

# In Vitro Antimicrobial Activity of Dimethylsulfoxide

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Solubilization of drugs in dimethylsulfoxide (DMSO) before the determination of their antimicrobial activity is a common practice in the pharmaceutical industry. Since DMSO (Crown Zellerbach, Camas, Wash.) has been shown to possess bacteriostatic properties (2-4), a systematic documentation of its antimicrobial spectrum and level of activity was indicated.

Conventional, serial twofold tube dilution tests were performed with double-strength Penassay Broth (Difco) for most bacteria and fungi; thioglycolate broth was used for *Clostridia*; Kirchner's medium (1) was used for *Mycobacterium tuberculosis*; