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(54) Benævnelse: **Induction of a physiological dispersion response in bacterial cells in a biofilm**

(57) Sammendrag:

(57) Abstract: One aspect of the present invention is directed to a composition. The composition includes a dispersion inducer comprising: H_3C-$(CH2)_n$-CH_m CH_mR, where is a single or double carbon-carbon bond, m is 1 or 2, n is 2 to 15, and R is a carboxylic acid, a salt, an ester, or an amide, where the ester or amide is an isostere or biostere of the carboxylic acid. The composition additionally contains an additive component selected from one or more of the group consisting of biocides, surfactants, antibiotics, antiseptics, detergents, chelating agents, virulence factor inhibitors, gels, polymers, pastes, edible products, and chewable products. The composition is formulated so that when it is contacted with a biofilm produced by a microorganism, where the biofilm comprises a matrix and microorganism on a surface, the dispersion inducer selectively acts on the microorganism and has a suitable biological response without a required direct effect on the matrix to disperse the biofilm. The present invention is also directed to methods of using this compound.

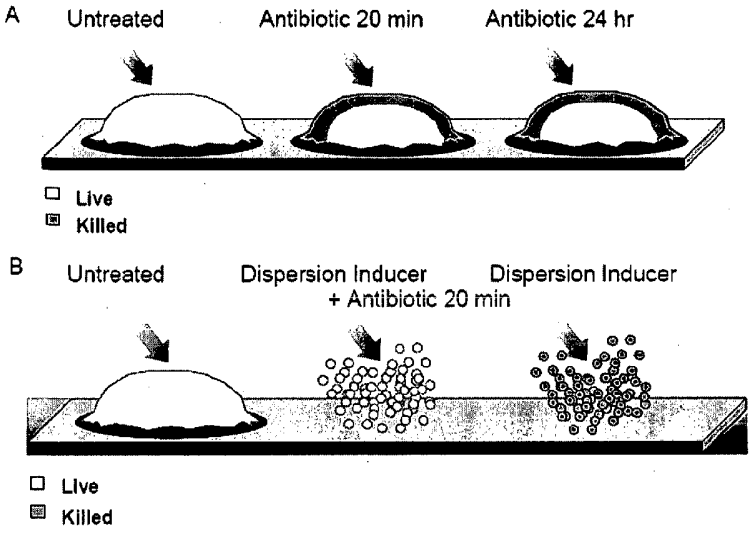


Figure 1

WHAT IS CLAIMED:

1. A composition comprising:

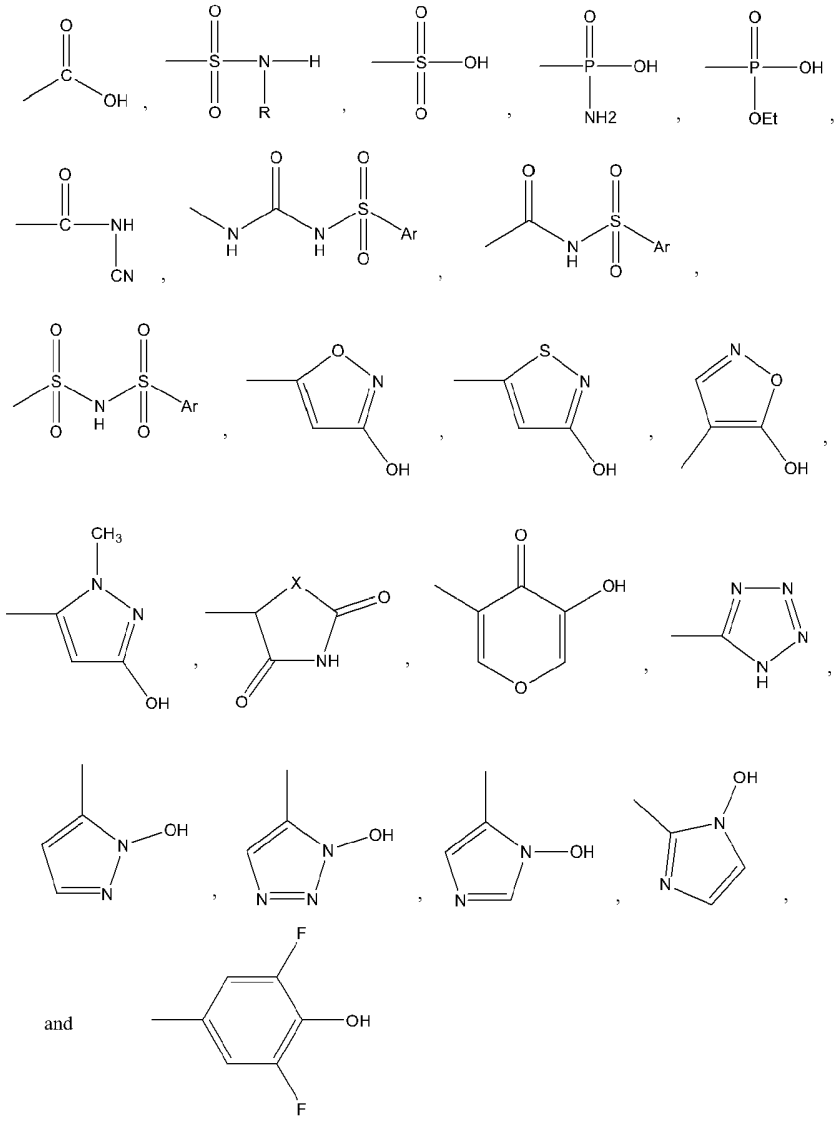
one or more dispersion inducers comprises:

$$H_3C\text{-}(CH_2)_n\text{-}CH_m \text{======} CH_mR,$$

wherein ====== is a single or double carbon-carbon bond, m is 1 or 2, n is 2 to 15, and R is a carboxylic acid, a salt, an ester, or an amide, wherein the ester or amide is an isostere or biostere of the carboxylic acid and

one or more additive components selected from the group consisting of biocides, surfactants, antibiotics, antiseptics, detergents, chelating agents, virulence factor inhibitors, gels, polymers, pastes, edible products, and chewable products, said composition being formulated so that when it is contacted with a biofilm produced by a microorganism, where the biofilm comprises a matrix and microorganism on a surface, the dispersion inducer selectively acts on the microorganism and has a suitable biological response without a required direct effect on the matrix to disrupt the biofilm.

2. The composition of claim 1, wherein R is selected from the group consisting of:



and

a homoserine lactone or a furanone group.

