

**Background:**

The minimum inhibitory concentration (MIC) of a chemical is the lowest concentration of the test agent that will inhibit the growth of the test bacteria i.e. it measures the bacteriostatic activity of the test agent.

The minimum bactericidal concentration (MBC) of a chemical is the lowest concentration of the test agent that will kill the test bacteria i.e. it measures the bacteriocidal activity of the test agent.

**1. Susceptibility Testing of Bacteria Using a Standard Broth Dilution Method**

**Aim:**

To determine the MIC and MBC of solutions of Tea Tree oil and BIO BACTERIAL GEL (BBG) on a wide range of airborne bacteria.

**Method:**

Susceptibility tests were carried out on a range of common airborne bacteria. Bacterial species were chosen to compare the results for Tea Tree oil and BBG to previous published results (Product information sheet). Cultures were obtained from a standard reference collection of bacteria (Australian Collection of Microorganisms).

Enteric bacteria (found in animal faeces)

*Escherichia coli* ATCC 25922  
*Klebsiella pneumoniae* ATCC 13883  
*Proteus vulgaris* ATCC 6280  
*Serratia marcescens* NCTC 1377  
*Enterococcus faecalis* ATCC 29212  
*Enterobacter aerogenes* ATCC 13048  
*Pseudomonas putida* ATCC 49128

Skin bacteria

*Staphylococcus epidermidis* ATCC 14990  
*Staphylococcus aureus* NCTC 6571  
*Corynebacterium sp.* ATCC 19409  
*Propionibacterium acnes* ACM 821 (Australian Collection of Microorganisms)

Bacteria common in dust

*Bacillus subtilis* ATCC 11774  
*Bacillus cereus* ATCC 10876A  
*Micrococcus luteus* ATCC 10240

Environmental pathogens  
*Legionella pneumophila*

Bacteria were tested against the following concentrations of tea tree oil/ BBG:  
1.0%, 0.75%, 0.5%, 0.4%, 0.3%, 0.2% and 0.1% mg/mL

The concentration of the BBG gel was set up to obtain the same concentration of the oil component as the pure oil. Each organism was tested against all concentrations of the oil taken from both 100% pure tea tree oil and from the BBG gel using the macrodilution method described in the NCCLS reference. For each organism a full set of controls was also set up with equivalent amounts of 85% ethanol (used for dissolving both the pure oil and the gel) to those that would have been present in each of the dilutions. A control with neither tea tree oil nor ethanol was also included for each organism to check the viability of the inoculum.

Tea tree oil

MICs were determined by recording presence or absence of turbidity in the broth dilutions. After initial reading of the tubes the last three dilutions showing no apparent growth were plated out onto a suitable agar medium to determine the minimum bactericidal concentration (MBC).

BBG

MICs of the gel-associated oil could not be read because the cloudiness of the diluted gel solutions made it impossible to read turbidity changes. MBCs were determined by plating out the diluted gel solutions.

**Results:**

In the table below G = growth, N = no growth and NAG = no apparent growth. No distinction has been made between levels of growth as this is difficult in broth cultures.

Organism	Chemical	Concentration of tea tree oil/ BBG gel (w/v%)								
		1.0	0.75	0.5	0.4	0.3	0.2	0.1	0	
<i>Escherichia coli</i>	100% oil	NAG	NAG	NAG	NAG	NAG	G	G	G	
	After plating	N	N	N	N	N	G	G		
	Ethanol only	G	G	G	G	G	G	G		
	Gel-assoc. oil	N	N	N	N	N	G	G		
	Ethanol only	NAG	NAG	G	G	G	G	G		

MIC = 0.3%    MBC = 0.3%(oil), 0.3%(gel)

<i>Klebsiella pneumoniae</i>	100% oil	NAG	NAG	NAG	NAG	NAG	NAG	G	G	
	After plating	N	N	N	N	N	N	G		
	Ethanol only (oil dil. equiv.)	G	G	G	G	G	G	G		
	Gel-assoc. oil	N	N	N	N	N	N	G		
	Ethanol only (gel dil. equiv.)	NAG	NAG	G	G	G	G	G		

MIC = 0.2%    MBC = 0.2%(oil), 0.2%(gel) Note that the gel-associated oil has a greater quantity of ethanol added with it, due to the low concentration of oil in the gel. This explains the difference between the results from the two sets of ethanol controls.

		1.0	0.75	0.5	0.4	0.3	0.2	0.1	0
<i>Proteus vulgaris</i>	100% oil	NAG	NAG	NAG	NAG	NAG	NAG	G	G
	After plating	N	N	N	N	N	N	G	
	Ethanol only	G	G	G	G	G	G	G	
	Gel-assoc. oil	N	N	N	N	N	N	G	
	Ethanol only	NAG	G	G	G	G	G	G	

MIC = 0.2% MBC = 0.2% (oil), 0.2% (gel)

		1.0	0.75	0.5	0.4	0.3	0.2	0.1	0
<i>Serratia marcescens</i>	100% oil	NAG	NAG	NAG	NAG	NAG	G	G	G
	After plating	N	N	N	N	N	G	G	
	Ethanol only	NAG	G	G	G	G	G	G	
	Gel-assoc. oil	N	N	G	G	G	G	G	
	Ethanol only	G	G	G	G	G	G	G	

		1.0	0.75	0.5	0.4	0.3	0.2	0.1	0
<i>Enterococcus faecalis</i>	100% oil	NAG	NAG	NAG	G	G	G	G	G
	After plating	N	G	G	G	G	G	G	
	Ethanol only	G	G	G	G	G	G	G	
	Gel-assoc. oil	N	N	G	G	G	N	G	
	Ethanol only	NAG	G	G	G	G	G	G	

MIC = 0.5% MBC = 1.0% (oil), 0.75% (gel)

		1.0	0.75	0.5	0.4	0.3	0.2	0.1	0
<i>Enterobacter aerogenes</i>	100% oil	NAG	NAG	NAG	NAG	NAG	NAG	G	G
	After plating	N	N	N	N	N	G	G	
	Ethanol only	G	G	G	G	G	G	G	
	Gel-assoc. oil	N	N	N	N	G	G	G	
	Ethanol only	NAG	NAG	G	G	G	G	G	

MIC = 0.2% MBC = 0.3% (oil), 0.4% (gel)

		1.0	0.75	0.5	0.4	0.3	0.2	0.1	0
<i>Pseudomonas putida</i>	100% oil	NAG	NAG	NAG	NAG	NAG	NAG	NAG	G
	After plating	N	N	N	N	N	N	G	
	Ethanol only	N	N	N	N	N	G	G	
	Gel-assoc. oil	N	N	N	N	N	N	G	
	Ethanol only	N	N	N	N	N	N	G	

MIC = 0.1% MBC = 0.2% (oil), 0.2% (gel) The gel results are inconclusive because the ethanol appears to be inhibiting growth at the same concentration.

		1.0	0.75	0.5	0.4	0.3	0.2	0.1	0
<i>Staphylococcus epidermidis</i>	100% oil	NAG	NAG	NAG	NAG	NAG	NAG	NAG	G
	After plating	N	N	N	N	N	N	N	
	Ethanol only	G	G	G	G	G	G	G	
	Gel-assoc. oil	N	N	N	N	N	N	G	
	Ethanol only	NAG	G	G	G	G	G	G	

MIC = 0.1% MBC = 0.1 (oil), 0.2% (gel)

		1.0	0.75	0.5	0.4	0.3	0.2	0.1	0
<i>Staphylococcus aureus</i>	100% oil	NAG	NAG	NAG	NAG	NAG	NAG	NAG	G
	After plating	N	N	N	N	N	N	N	
	Ethanol only	G	G	G	G	G	G	G	
	Gel-assoc. oil	N	N	N	N	N	N	G	
	Ethanol only	NAG	NAG	G	G	G	G	G	

MIC = 0.1%    MBC = 0.1% (oil), 0.2% (gel)

		1.0	0.75	0.5	0.4	0.3	0.2	0.1	0
<i>Corynebacterium sp.</i>	100% oil	NAG	NAG	NAG	NAG	NAG	NAG	NAG	G
	After plating	N	N	N	N	N	G	G	
	Ethanol only	NAG	NAG	G	G	G	G	G	
	Gel-assoc.oil	N	N	N	N	N	N	N	
	Ethanol only	NAG	NAG	NAG	NAG	G	G	G	

MIC = 0.3%    MBC = 0.3%(oil), <0.1%(gel)

		1.0	0.75	0.5	0.4	0.3	0.2	0.1	0
<i>Propionibacterium acnes</i>	100% oil	NAG	NAG	NAG	NAG	NAG	NAG	NAG	G
	After plating	N	N	N	N	N	N	G	
	Ethanol only	G	G	G	G	N	G	G	
	Gel-assoc. oil	N	N	N	N	N	N	N	
	Ethanol only	NAG	NAG	G	G	G	G	G	

