FIG. 1

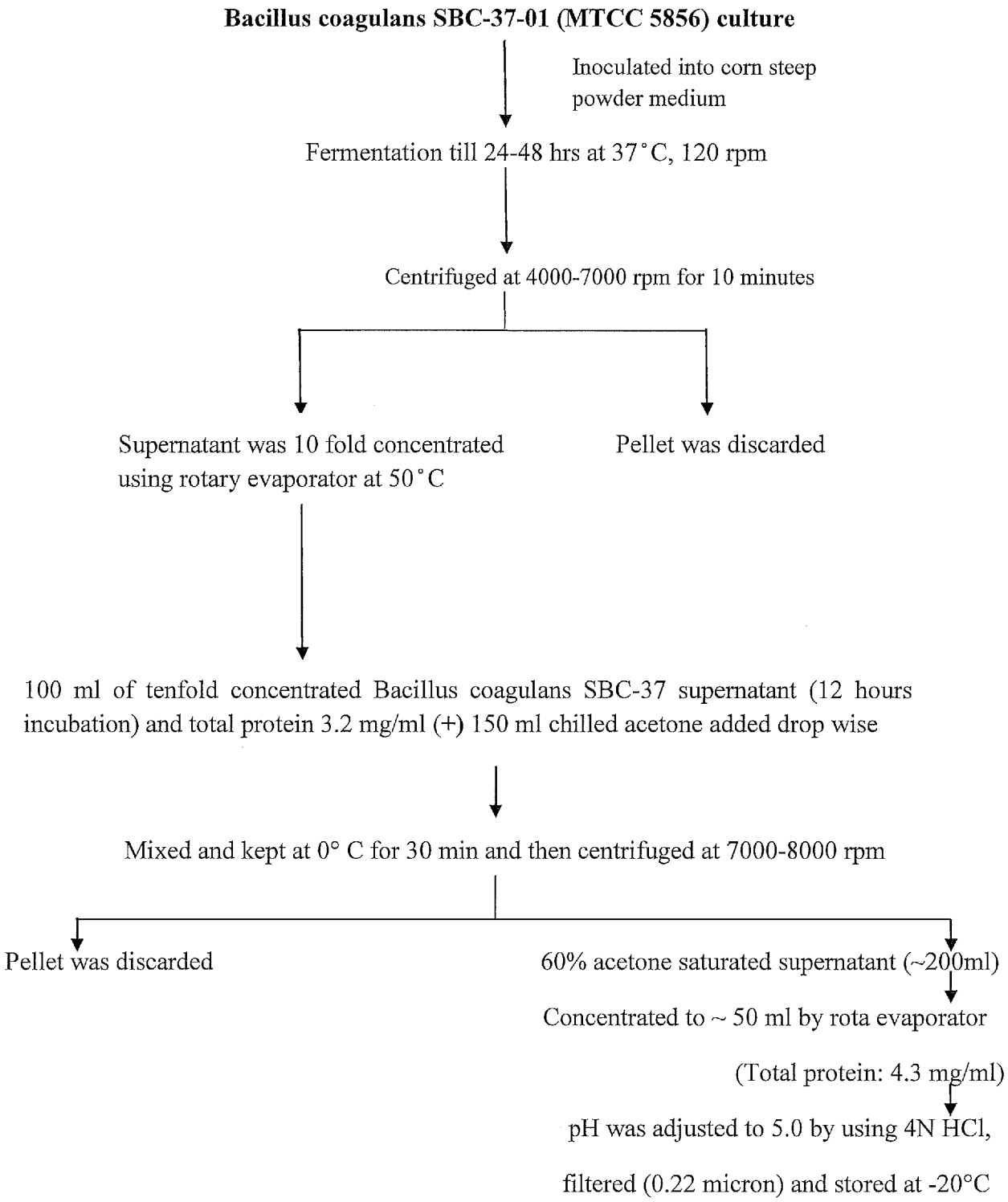

Bacillus coagulans SBC-37-01 (MTCC 5856) culture
Inoculated into corn steep powder medium
Fermentation till 24-48 hrs at 37°C, 120 rpm
Centrifuged at 4000-7000 rpm for 10 minutes
Supernatant was 10 fold concentrated using rotary evaporator at 50°C
Pellet was discarded
100 ml of tenfold concentrated Bacillus coagulans SBC-37 supernatant (12 hours incubation) and total protein 3.2 mg/ml (+) 150 ml chilled acetone added drop wise
Mixed and kept at 0° C for 30 min and then centrifuged at 7000-8000 rpm
Pellet was discarded
60% acetone saturated supernatant (~200ml)
Concentrated to ~ 50 ml by rota evaporator
(Total protein: 4.3 mg/ml)
pH was adjusted to 5.0 by using 4N HCl,
filtered (0.22 micron) and stored at -20°C

Fig.2

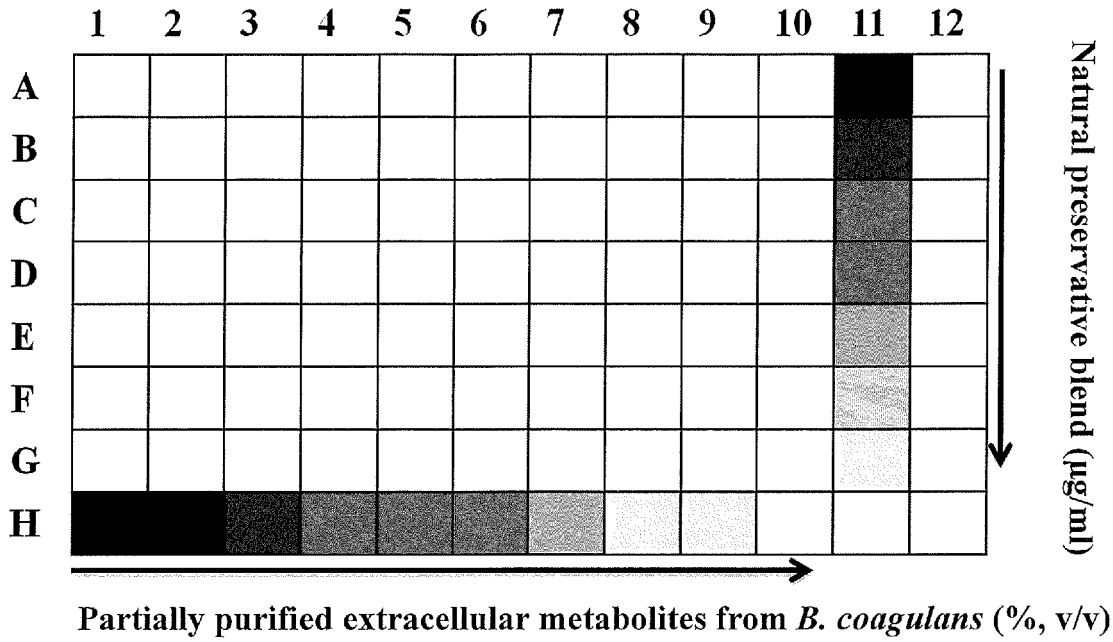

1 2 3 4 5 6 7 8 9 10 11 12
A
B
C
D
E
F
G
H
Natural preservative blend (µg/ml)
Partially purified extracellular metabolites from B. coagulans (%, v/v)

