lost the ability to inhibit *E. coli* biofilm formation, which correlated with a reduced amount of precipitated sugars in the supernatant (FIGS. **3**B, **3**C). While a ferritin-stained capsule could still be detected around CFT073 cells (FIG. **3**D), these results indicated that the CFT073 capsule nevertheless undergoes a significant release into the medium supernatant that is responsible for the observed anti-biofilm effect.

[0077] In order to determine whether biofilm inhibition was an exclusive property of *E. coli* CFT073 supernatant, the inventors screened several clinical uropathogenic bacterial isolates of *Klebsiella, Proteus, Enterobacter, Morganella, Citrobacter* and *Serratia*, as well as a collection of 110 *E. coli* strains of diverse origins. They found that only the filtered supernatant of 40 *E. coli*, including 17 UPEC, inhibited biofilm formation on a wide range of bacteria without affecting growth rate (FIG. **4**A). Moreover, as CFT073 *E. coli* strain, all active strains are able to inhibit biofilm formation of adherent bacteria other than *E. coli* (in FIG. **4**A see 15981 *S. aureus* biofilm data). Using specific PCR probes (Johnson and O'Bryan, 2004), they showed that 39 of the 40 active *E. coli* strains carried group II capsule genes. The 40^{th} bacterium, EcoR47, seems in fact to produce a hybrid group II/group III capsule. This strain has been shown to carry group II KPS genes (Boyd and Hartl, 1998). Consistently, the introduction of a kpsD mutation into the clinical UPEC isolates U-9 and U-15 abolished the biofilm-inhibitory effect of their supernatants (FIG. **4**B). Interestingly, although CFT073, U-9 and U-15 strains displayed a very limited ability to form biofilm in the microfermentor biofilm model, their respective kpsD mutants displayed an increased biofilm phenotype. This phenotype could be reverted upon the addition of CFT073 supernatant, suggesting that these strains could also self-inhibit their own adhesion (FIG. **4**C).

[0078] A biofilm formation inhibition test was also performed with a strain of *Neisseria meningitidis*, the capsule of which is biochemically very similar to the group II capsule of *E. coli*. Interestingly, the results show that the supernatant of *N. meningitidis* also inhibits the biofilm formation of *E. coli* MG1655F' (FIG. **5**), demonstrating that anti-biofilm activity is a property not only of the group II capsule from *E. coli* but also of capsules known to be similar to the latter (i.e., group II-like capsules).

Example 4

Physico-Chemical Properties of the CFT073 Supernatant

[0079] When the inventors analyzed the composition of the polysaccharidic fractions precipitated from the active supernatants of different group II capsule *E. coli* serotypes, including CFT073 (K2), U-9 (non-K2) and IHE3034 (K1), they observed, in agreement with previous studies (Jann et al., 1980; Silver and Vimr, 1984), that these fractions displayed significantly different compositions (data not shown). This suggested that, although biochemically distinct, group II capsules released by these strains could share a similar mode of action leading to biofilm inhibition. To further study the mechanisms by which group II capsule inhibit bacterial biofilm formation, these fractions were brought into contact with cationic colloids composed of 10 μm in diameter latex particles bearing permanent net positive charge due to their polyethylenimine coating. The determination of the interface ζ (Zeta) potential showed that the wild-type supernatants induced a strong charge inversion of the cationic colloids, indicative of their highly anionic nature as compared to the supernatants of their respective capsule mutants (FIG. **6***a*). Moreover, the treatment of acid-cleaned glass slides with active supernatant lowered the water-slide interfacial energy, which is indicative of their hydrophilic nature (FIG. **6***b*).

[0080] To analyze whether group II capsule could induce surface modifications and affect intermolecular forces on the treated surfaces, the inventors monitored the adsorption of propidium iodide, a fluorescent amphiphillic cationic ion, on colloids coated with active or inactive supernatants. They first showed that anionic but inactive supernatant of the non-group II capsulated *E. coli* EcoR72 displayed strong affinity for the cationic fluorescent probe (FIG. **6***c*). Despite their high negative charge, active supernatants displayed significantly lower probe affinity than inactive but less negatively charged capsule mutant supernatants (FIGS. **6***c* and **6***d*). This effect was even more pronounced with the 500 kDa K2 capsular active fraction (FR2) purified from CFT073 by anion exchange-chromatography containing galactose, glycerol, phosphate and acetate in the molar ratio of 1:2:1:1 (Jann et al., 1980) (FIGS. **6***c* and **6***d*). Therefore, these results showed that, besides strong electrostatic modifications, active supernatants also induced a profound remodelling of the colloid surface properties, possibly including surface hydration and steric repulsion. These analyses confirm that the surface modifications induced by group II capsule are more critical for the biofilm inhibition activity than the capsule primary composition.