MIC = 0.1%    MBC = 0.2% (oil), <0.1%(gel)

		1.0	0.75	0.5	0.4	0.3	0.2	0.1	0
<i>Bacillus subtilis</i>	100% oil	NAG	NAG	NAG	NAG	NAG	G	G	G
	After plating	G	G	G	G	G	G	G	
	Ethanol only	G	G	G	G	G	G	G	
	Gel-assoc. oil	N	G	G	G	G	G	G	
	Ethanol only	NAG	G	G	G	G	G	G	

MIC = 0.3%    MBC = >1.0% (oil), >1.0% (gel)

MIC = 0.3%    MBC = 0.3% (oil), 0.75%(gel)

		1.0	0.75	0.5	0.4	0.3	0.2	0.1	0
<i>Bacillus cereus</i>	100% oil	NAG	NAG	NAG	NAG	?G	?G	G	G
	After plating	G	G	G	G	G	G	G	
	Ethanol only	G	G	G	G	G	G	G	
	Gel-assoc. oil	G	G	G	G	G	G	G	
	Ethanol only	G	G	G	G	G	G	G	

MIC = 0.2 - 0.4%    MBC = >1.0% (oil), >1.0 % (gel)

		1.0	0.75	0.5	0.4	0.3	0.2	0.1	0
<i>Micrococcus</i>	100% oil	NAG	NAG	NAG	NAG	NAG	NAG	NAG	G
<i>luteus</i>	After plating	N	N	N	N	N	N	N	
	Ethanol only	G	G	G	G	G	G	G	
	Gel-assoc. oil	N	N	N	N	N	N	G	
	Ethanol only	G	G	G	G	G	G	G	

MIC = 0.1%    MBC = 0.1% (oil), 0.2% (gel)

		1.0	0.75	0.5	0.4	0.3	0.2	0.1	0
<i>Legionella</i>	100% oil	NAG	NAG	NAG	NAG	NAG	NAG	NAG	G
<i>pneumophila</i>	After plating	N	N	N	N	N	N	N	
	Ethanol only	N	N	G	G	G	G	G	
	Gel-assoc. oil	N	N	N	N	N	N	N	
	Ethanol only	N	N	N	N	G	G	G	

MIC = <0.1%    MBC = <0.1% (oil), <0.1% (gel)

## Summary:

Bacteria	Minimum inhibitory concentration (MIC) % mg/mL		Minimum bactericidal concentration (MBC) % mg/mL	
	Tea tree oil	BBG gel#	Tea tree oil	BBG gel#
<i>Escherichia coli</i>	0.3	*	0.3	0.3
<i>Klebsiella pneumoniae</i> ®	0.2	*	0.2	0.2
<i>Proteus vulgaris</i> ®	0.2	*	0.2	0.2
<i>Enterococcus faecalis</i>	0.5	*	1.0	0.75
<i>Serratia marcescens</i> ®	0.3	*	0.3	0.75
<i>Enterobacter aerogenes</i> ®	0.2	*	0.3	0.4
<i>Pseudomonas putida</i> ®	0.1	*	0.2	~
<i>Staphylococcus epidermidis</i>	0.1	*	0.1	0.2
<i>Staphylococcus aureus</i>	0.1	*	0.1	0.2
<i>Corynebacterium sp.</i>	0.1	*	0.3	<0.1
<i>Propionebacterium acnes</i>	0.1	*	0.2	<0.1
<i>Bacillus subtilis</i>	0.3	*	>1.0	>1.0
<i>Bacillus cereus</i>	0.2	*	>1.0	>1.0
<i>Micrococcus luteus</i>	0.1	*	0.1	0.2
<i>Legionella pneumophila</i> ®	<0.1	*	<0.1	<0.1

~ inconclusive result

# concentration of oil in the gel solution

\* not readable due to cloudiness of gel solution

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## Conclusion:

### Minimum Inhibitory Concentration (MIC)

The growth of all bacteria tested was inhibited by tea tree oil at concentrations varying from 0.1 to 0.5%.

### Minimum Bactericidal Concentration (MBC)

Both tea tree oil and BBG gel were shown to have a bactericidal effect on most test bacteria at concentrations of 0.1 to 0.4%. The results for tea tree oil confirm earlier studies published.

Three species were shown to be more resistant than the others:

*Enterococcus faecalis*    MBC (oil) 1.0%    MBC (gel) 0.75%  
*Bacillus subtilis*        MBC (oil) >1.0%    MBC (gel) >1.0%  
*Bacillus cereus*        MBC (oil) >1.0%    MBC (gel) >1.0%

The resistance of *Bacillus subtilis* and *Bacillus cereus* is probably related to their ability to form resistant endospores.