Fig. 3A

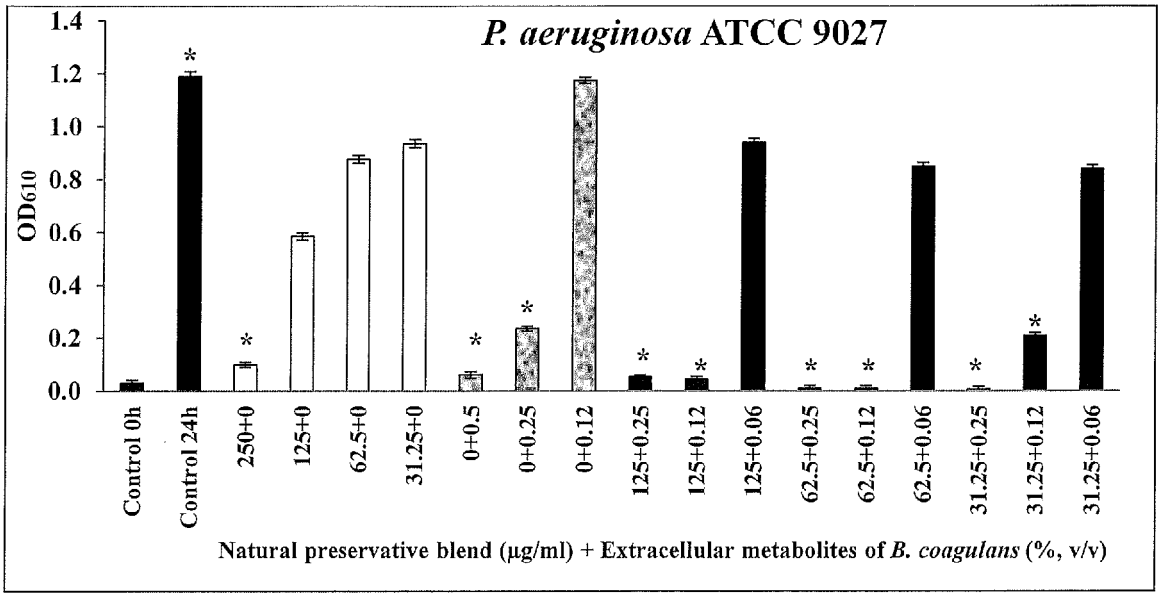

P. aeruginosa ATCC 9027
OD610
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
Control 0h
Control 24h
250+0
125+0
62.5+0
31.25+0
0+0.5
0+0.25
0+0.12
125+0.25
125+0.12
125+0.06
62.5+0.25
62.5+0.12
62.5+0.06
31.25+0.25
31.25+0.12
31.25+0.06
Natural preservative blend (µg/ml) + Extracellular metabolites of B. coagulans (%, v/v)

Fig. 3B

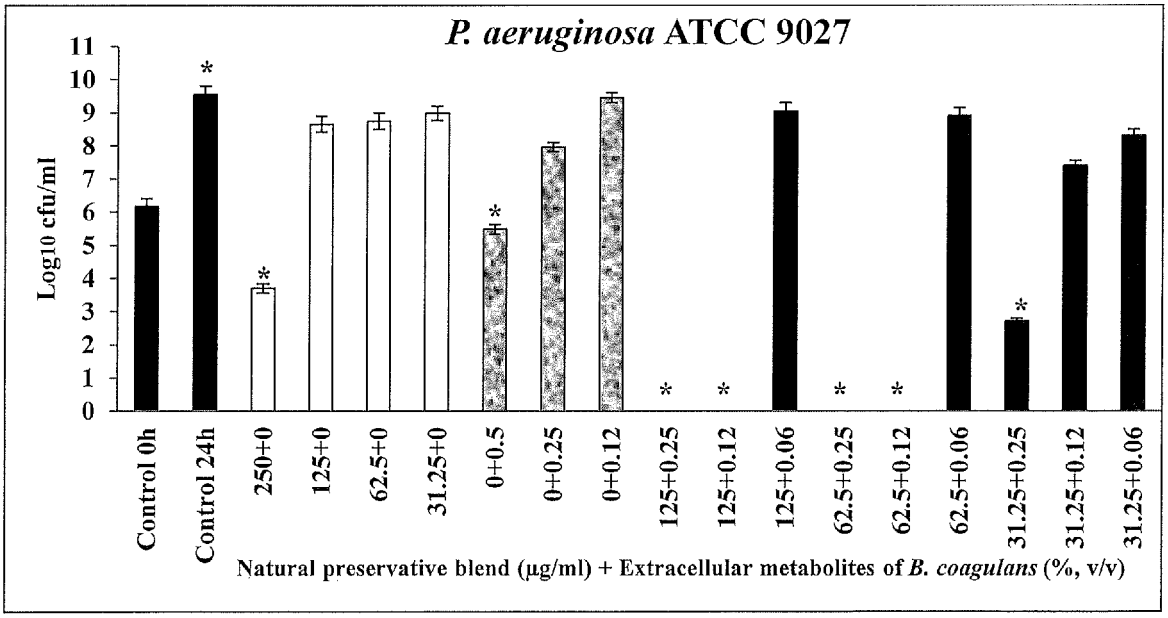

P. aeruginosa ATCC 9027
Log10 cfu/ml
11
10
9
8
7
6
5
4
3
2
1
0
Control 0h
Control 24h
250+0
125+0
62.5+0
31.25+0
0+0.5
0+0.25
0+0.12
125+0.25
125+0.12
125+0.06
62.5+0.25
62.5+0.12
62.5+0.06
31.25+0.25
31.25+0.12
31.25+0.06
Natural preservative blend (µg/ml) + Extracellular metabolites of B. coagulans (%, v/v)

Fig. 3C

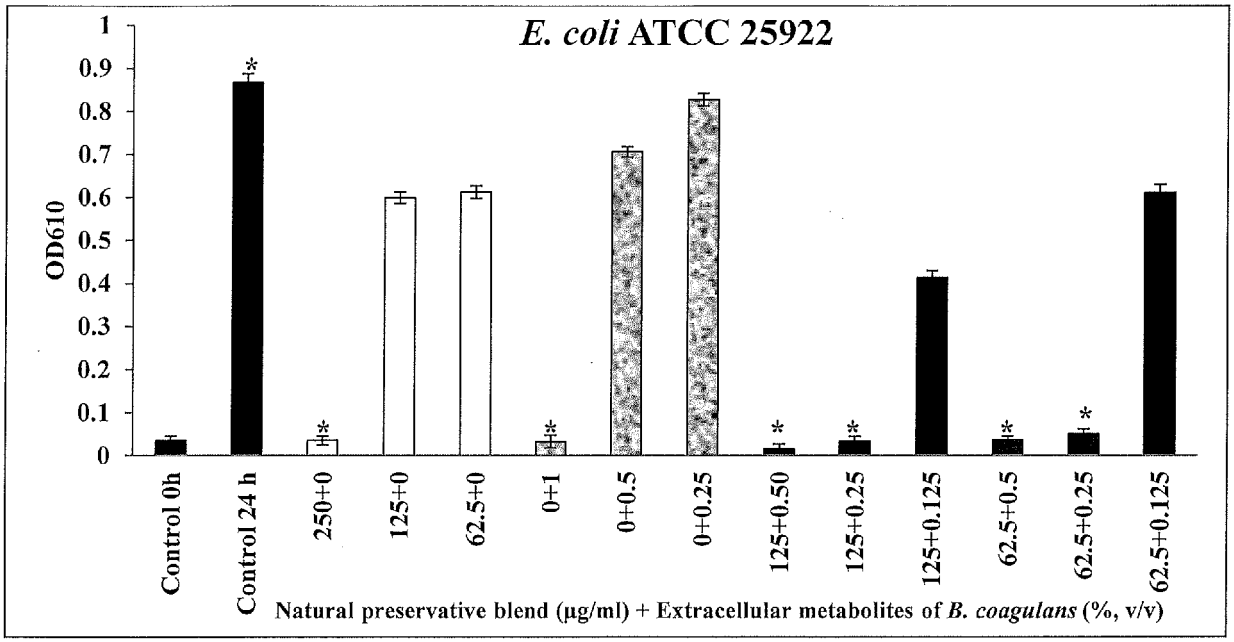

E. coli ATCC 25922
OD610
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
Control 0h
Control 24 h
250+0
125+0
62.5+0
0+1
0+0.5
0+0.25
125+0.50
125+0.25
125+0.125
62.5+0.5
62.5+0.25
62.5+0.125
Natural preservative blend (µg/ml) + Extracellular metabolites of B. coagulans (%, v/v)