Example 5

Prevention of Biofilm Development

[0081] The physico-chemical properties displayed by group II capsule might deeply alter bacterial ability to interact with surfaces and therefore drastically reduce adhesion (Neu, 1996). To test this hypothesis, the capacity of both MG1655 F' and *S. aureus* to adhere to glass surfaces pre-treated with CFT073 supernatant was analysed. After 1 hour of incubation, *E. coli* MG1655 F' and *S. aureus* 15981 exhibited a 3-fold reduction in their initial adhesion on treated surface (data not shown). Consistently, pre-treatment of the internal microfermentor glass slide with CFT073 supernatant drastically reduced biofilm formation by *E. coli* and a wide range of Gram-positive and Gram-negative bacteria (FIG. **7**). The same effect was observed when CFT073 supernatant was perfused in the microfermentor (FIG. **2**B). No effect was observed when a similar treatment was performed with CFT073ΔkpsD supernatant (FIG. **7**). These results therefore suggested that the surface modifications induced by capsular polysaccharides released in the CFT073 supernatant could interfere with biofilm formation by impairing initial bacterial-surface interactions.

[0082] Remarkably, the anti-biofilm effect of the CFT073 supernatant persisted even after drastic treatments of the glass slide (FIG. **8**), which suggests that the group II capsule could be used in applications which necessitate a sterilisation step (such as agro-industrial or medical applications).

[0083] In order to investigate the effect of CFT073 supernatant on already existing biofilms, microfermentors inoculated with MG1655 F' at different stages of biofilm maturation were supplemented with filtered CFT073 supernatant. This analysis showed that, whereas the treatment of a mature

24 h biofilm did not induce biofilm dispersal, addition of the CFT073 supernatant at 0, 1 and 6 h after MG1655 F' biofilm initiation blocked its further development (FIG. **9**A). The inventors then examined the in vitro biofilm characteristics of a GFP-tagged MG1655F' after addition of CFT073 supernatant and confocal laser scanning microscopy (CLSM). After 3 h post initial inoculation, the addition of active CFT073 exogenous supernatant on a regularly covered surface profoundly affected MG1655F' mature biofilm structure development (FIG. **9**B). This effect was not observed upon control KS272 supernatant treatment.

[0084] The direct contribution of bacterial surface structures to the tri-dimensional *E. coli* biofilm structure has been amply demonstrated (Beloin et al., 2005). These structures have also been shown to mediate bacterial aggregation and clumping in standing cultures. To further characterize the role of group II capsule in biofilm maturation, the inventors tested its effects on bacterial aggregation mediated by several different surface-exposed factors also involved in biofilm formation. It was shown that CFT073 supernatant prevents formation of bacterial aggregates induced by different types of bacterial surface structures (FIG. **9**C).

[0085] The anti-biofilm activity of different concentrations of the FR2 fraction was tested in microtiter plate assays. This showed that the purified FR2 fraction is active at concentrations starting from 50 μg/ml (FIG. **10**).

[0086] Taken together, these results suggest that the physico-chemical properties of the group II capsular polysaccharides affect biofilm formation by weakening cell-surface contacts (initial adhesion) but also by reducing cell-cell interactions (biofilm maturation).

[0087] In conclusion, the inventors demonstrated that group II-like capsular polysaccharides are released in the culture supernatant and display anti-adhesion properties against a wide range of bacteria, including important nosocomial pathogens. This study reveals a novel property of the group II capsular polysaccharides that are commonly expressed by extra-intestinal *E. coli*, but also by other pathogens such as *Neisseria meningitides* (Kaijser, 1973; Sandberg et al., 1988), which supernatant could also inhibit *E. coli* biofilm formation (data not shown). Group II capsule has been shown to be involved in UPEC virulence by increasing their resistance to phagocytosis and to the bactericidal effects of human serum (Cross et al., 1986; Kaper et al., 2004; Pluschke et al., 1983; Russo et al., 1995). Capsule could also play an important biological role in UPEC interactions with living and inert surfaces. In particular, besides bacterial competition, the inhibition of UPEC own adhesion by group II capsule secretion may contribute to gastrointestinal tract colonisation by reducing bacteria-bacteria interactions (Schembri et al., 2004), thus avoiding bacterial clearance due to clump formation (Favre-Bonte et al., 1999). Consistently, it was observed that an uncapsulated CFT073ΔR1 mutant is unable to colonize the mouse intestine (FIG. **11**).