## 2. Susceptibility Testing of Fungi Using a Standard Broth Dilution Method

### Aim:

To determine the MIC and MFC of solutions of Tea Tree Oil on a wide range of airborne yeasts and moulds.

### Test fungi:

Cultures were obtained from a standard reference collection of fungi (Australian Collection of Microorganisms).

Yeasts	<i>Candida albicans</i>	ATCC 2091
Moulds	<i>Trichophyton mentagrophytes</i>	ATCC 9533
	<i>Trichophyton rubrum</i>	ATCC 28188
	<i>Aspergillus niger</i>	ATCC 6275
	<i>Aspergillus flavus</i>	ATCC 15546

Each organism was tested against all concentrations of the oil taken from 100% pure tea tree oil using a modified macrodilution method described by Arzeni et al(1998) and McGinnis & Pasarell (1998). For each organism a full set of controls was also set up with equivalent amounts of 85% ethanol to those that would have been present in each of the dilutions. A control with neither tea tree oil nor ethanol was also included for each organism to check the viability of the inoculum.

After initial reading of the tubes the last three dilutions showing no apparent growth were plated out onto a suitable agar medium to distinguish between the minimum inhibitory concentration (MIC) and the minimum fungicidal concentration (MFC).

The tests with the gel dilutions could not be performed due to clouding of the broth caused by the gel.

### Results:

In the table below G = growth, N = no growth and NAG = no apparent growth. No distinction has been made between levels of growth as this is difficult in broth cultures.

Organism	Chemical	Concentration of tea tree oil (w/v%)								
		1.0	0.75	0.5	0.4	0.3	0.2	0.1	0	
<i>Candida albicans</i>	100% oil	NAG	NAG	NAG	NAG	NAG	NAG	NAG	G	G
	After plating	N	N	N	N	G	G	G		
	Ethanol only	G	G	G	G	G	G	G		

MIC = 0.2%      MFC = 0.4%

		1.0	0.75	0.5	0.4	0.3	0.2	0.1	0
<i>Aspergillus flavus</i>	100% oil	NAG	NAG	NAG	G	G	G	G	G
	After plating	N	N	G	G	G	G	G	
	Ethanol only	G	G	G	G	G	G	G	

MIC = 0.5%    MFC = 0.75%

		1.0	0.75	0.5	0.4	0.3	0.2	0.1	0
<i>Aspergillus niger</i>	100% oil	NAG	NAG	NAG	NAG	NAG	NAG	NAG	G
	After plating	N	N	G	G	G	G	G	
	Ethanol only	G	G	G	G	G	G	G	

MIC = 0.1%    MFC = 0.75%

		1.0	0.75	0.5	0.4	0.3	0.2	0.1	0
<i>Trichophyton mentagrophytes</i>	100% oil	NAG	NAG	NAG	NAG	NAG	NAG	G	G
	After plating	N	N	N	N	G	G		
	Ethanol only	G	G	G	G	G	G	G	

MIC = 0.2%    MFC = 0.4%

		1.0	0.75	0.5	0.4	0.3	0.2	0.1	0
<i>Trichophyton rubrum</i>	100% oil	NAG	NAG	NAG	NAG	NAG	NAG	NAG	G
	After plating	N	N	N	N	N	N	G	
	Ethanol only	NAG	G	G	G	G	G	G	

MIC = 0.1%    MFC = 0.2%

### Summary:

Fungi	Minimum inhibitory concentration (MIC) % mg/mL	Minimum fungicidal concentration (MBC) % mg/mL
	Tea tree oil	Tea tree oil
<i>Candida albicans</i>	0.2	0.4
<i>Aspergillus flavus</i>	0.5	0.75
<i>Aspergillus niger</i>	0.1	0.75
<i>Trichophyton mentagrophytes</i>	0.2	0.4
<i>Trichophyton rubrum</i>	0.1	0.2

### Conclusion:

The MIC of the chemical is the lowest concentration of the test agent that will inhibit the growth of the test fungi i.e. it measures the fungistatic activity of the test agent. The growth of all fungi tested was inhibited by tea tree oil at concentrations varying from 0.1 to 0.5%.

The MFC of the chemical is the lowest concentration of the test agent that will kill the test fungi i.e. it measures the fungicidal activity of the test agent. Tea tree oil was shown to have a fungicidal effect on the test fungi at concentrations of 0.2 to 0.75%.



**References:**

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27/02/01