Fig. 3D

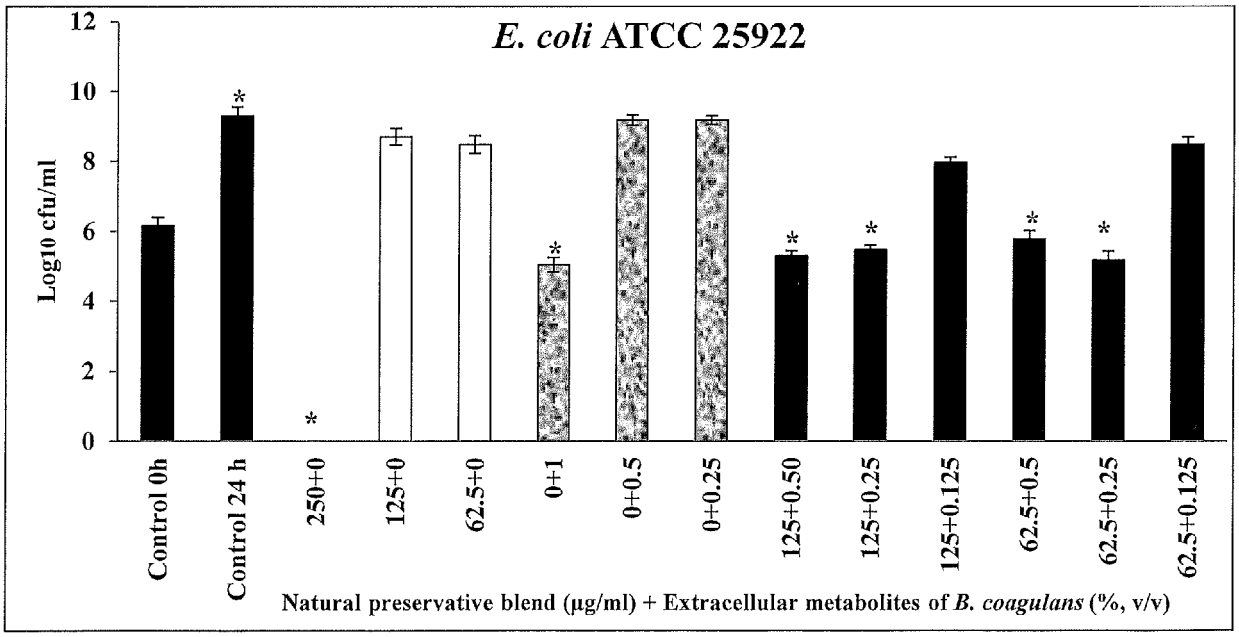

E. coli ATCC 25922
Log10 cfu/ml
12
10
8
6
4
2
0
Control 0h
Control 24 h
250+0
125+0
62.5+0
0+1
0+0.5
0+0.25
125+0.50
125+0.25
125+0.125
62.5+0.5
62.5+0.25
62.5+0.125
Natural preservative blend (µg/ml) + Extracellular metabolites of B. coagulans (%, v/v)

Fig.3E

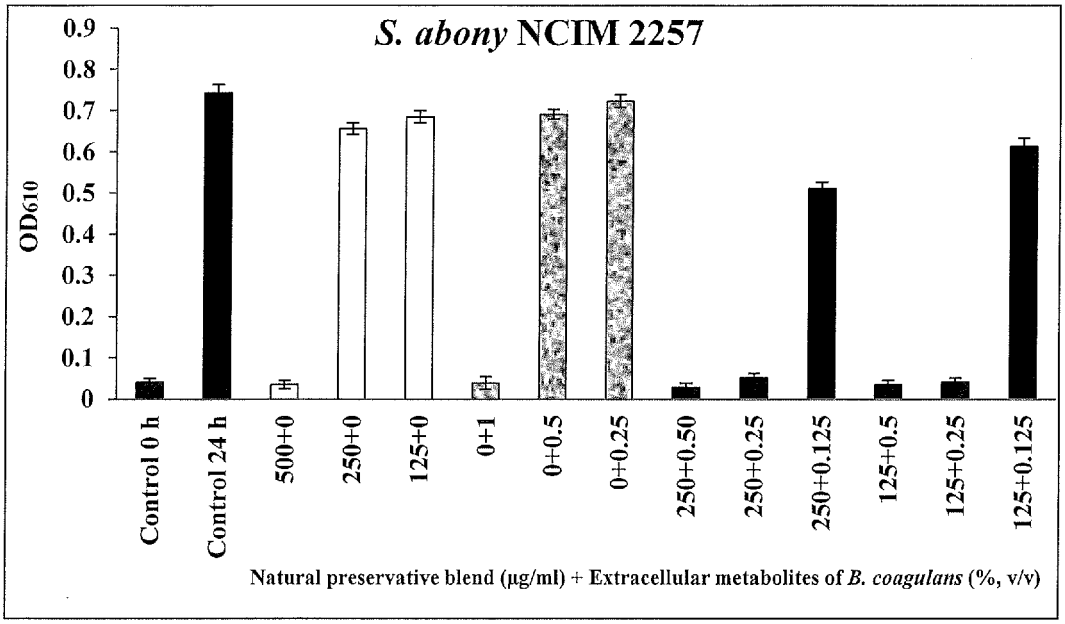

S. abony NCIM 2257
OD610
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
Control 0 h
Control 24 h
500+0
250+0
125+0
0+1
0+0.5
0+0.25
250+0.50
250+0.25
250+0.125
125+0.5
125+0.25
125+0.125
Natural preservative blend (µg/ml) + Extracellular metabolites of B. coagulans (%, v/v)

Fig. 3F

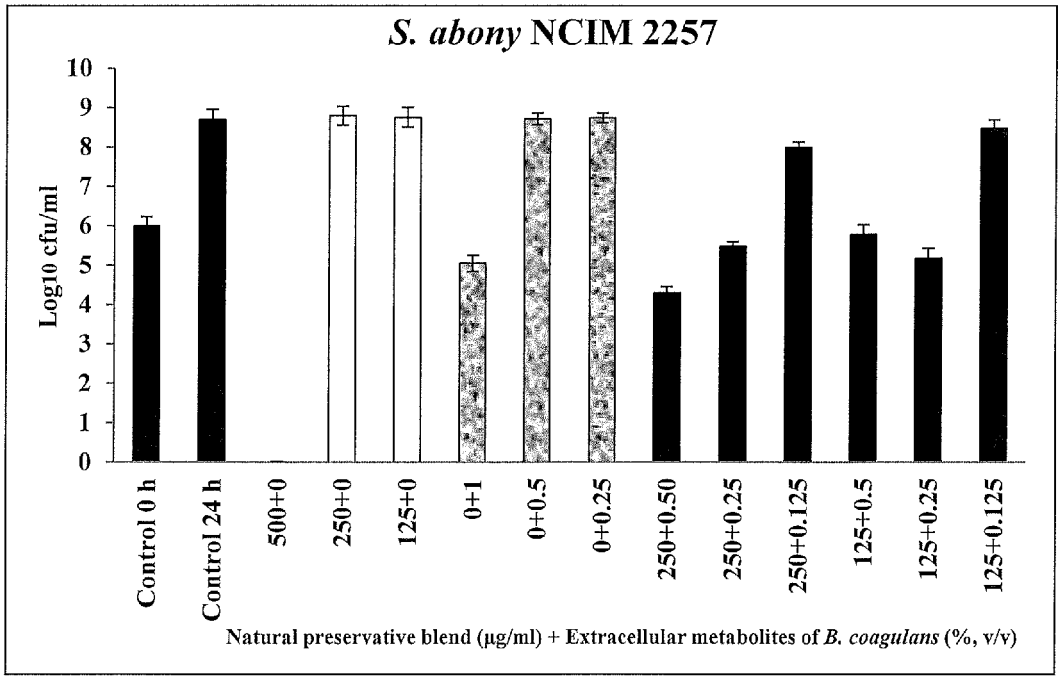

S. abony NCIM 2257
Log10 cfu/ml
10
9
8
7
6
5
4
3
2
1
0
Control 0 h
Control 24 h
500+0
250+0
125+0
0+1
0+0.5
0+0.25
250+0.50
250+0.25
250+0.125
125+0.5
125+0.25
125+0.125
Natural preservative blend (µg/ml) + Extracellular metabolites of B. coagulans (%, v/v)

Fig. 3G

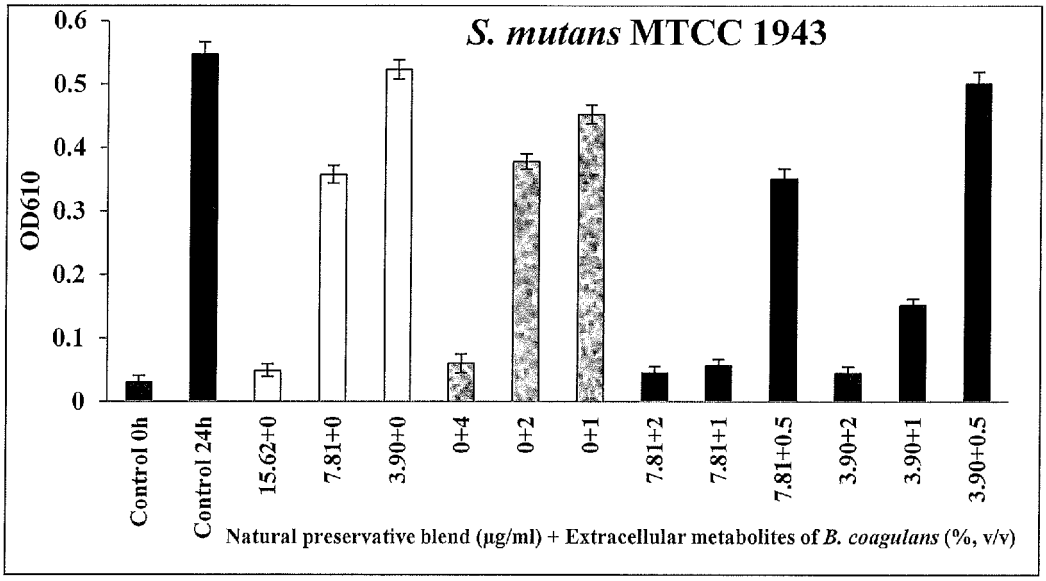

S. mutans MTCC 1943
OD610
0.6
0.5
0.4
0.3
0.2
0.1
0
Control 0h
Control 24h
15.62+0
7.81+0
3.90+0
0+4
0+2
0+1
7.81+2
7.81+1
7.81+0.5
3.90+2
3.90+1
3.90+0.5
Natural preservative blend (µg/ml) + Extracellular metabolites of B. coagulans (%, v/v)