[0088] The in vitro analyses indicate that group II capsule can induce surface modifications such as charge inversion of cationic surface, increased surface wettability and molecular repulsion, leading to non-specific anti-adhesion properties. Since this inhibitory effect was observed in both exponential and stationary growth phase supernatants as well as in a quorum-sensing ΔluxS mutant of CFT073 (FIG. **12**), this suggests that the anti-biofilm effect does not involve cell-signaling (Waters and Bassler, 2005), but rather acts through physico-chemical alteration, of either abiotic or bacterial surfaces. Polymers assembling on surfaces are known to cause strong physical repulsion depending on their density, size, solvation and structure (de Gennes, 1987). Such repulsive forces created by capsule polymers could limit initial bacterial adhesion and biofilm development by interfering with subsequent cell-cell contacts. Finally, the inventors showed that the application of group II capsular polysaccharides on abiotic surfaces reduces bacterial initial adhesion, and has enough long-lasting effect to significantly inhibit mature biofilm development of a broad-spectrum of bacteria. This finding may have far reaching implications in the design of therapeutic strategies to limit the formation of pathogenic biofilms, for example, on medical implants.

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1. A method for preventing or inhibiting bacterial adhesion and/or bacterial biofilm development on a substrate, comprising:

treating said substrate with a composition of a soluble group II-like capsular polysaccharide obtained from a bacterial strain.

2. The method of claim **1**, wherein said soluble group II-like capsular polysaccharide is obtained from the supernatant of a culture of bacteria selected from the group consisting of *Escherichia coli, Hemophilus influenzae* and *Neisseria meningitidis*.

3. The method of claim **1**, wherein said soluble group II-like capsular polysaccharide is obtained as a purified fraction.

4. A composition, comprising:

a soluble group II-like capsular polysaccharide obtained from a bacterial strain, wherein said composition inhibits bacterial adhesion and/or bacterial biofilm development.

5. The composition of claim **4**, which comprises a purified fraction of the supernatant of a culture of bacteria selected from the group consisting of *E. coli, H. influenzae* and *N. meningitidis*.

6. A process for purifying an anti-biofilm group II-like capsular polysaccharide obtained from a bacterial strain, comprising the following steps:

(i) separating the supernatant of a culture of a bacterial strain expressing a group II-like capsule from the bacterial cells,

(ii) precipitating the polysaccharides present in the obtained supernatant, and

(iii) optionally resuspending the precipitate.

7. The process of claim **6**, wherein said bacterial strain expressing a group II-like capsule is selected from the group consisting of *E. coli, H. influenzae* and *N. meningitidis*.

8. The process of claim **7**, wherein said bacterial strain is a uropathogenic *E. coli*.

9. The process of any of claim **6**, wherein the separation step (i) is performed by filter-sterilization and/or by centrifugation of the culture.

10. The process of any of claim **6**, wherein the precipitation in step (ii) is performed with three volumes of ethanol for one volume of supernatant.

11. The process of any of claim **6**, wherein the precipitate obtained in step (ii) is resuspended in water, dialyzed against deionised water, and then lyophilized before step (iii).

12. The process of any of claim **6**, further comprising an additional step (iv) of purification by ion exchange chromatography.

13. The process of claim **12**, wherein step (iv) is performed with a DEAE-Sepharose column.

14. The process of claim **12**, wherein the resuspension in step (iii) is done in TrisHCl 20 mM, pH 7.5, with 25% propanol-1, and the column of step (iv) is equilibrated with the same buffer.

15. The process of claim **12**, wherein a centrifugation step is performed between step (iii) and step (iv) to discard an insoluble fraction.

16. The process of claim **12**, wherein said group II-like capsular polysaccharide is eluted with 300 mM NaCl in TrisHCl 20 mM, pH 7.5, 25% propanol-1.

17. A method for preventing or inhibiting bacterial adhesion and/or bacterial biofilm development on a substrate, comprising:

treating said substrate with a composition of a soluble group II capsular polysaccharide obtained from a bacterial strain as prepared by the process according to claim **6**.

18. The composition of claim **4**, which is formulated for preventive or therapeutic administration to a subject in need thereof.

19. An anti-biofilm coating, comprising:

a group II-like capsular polysaccharide obtained from a bacterial strain.

20. The anti-biofilm coating of claim **19**, wherein said group II-like capsular polysaccharide is obtained from a bacterial strain selected from the group consisting of *Escherichia coli, Hemophilus influenzae* and *Neisseria meningitidis*.

21. An anti-biofilm coating, comprising: an applied film of the composition of claim **4**.

22. A medical or industrial device which is at least partly coated with the anti-biofilm coating according to claim **19**.

23. A composition, comprising:

a soluble group II capsular polysaccharide obtained from a bacterial strain obtained through the process according to claim **6**.

24. The composition of claim **23**, which is formulated for preventive or therapeutic administration to a subject in need thereof.

* * * * *