3. The composition of claim 1, wherein the composition is a dentifrice, mouthwash, or surgical glue.

4. A surface coated with the composition according to claim 1.

5. The surface of claim 4, wherein the surface is a dental floss, a contact lens, or a bone implant.

6. A method of treating or preventing a condition mediated by a biofilm in a subject, said method comprising:

providing a subject having, or susceptible to, a condition mediated by a biofilm produced by a microorganism, whereby the biofilm comprises a matrix and the micro-organism on a surface and

administering to the subject a dispersion inducer comprising:

$$H_3C\text{-}(CH_2)_n\text{-}CH_m \text{======} CH_mR,$$

wherein ====== is a single or double carbon-carbon bond, m is 1 or 2, n is 2 to 15, and R is a carboxylic acid, a salt, an ester, or an amide, wherein the ester or amide is an isostere or biostere of the carboxylic acid, under conditions effective for the dispersion inducer to selectively act on the microorganism and have a suitable biological response without a required direct effect on the matrix, whereby the condition mediated by a biofilm in the subject is treated or prevented.

7. The method of claim 6, wherein a subject with burns, dental plaque, dental caries, gingival disease, oral infection, acne or other biofilm-associated skin infections on the skin, or a chronic biofilm-associated disease is treated.

8. The method of claim 7, wherein said administering is carried out with a dentifrice, mouthwash, dental floss, gum, strip, or brush.

9. The composition of claim 1 or the method of claim 6, wherein said dispersion inducer is administered at a concentration of 0.01 μM to mM.

10. The composition of claim 1 or the method of claim 6, wherein said dispersion inducer is administered as a composition formulated so that said dispersion inducer is non-bacteriocidal.

11. The composition of claim 1 or the method of claim 6, wherein said dispersion inducer is administered at a concentration of less than 0.5 percent by weight.

12. A method of treating or inhibiting formation of a biofilm on a surface, said method comprising:

providing a surface having or being susceptible to formation of a biofilm produced by a microorganism, whereby the biofilm comprises a matrix and the micro-organism on the surface and

administering to the surface a dispersion inducer comprising:

$$H_3C\text{-}(CH_2)_n\text{-}CH_m \text{======} CH_mR,$$

wherein ====== is a single or double carbon-carbon bond, m is 1 or 2, n is 2 to 15, and R is a carboxylic acid, a salt, an ester, or an amide, wherein the ester or amide is an isostere or biostere of the carboxylic acid, under conditions effective for the dispersion inducer to selectively act on the microorganism and have a suitable biological effect without a required direct effect on the matrix, whereby formation of the biofilm on the surface is treated or inhibited.

13. The method of claim 12, wherein the surface is a contact lens, an indwelling medical device, an implanted medical device, drains, tubs, kitchen appliances, countertops, shower curtains, grout, toilets, industrial food and beverage production facilities, flooring, food processing equipment, a heat exchanger surface, a filter surface, a marine structure, a system for water treatment and/or distribution, or a surface associated with a system for petroleum drilling or storage or separation or refining or distribution or porous medium from which the petroleum is extracted,

14. The method of claim 6 or claim 12 further comprising:

administering to the surface, in conjunction with said administering the dispersion inducer, at least one antimicrobial treatment selected from the group consisting of biocides, surfactants, antibiotics, antiseptics, detergents,

chelating agents, virulence factor inhibitors, ultrasonic treatment, radiation treatment, thermal treatment, and mechanical treatment.

15. The method of claim 14, wherein the dispersion inducer is impregnated in the surface or administered in a copolymer or a gel coating the surface.

16. A solution comprising:

a dispersion inducer comprising:

$$H_3C\text{-}(CH_2)_n\text{-}CH_m \text{======} CH_mR,$$

wherein ====== is a single or double carbon-carbon bond, m is 1 or 2, n is 4 to 7, and R is a carboxylic acid, wherein said inducer is present at a concentration less than 0.5 percent by weight, and wherein said solution has a pH greater than 5.

17. The solution of claim 16, further comprising a component selected from one or more of the group consisting of biocides, surfactants, antibiotics, antiseptics, detergents, chelating agents, virulence factor inhibitors, gels, polymers, pastes, edible products, and chewable products.

18. A composition comprising:

a component selected from one or more of the group consisting of biocides, surfactants, antibiotics, antiseptics, detergents, chelating agents, virulence factor inhibitors, gels, polymers, pastes, edible products, and chewable products, and

a dispersion inducer having the following formula:

$$H_3C\text{-}(CH_2)_n\text{-}CH_m \text{======} CH_mR,$$

wherein ====== is a single or double carbon-carbon bond, m is 1 or 2, n is 4 to 7, and R is a carboxylic acid, said inducer being formulated in a non-salt form.

19. The solution of claim 16 or the composition of claim 18, wherein said composition is in a formulation selected from the group consisting of a skin cream, a toothpaste, and a mouthwash.

20. A method comprising:

a) providing contact lenses and a solution comprising a dispersion inducer at a concentration less than 0.5% by weight, said inducer comprises:

$$H_3C\text{-}(CH_2)_n\text{-}CH_m \text{======} CH_mR,$$

wherein ====== is a single or double carbon-carbon bond, m is 1 or 2, n is 4 to 7, and R is a carboxylic acid, a salt, an ester, or an amide, wherein the ester or amide is an isostere or biostere of the carboxylic acid; and

b) treating said contact lenses with said solution.

21. A method comprising:

a) providing a subject with a skin condition and a solution having a pH greater than 5, said solution comprising a dispersion inducer at a concentration less than 0.5% by weight, said inducer comprises:

$$H_3C\text{-}(CH_2)_n\text{-}CH_m \text{======} CH_mR,$$

wherein ====== is a single or double carbon-carbon bond, m is 1 or 2, n is 4 to 7, and R is a carboxylic acid, a salt, an ester, or an amide, wherein the ester or amide is an isostere or biostere of the carboxylic acid; and

b) treating said skin condition with said solution.

22. The method of claim 21 or claim 20, the composition of claim 1 or claim 18, or the solution of claim 16, wherein said dispersion inducer comprises:

$$CH_3\text{-}(CH_2)_n\text{-}CH{=}CHCOOH.$$

23. The method of claim 20 or claim 21, wherein said solution further comprises at least one component selected from the group consisting of biocides, surfactants, antibiotics, antiseptics, detergents, chelating agents, and virulence factor inhibitors.

24. A composition comprising:

one or more dispersion inducers and

one or more additive components selected from the group consisting of biocides, surfactants, antibiotics, antiseptics, detergents, chelating agents, virulence factor inhibitors, gels, polymers, pastes, edible products, and chewable products, said composition being formulated so that when it is contacted with a biofilm produced by a microorganism, where the biofilm comprises a matrix and microorganism on a surface, the dispersion inducer selectively acts on the microorganism and has a suitable biological response without a required direct effect to disrupt the matrix.

25. A method of treating or preventing a condition mediated by a biofilm in a subject, said method comprising:

providing a subject having, or susceptible to, a condition mediated by a biofilm produced by a microorganism, whereby the biofilm comprises a matrix and the micro-organism on a surface and

administering to the subject a dispersion inducer under conditions effective for the dispersion inducer to selectively act on the microorganism and have a suitable biological response without a required direct effect on the matrix, whereby the condition mediated by a biofilm in the subject is treated or prevented.