Fig. 3H

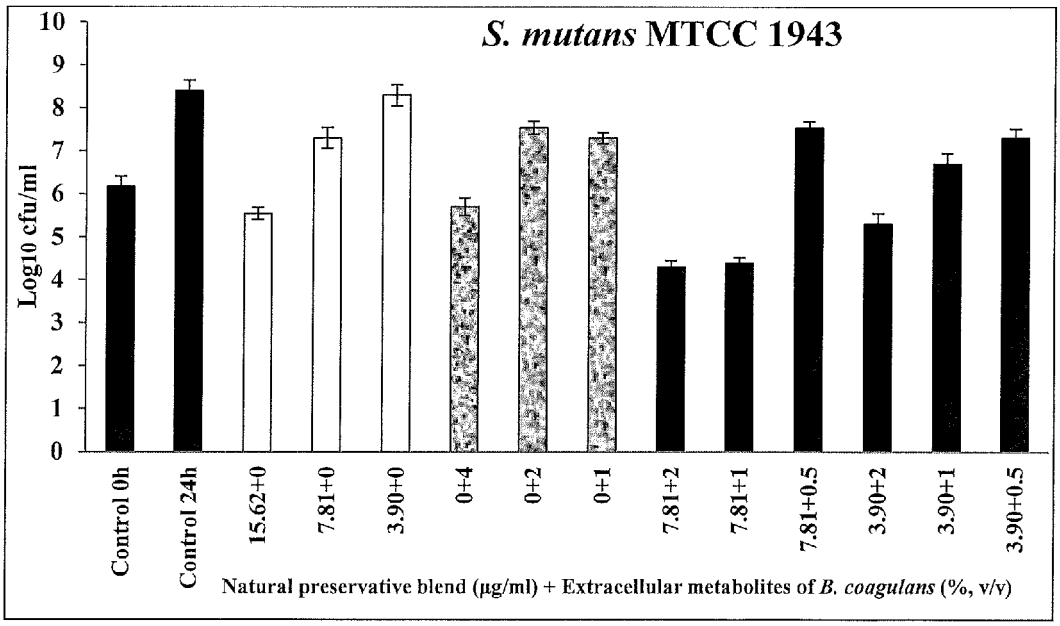

S. mutans MTCC 1943
Log10 cfu/ml
10
9
8
7
6
5
4
3
2
1
0
Control 0h
Control 24h
15.62+0
7.81+0
3.90+0
0+4
0+2
0+1
7.81+2
7.81+1
7.81+0.5
3.90+2
3.90+1
3.90+0.5
Natural preservative blend (µg/ml) + Extracellular metabolites of B. coagulans (%, v/v)

Fig. 3I

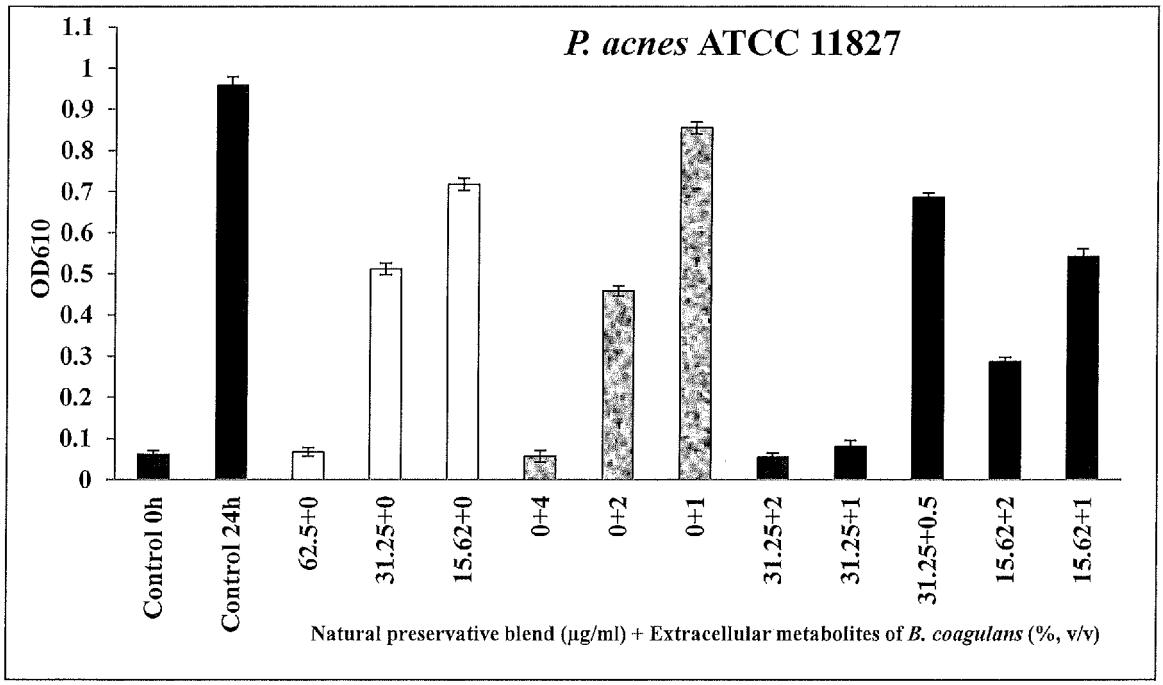

P. acnes ATCC 11827
OD610
1.1
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
Control 0h
Control 24h
62.5+0
31.25+0
15.62+0
0+4
0+2
0+1
31.25+2
31.25+1
31.25+0.5
15.62+2
15.62+1
Natural preservative blend (µg/ml) + Extracellular metabolites of B. coagulans (%, v/v)

Fig. 3J

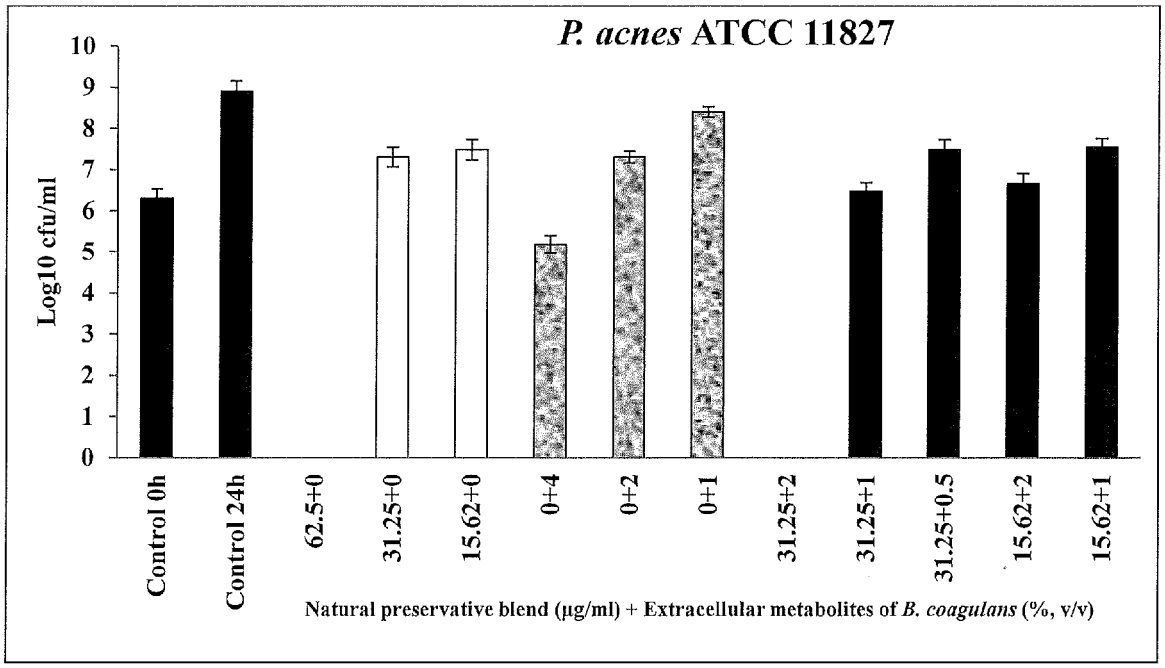

P. acnes ATCC 11827
Log10 cfu/ml
10
9
8
7
6
5
4
3
2
1
0
Control 0h
Control 24h
62.5+0
31.25+0
15.62+0
0+4
0+2
0+1
31.25+2
31.25+1
31.25+0.5
15.62+2
15.62+1
Natural preservative blend (µg/ml) + Extracellular metabolites of B. coagulans (%, v/v)

Fig. 3K

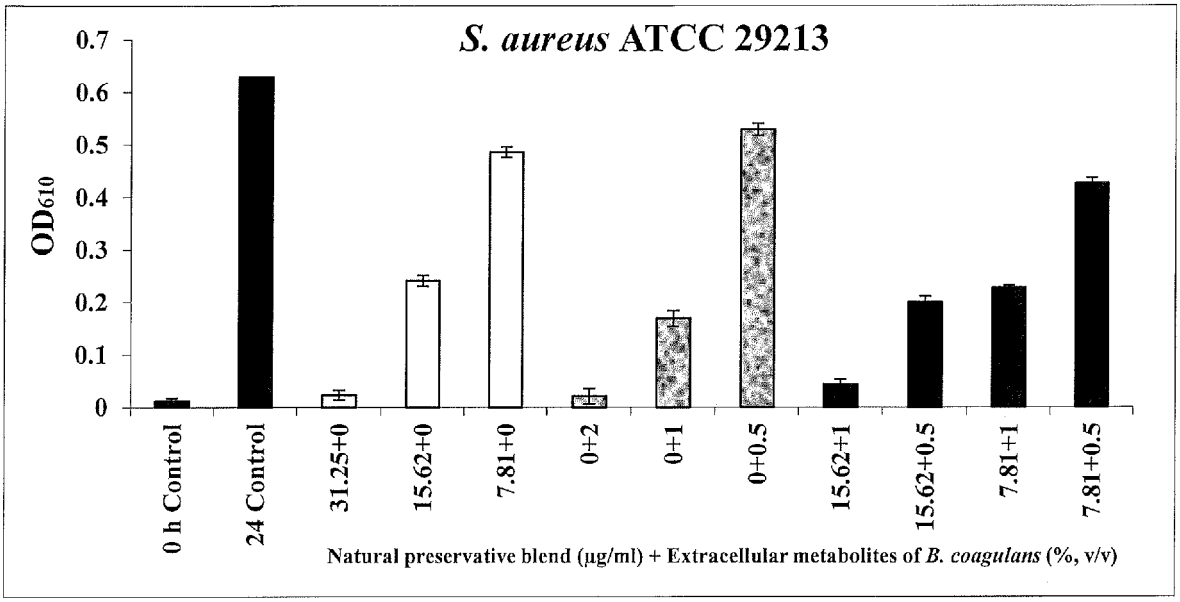

S. aureus ATCC 29213
OD610
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
0 h Control
24 Control
31.25+0
15.62+0
7.81+0
0+2
0+1
0+0.5
15.62+1
15.62+0.5
7.81+1
7.81+0.5
Natural preservative blend (µg/ml) + Extracellular metabolites of B. coagulans (%, v/v)

Fig. 3L

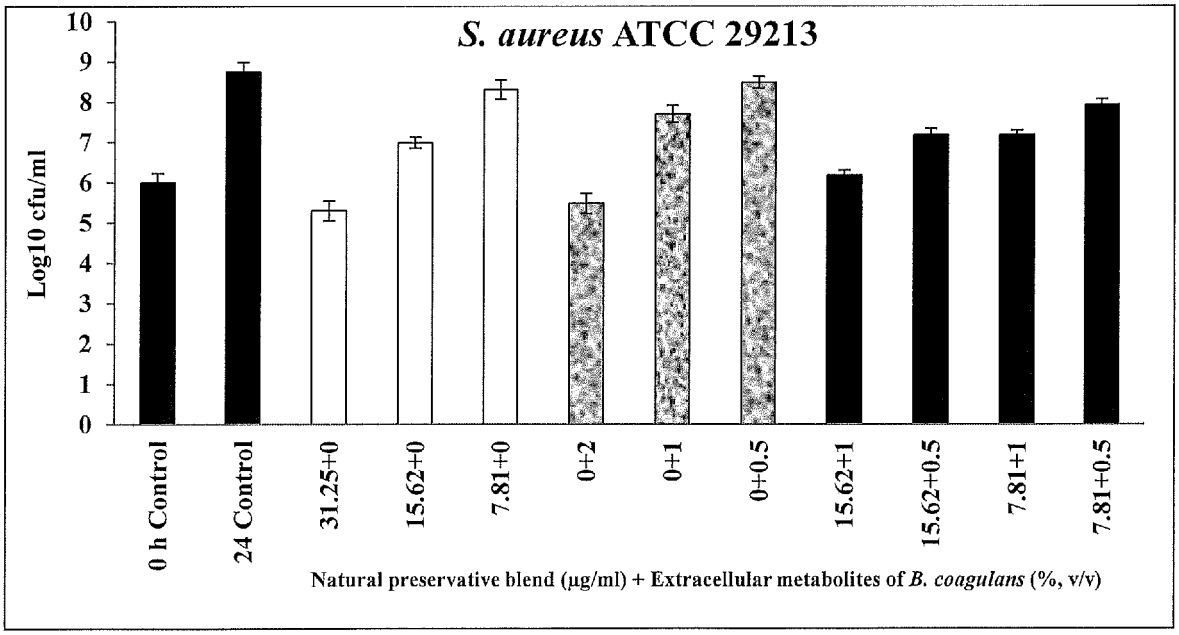

S. aureus ATCC 29213
Log10 cfu/ml
10
9
8
7
6
5
4
3
2
1
0
0 h Control
24 Control
31.25+0
15.62+0
7.81+0
0+2
0+1
0+0.5
15.62+1
15.62+0.5
7.81+1
7.81+0.5
Natural preservative blend (µg/ml) + Extracellular metabolites of B. coagulans (%, v/v)

Fig. 3M

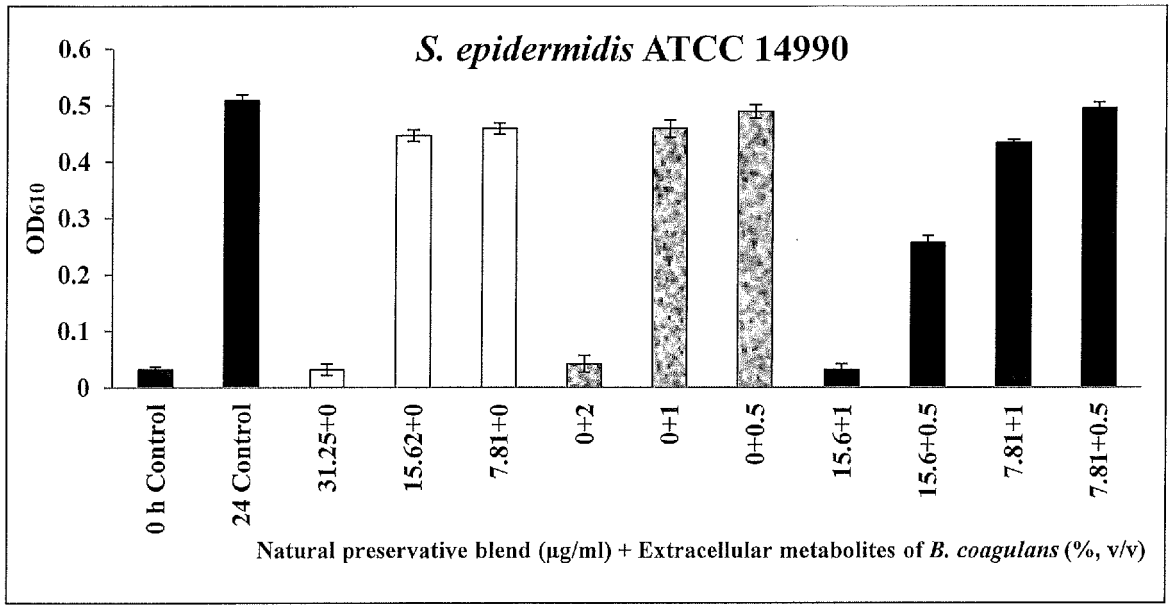

S. epidermidis ATCC 14990
OD610
0.6
0.5
0.4
0.3
0.2
0.1
0
0 h Control
24 Control
31.25+0
15.62+0
7.81+0
0+2
0+1
0+0.5
15.6+1
15.6+0.5
7.81+1
7.81+0.5
Natural preservative blend (µg/ml) + Extracellular metabolites of B. coagulans (%, v/v)