26. A method of treating or inhibiting formation of a biofilm on a surface, said method comprising:

providing a surface having or being susceptible to formation of a biofilm produced by a microorganism, whereby the biofilm comprises a matrix and the micro-organism on the surface and

administering to the surface a dispersion inducer under conditions effective for the dispersion inducer to selectively act on the microorganism and have a suitable biological response without a required direct effect on the matrix, whereby formation of the biofilm on the surface is treated or inhibited.

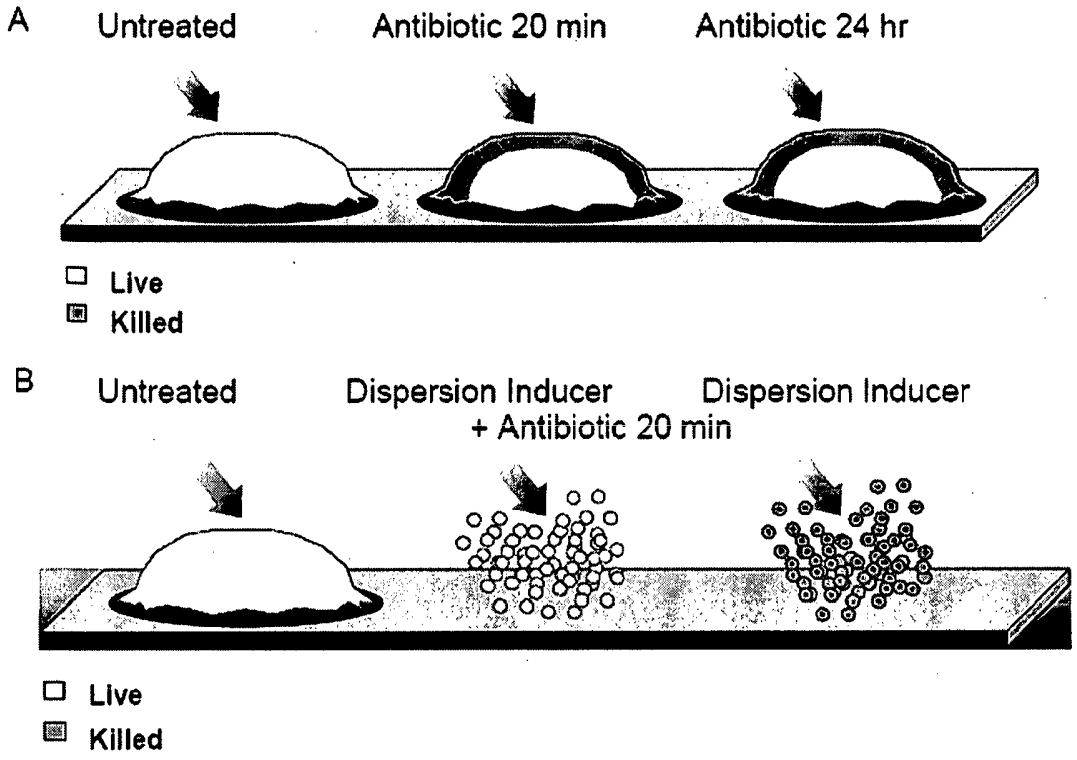

A
Untreated
Antibiotic 20 min
Antibiotic 24 hr
Live
Killed
B
Untreated
Dispersion Inducer
Dispersion Inducer
+ Antibiotic 20 min
Live
Killed

Figure 1

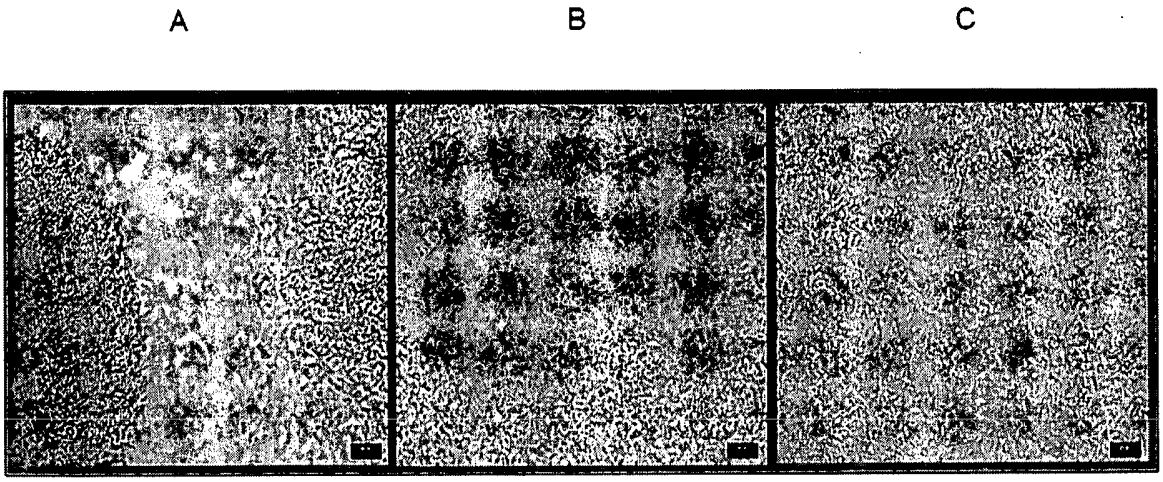
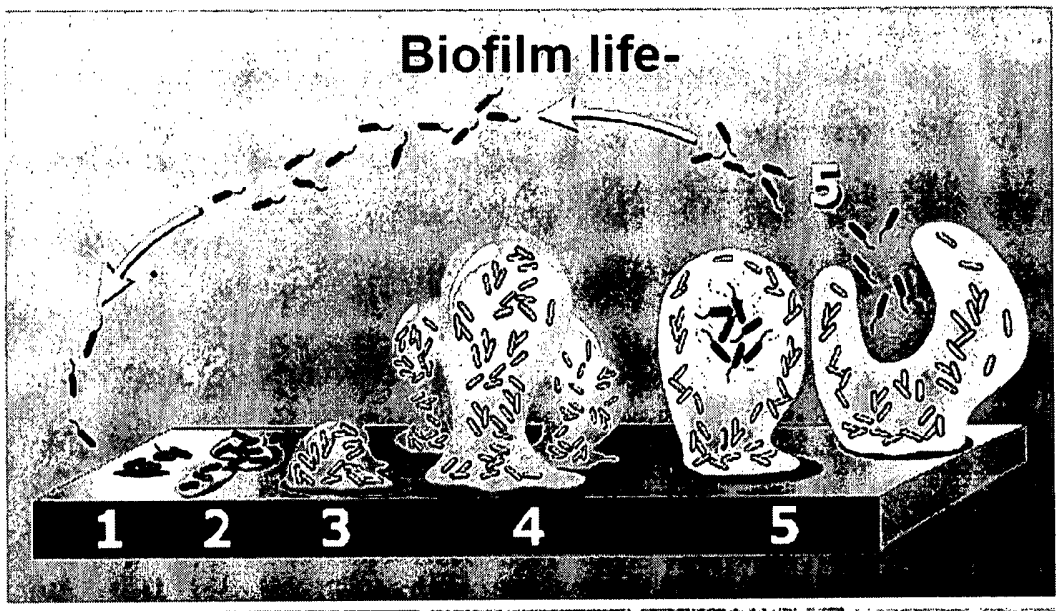