Fig. 3N

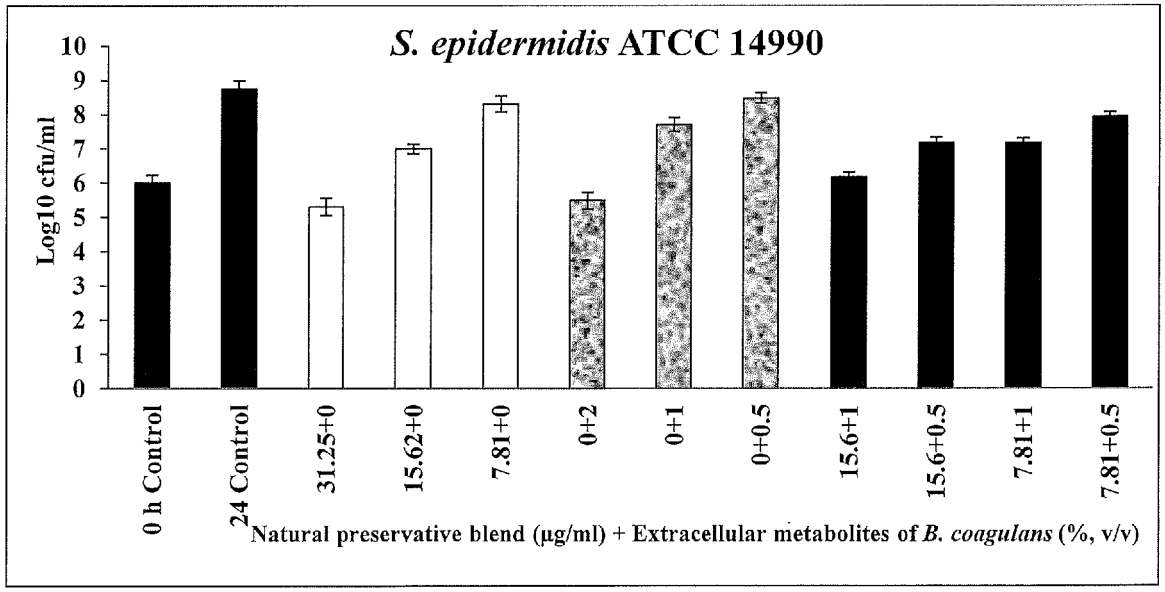

S. epidermidis ATCC 14990
Log10 cfu/ml
10
9
8
7
6
5
4
3
2
1
0
0 h Control
24 Control
31.25+0
15.62+0
7.81+0
0+2
0+1
0+0.5
15.6+1
15.6+0.5
7.81+1
7.81+0.5
Natural preservative blend (µg/ml) + Extracellular metabolites of B. coagulans (%, v/v)

Fig. 3O

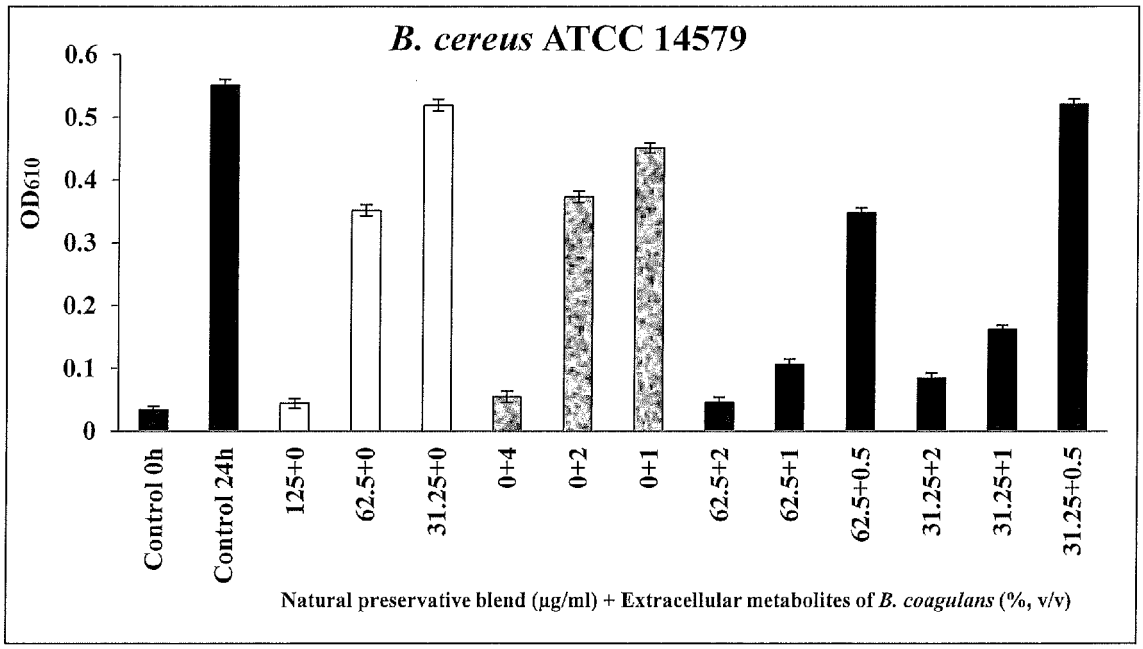

B. cereus ATCC 14579
OD610
0.6
0.5
0.4
0.3
0.2
0.1
0
Control 0h
Control 24h
125+0
62.5+0
31.25+0
0+4
0+2
0+1
62.5+2
62.5+1
62.5+0.5
31.25+2
31.25+1
31.25+0.5
Natural preservative blend (µg/ml) + Extracellular metabolites of B. coagulans (%, v/v)

Fig. 3P

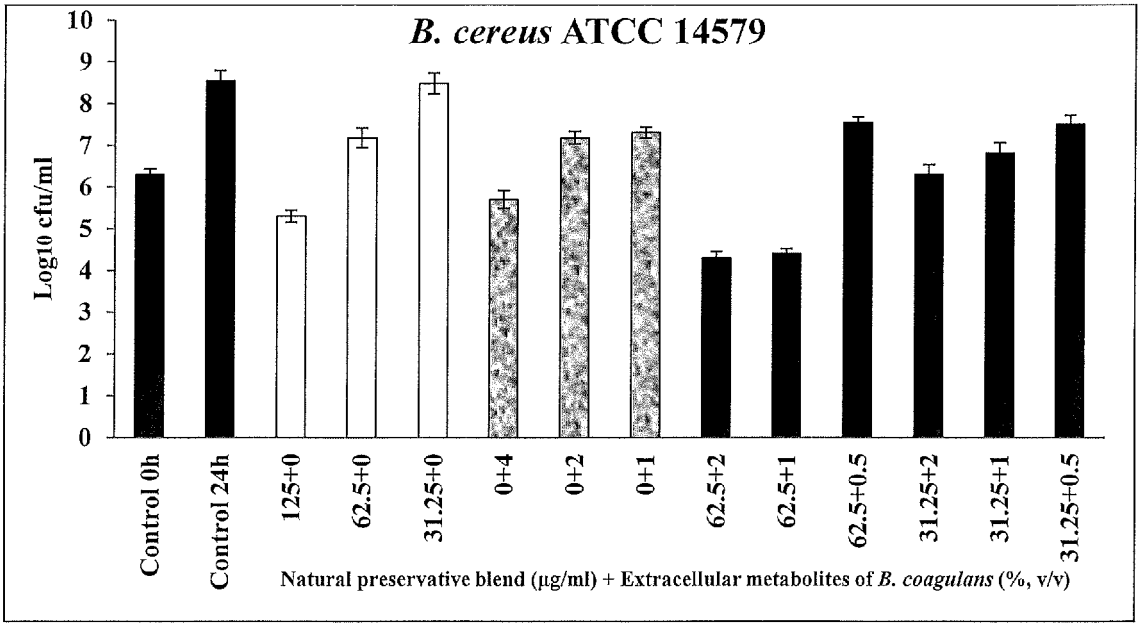

B. cereus ATCC 14579
Log10 cfu/ml
10
9
8
7
6
5
4
3
2
1
0
Control 0h
Control 24h
125+0
62.5+0
31.25+0
0+4
0+2
0+1
62.5+2
62.5+1
62.5+0.5
31.25+2
31.25+1
31.25+0.5
Natural preservative blend (µg/ml) + Extracellular metabolites of B. coagulans (%, v/v)

Fig. 4A

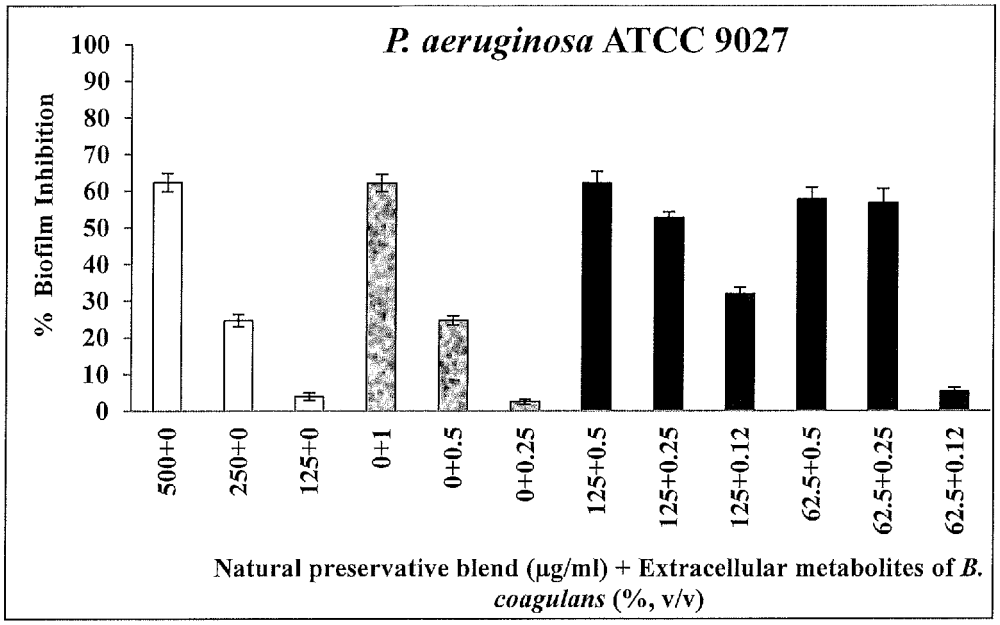

P. aeruginosa ATCC 9027
% Biofilm Inhibition
100
90
80
70
60
50
40
30
20
10
0
500+0
250+0
125+0
0+1
0+0.5
0+0.25
125+0.5
125+0.25
125+0.12
62.5+0.5
62.5+0.25
62.5+0.12
Natural preservative blend (µg/ml) + Extracellular metabolites of B. coagulans (%, v/v)

Fig. 4B

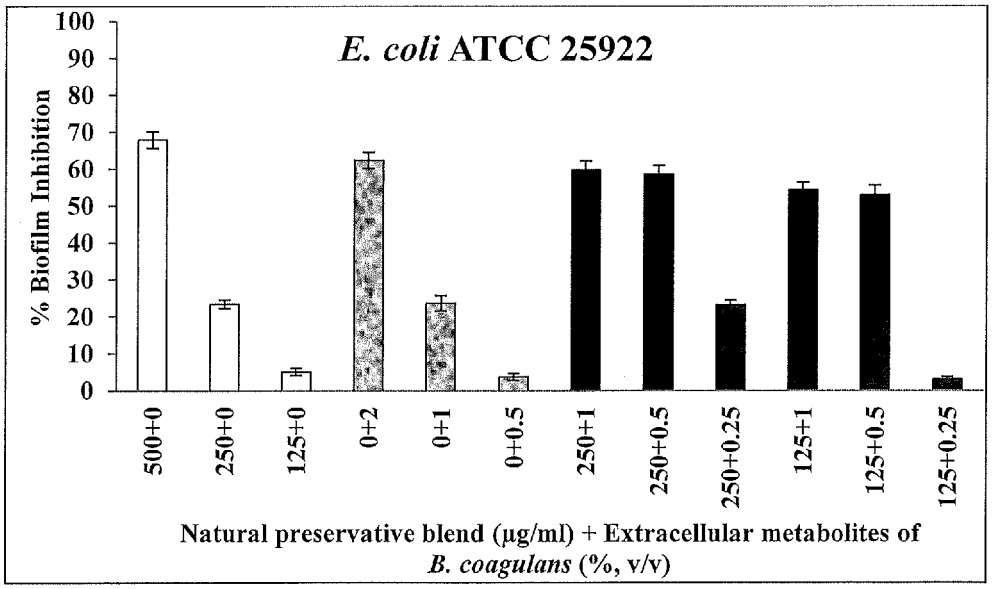

E. coli ATCC 25922
% Biofilm Inhibition
100
90
80
70
60
50
40
30
20
10
0
500+0
250+0
125+0
0+2
0+1
0+0.5
250+1
250+0.5
250+0.25
125+1
125+0.5
125+0.25
Natural preservative blend (µg/ml) + Extracellular metabolites of B. coagulans (%, v/v)

Fig. 4C

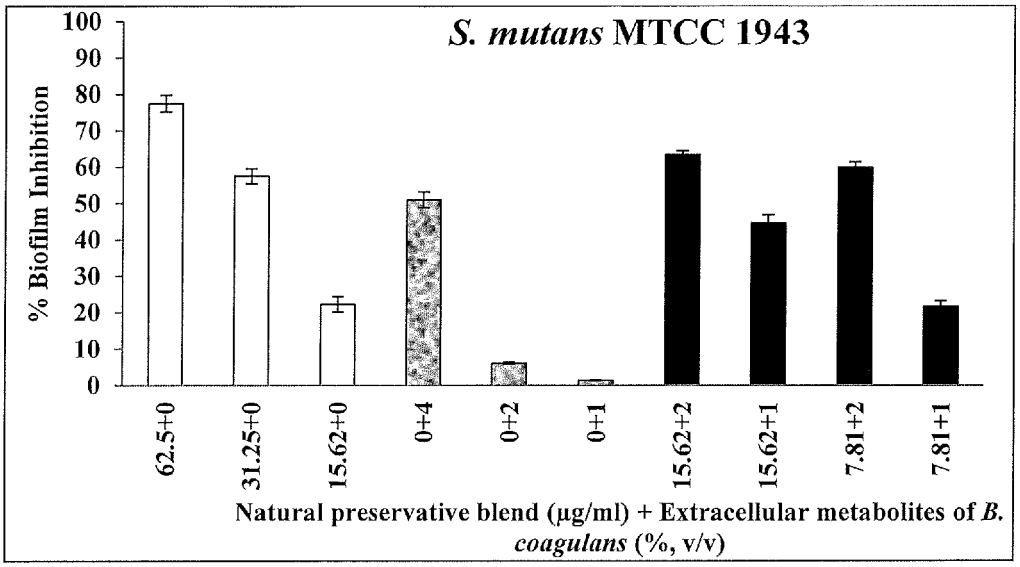

S. mutans MTCC 1943
% Biofilm Inhibition
100
90
80
70
60
50
40
30
20
10
0
62.5+0
31.25+0
15.62+0
0+4
0+2
0+1
15.62+2
15.62+1
7.81+2
7.81+1
Natural preservative blend (µg/ml) + Extracellular metabolites of B. coagulans (%, v/v)