A
B
C
Before Treatment
5 min post Treatment
30 min post Treatment

Figure 2

A



B

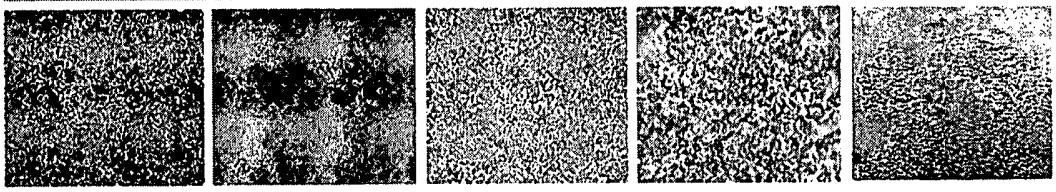
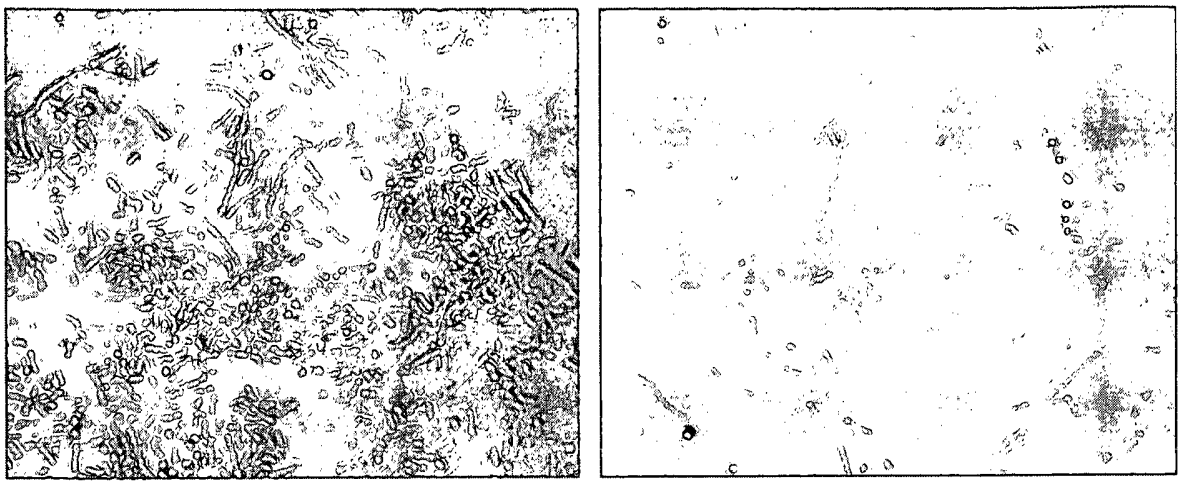


Figure 3



A B

Figure 4

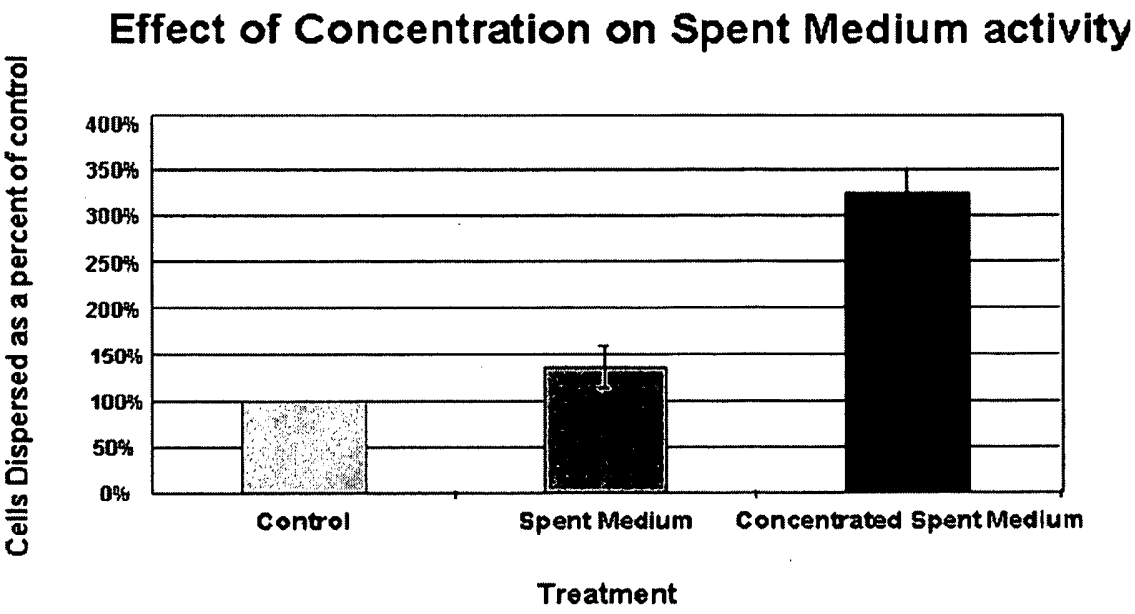

Effect of Concentration on Spent Medium activity
Cells Dispersed as a percent of control
400%
350%
300%
250%
200%
150%
100%
50%
0%
Control
Spent Medium
Concentrated Spent Medium
Treatment