Fig. 4D

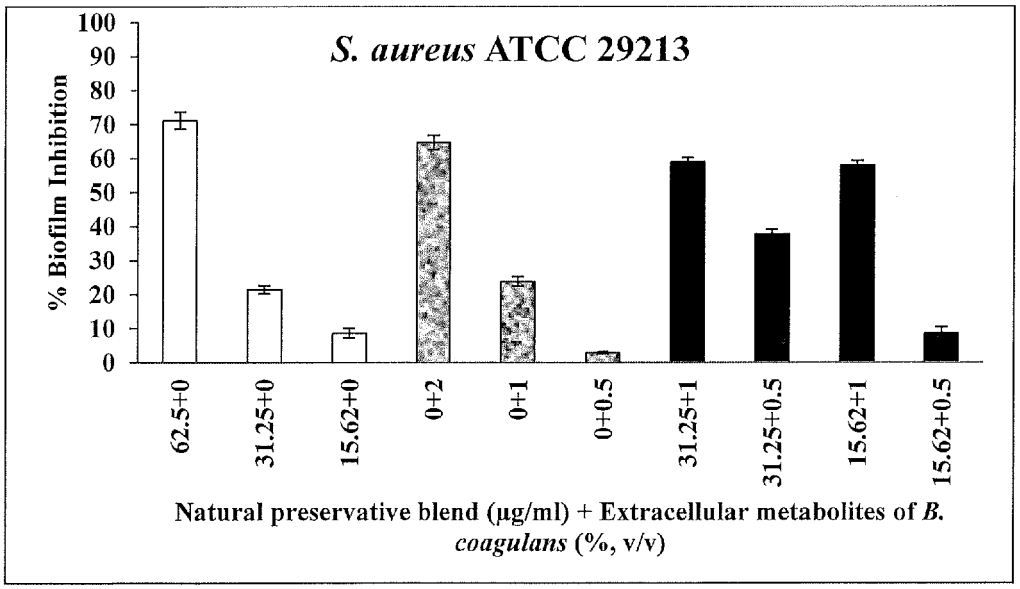

S. aureus ATCC 29213
% Biofilm Inhibition
100
90
80
70
60
50
40
30
20
10
0
62.5+0
31.25+0
15.62+0
0+2
0+1
0+0.5
31.25+1
31.25+0.5
15.62+1
15.62+0.5
Natural preservative blend (µg/ml) + Extracellular metabolites of B. coagulans (%, v/v)

Fig. 4E

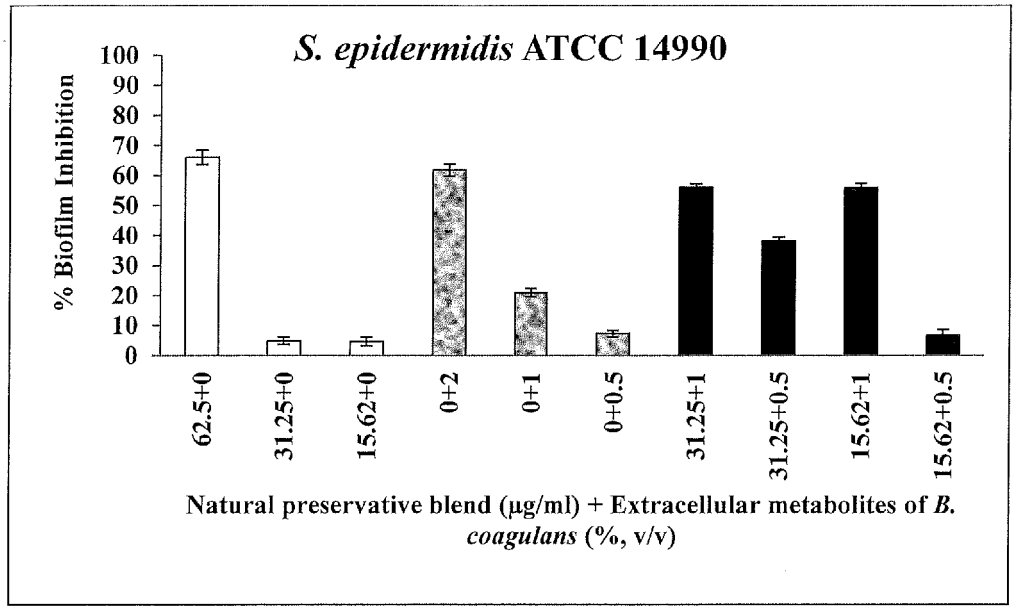

S. epidermidis ATCC 14990
% Biofilm Inhibition
100
90
80
70
60
50
40
30
20
10
0
62.5+0
31.25+0
15.62+0
0+2
0+1
0+0.5
31.25+1
31.25+0.5
15.62+1
15.62+0.5
Natural preservative blend (µg/ml) + Extracellular metabolites of B. coagulans (%, v/v)

Fig. 5A

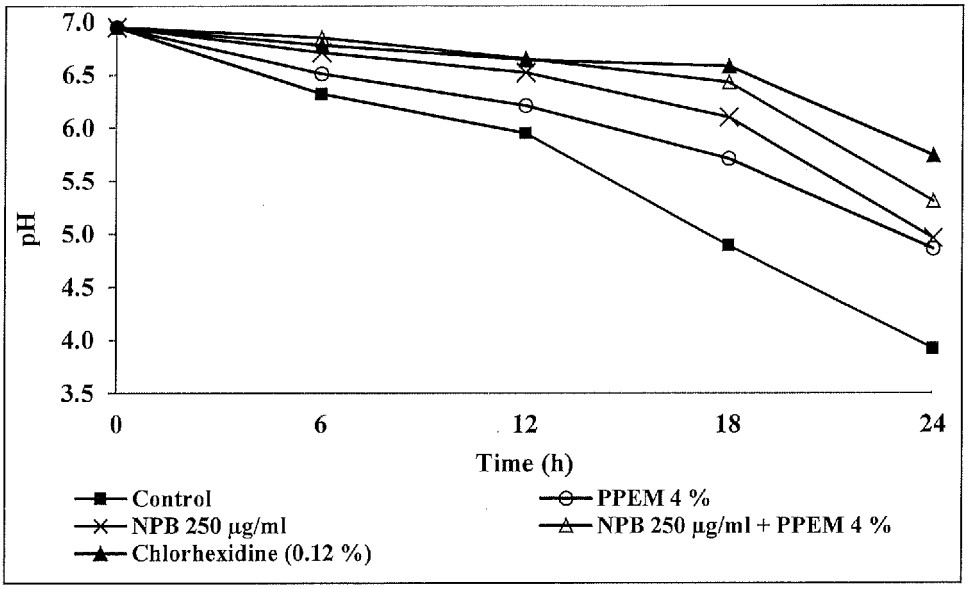
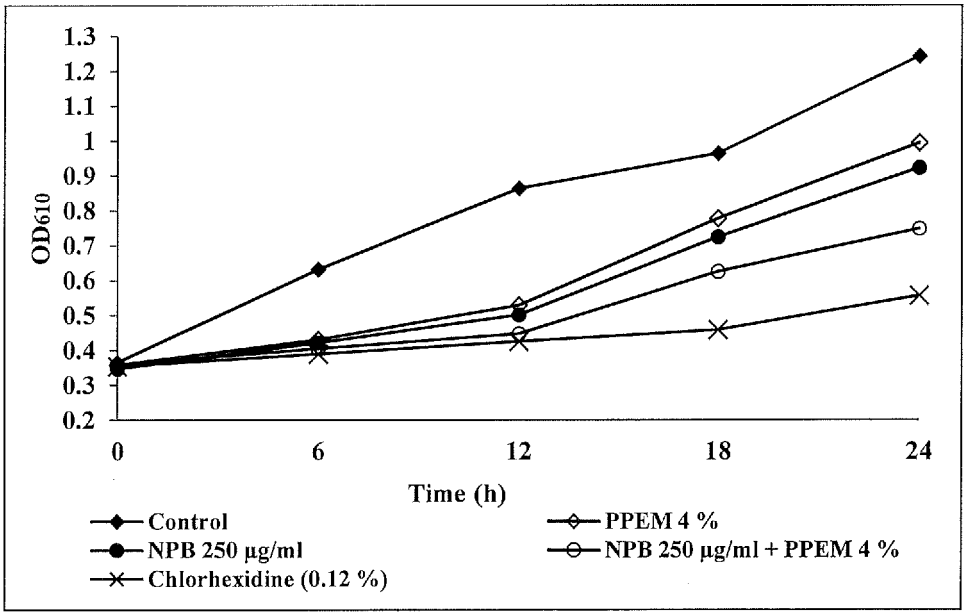

7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
pH
0
6
12
18
24
Time (h)
Control
NPB 250 µg/ml
Chlorhexidine (0.12 %)
PPEM 4 %
NPB 250 µg/ml + PPEM 4 %

Fig. 5B


1.3
1.2
1.1
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
OD610
0
6
12
18
24
Time (h)
Control
NPB 250 µg/ml
Chlorhexidine (0.12 %)
PPEM 4 %
NPB 250 µg/ml + PPEM 4 %