Figure 5

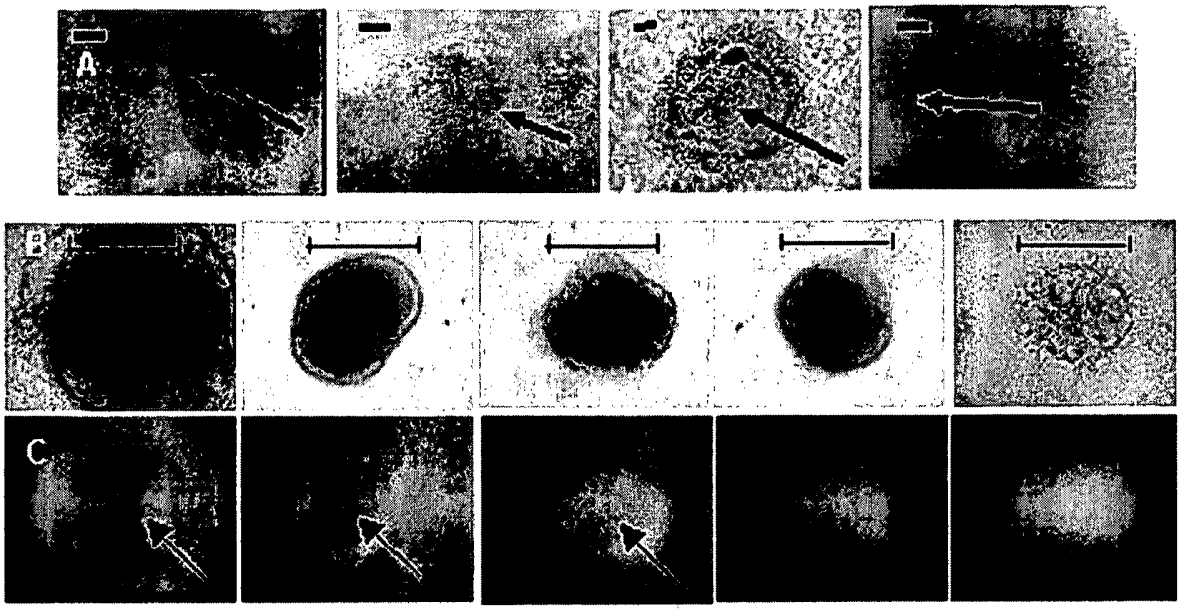


Figure 6

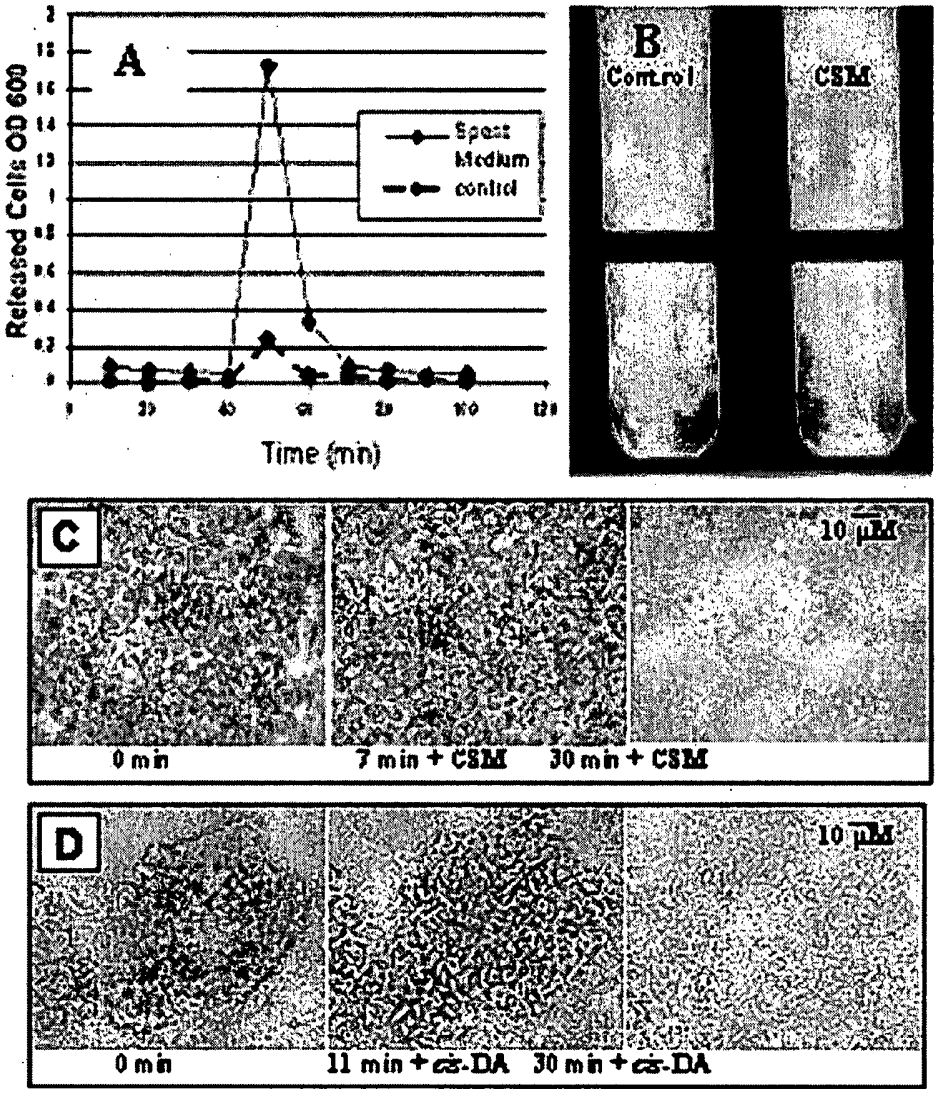

A
Released Cells OD 600
Time (min)
Spent Medium control
B
Control
CSM
C
10 µM
0 min
7 min + CSM
30 min + CSM
D
10 µM
0 min
11 min + α-DA
30 min + α-DA

Figure 7

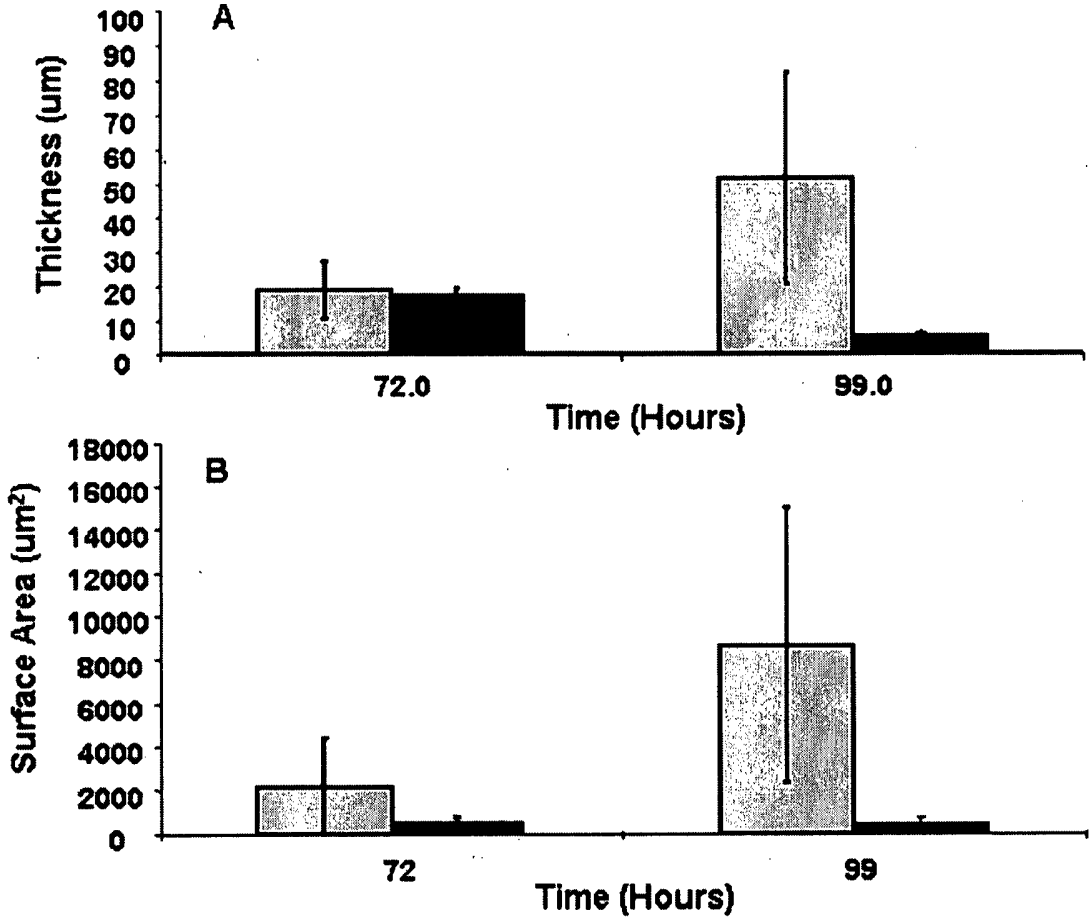

A
Thickness (um)
100
90
80
70
60
50
40
30
20
10
0
72.0
99.0
Time (Hours)
B
Surface Area (um²)
18000
16000
14000
12000
10000
8000
6000
4000
2000
0
72
99
Time (Hours)

Figure 8

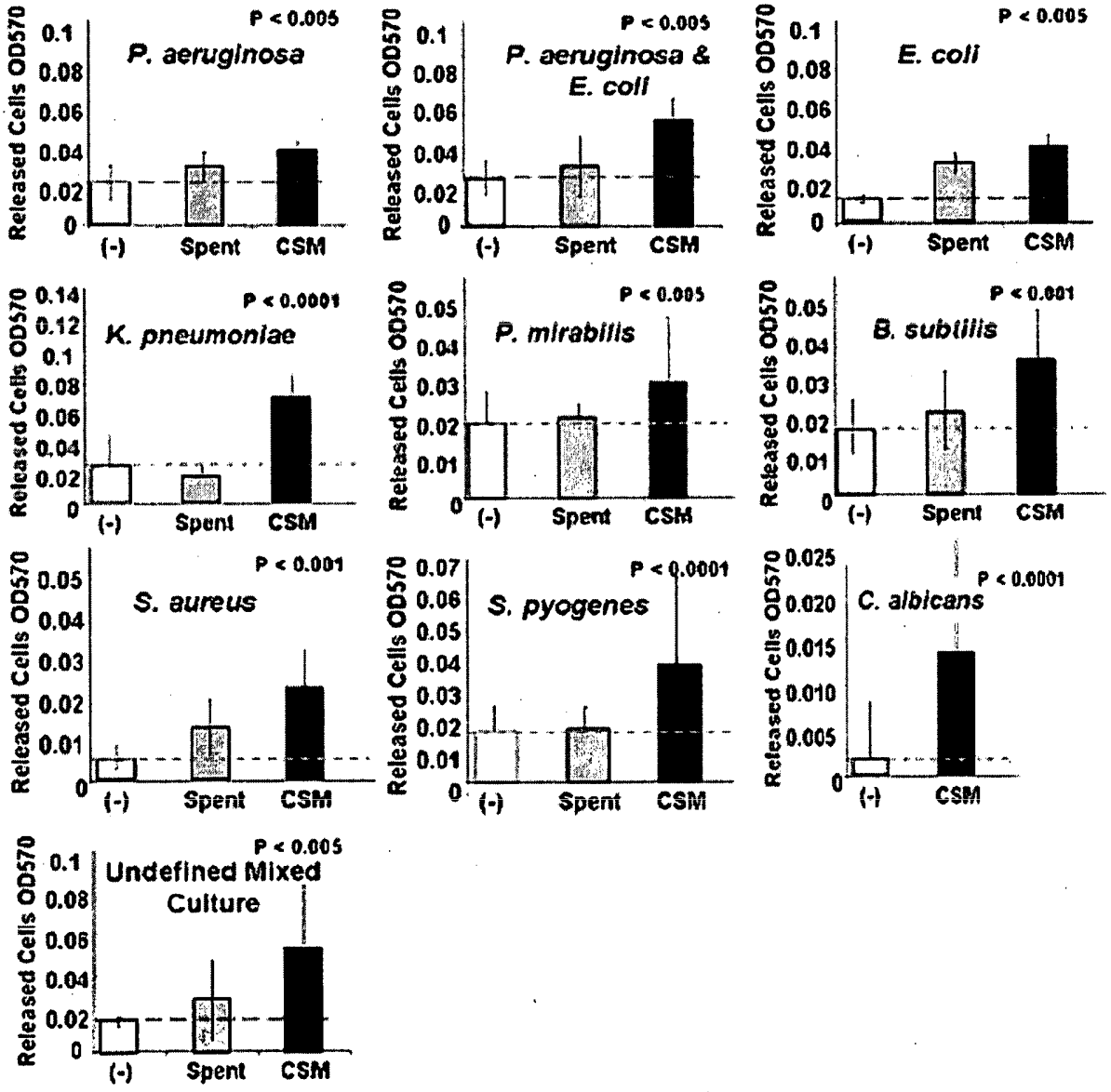

P. aeruginosa
P < 0.005
P. aeruginosa & E. coli
P < 0.005
E. coli
P < 0.005
K. pneumoniae
P < 0.0001
P. mirabilis
P < 0.005
B. subtilis
P < 0.001
S. aureus
P < 0.001
S. pyogenes
P < 0.0001
C. albicans
P < 0.0001
Undefined Mixed Culture
P < 0.005
Released Cells OD570
(-)
Spent
CSM

Figure 9

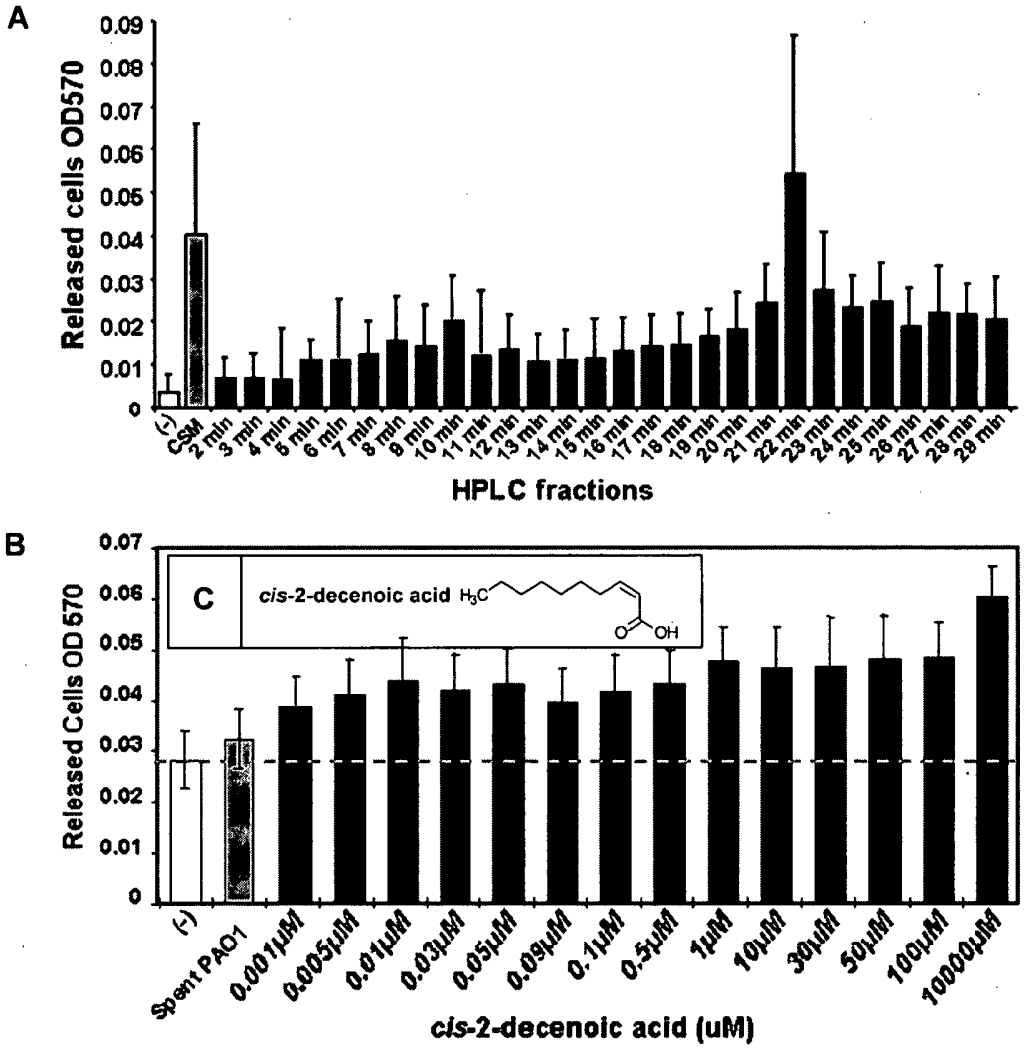

A
Released cells OD570
0.09
0.08
0.07
0.06
0.05
0.04
0.03
0.02
0.01
0
(-)
CSM
2 min
3 min
4 min
5 min
6 min
7 min
8 min
9 min
10 min
11 min
12 min
13 min
14 min
15 min
16 min
17 min
18 min
19 min
20 min
21 min
22 min
23 min
24 min
25 min
26 min
27 min
28 min
29 min
HPLC fractions
B
Released Cells OD 570
0.07
0.06
0.05
0.04
0.03
0.02
0.01
0
C
cis-2-decenoic acid
H3C
O
OH
(-)
Spent PAO1
0.001µM
0.005µM
0.01µM
0.03µM
0.05µM
0.09µM
0.1µM
0.5µM
1µM
10µM
30µM
50µM
100µM
1000µM
cis-2-decenoic acid (uM)

Figure 10

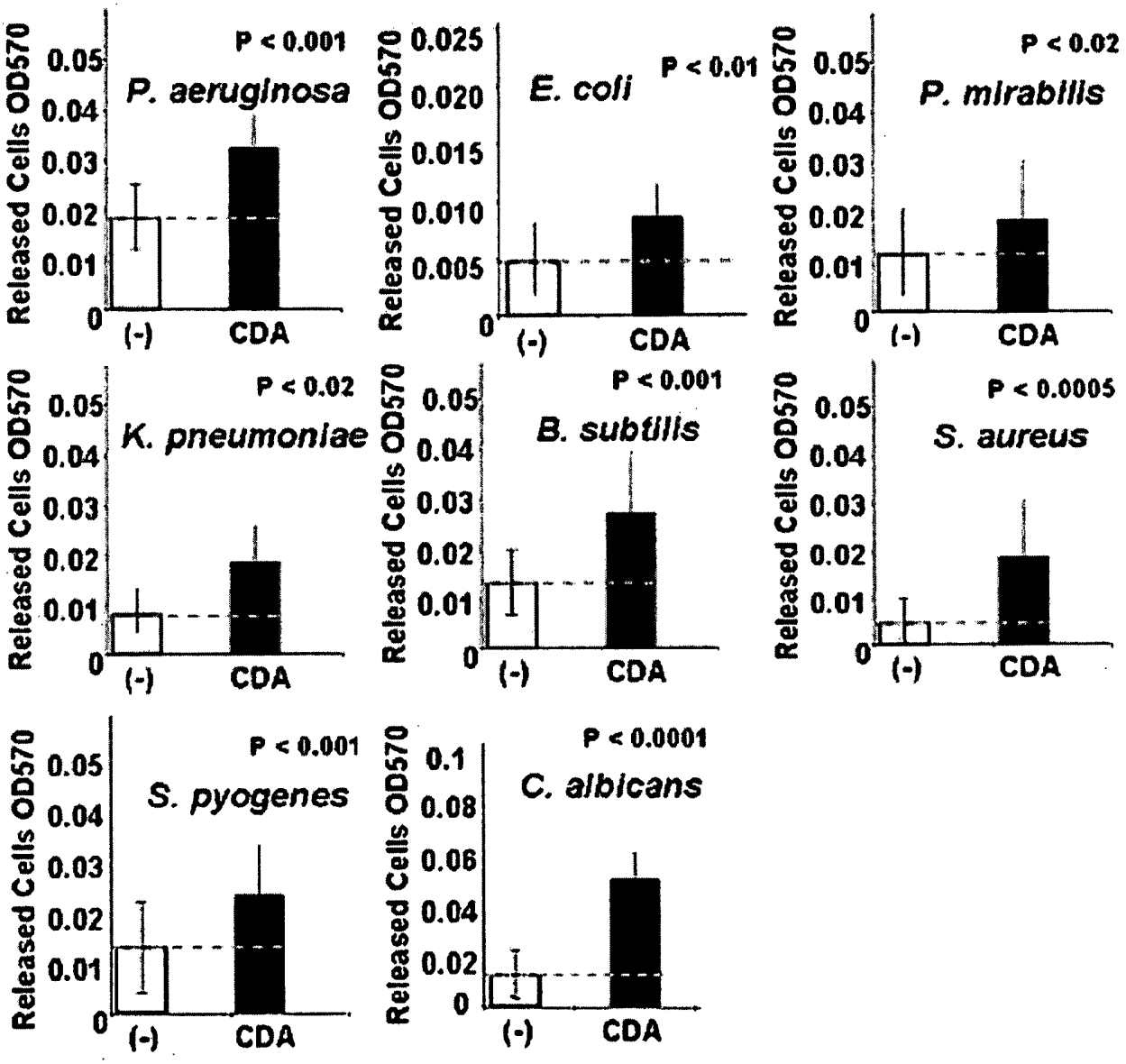

Released Cells OD570
P. aeruginosa
P < 0.001
E. coli
P < 0.01
P. mirabilis
P < 0.02
K. pneumoniae
P < 0.02
B. subtilis
P < 0.001
S. aureus
P < 0.0005
S. pyogenes
P < 0.001
C. albicans
P < 0.0001
(-)
CDA

Figure 11

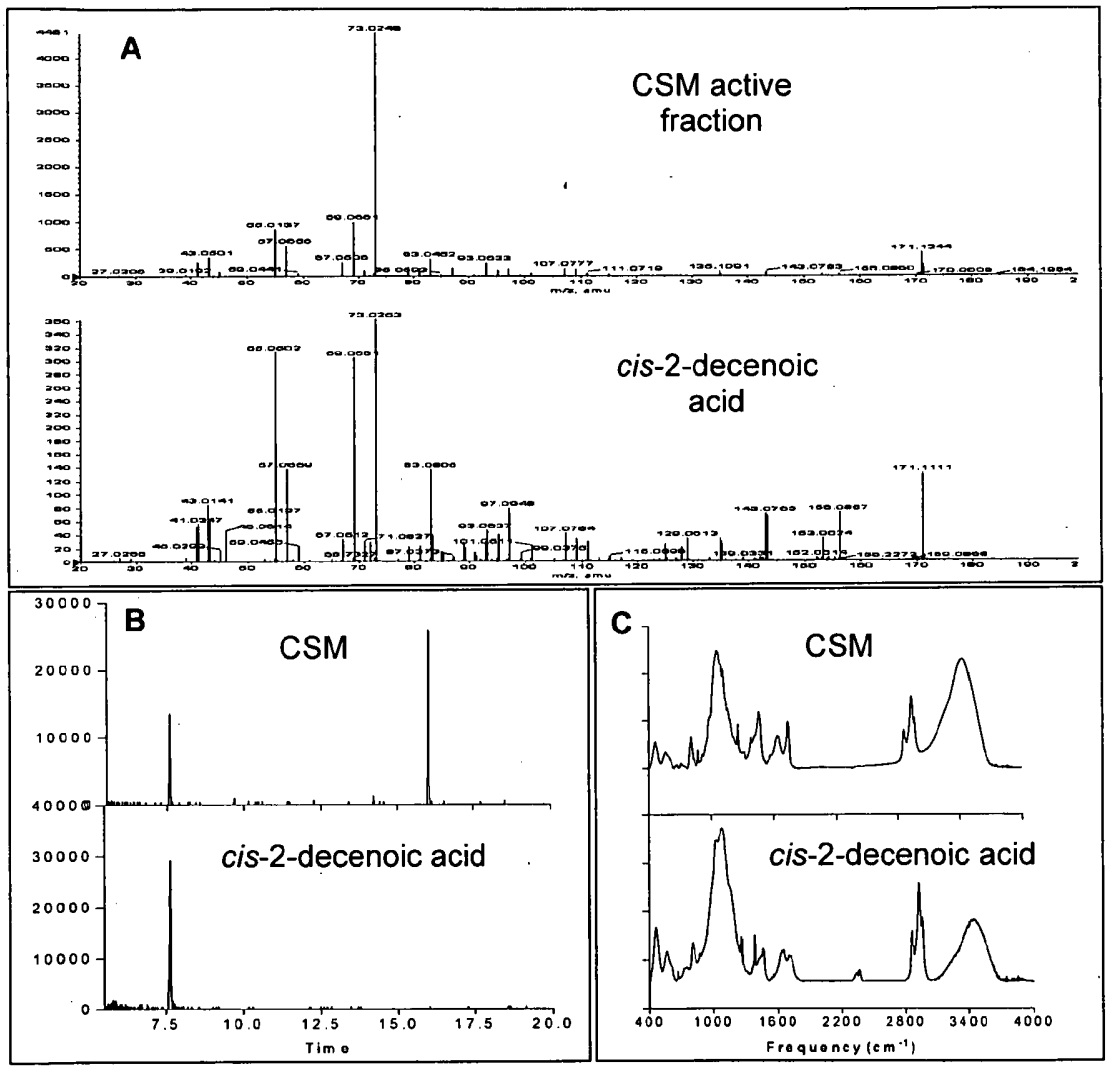

A
CSM active fraction
cis-2-decenoic acid
B
CSM
cis-2-decenoic acid
Time
C
CSM
cis-2-decenoic acid
Frequency (cm-1)

Figure 12

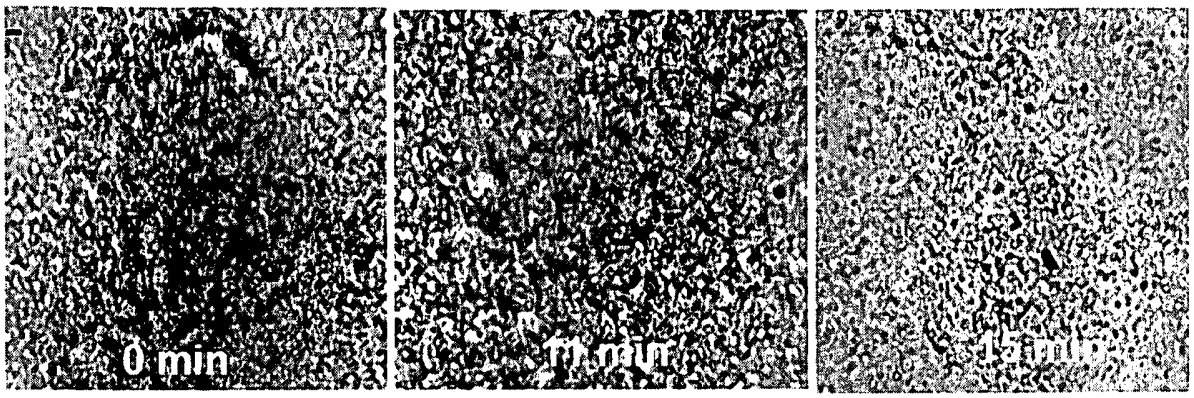


Figure 13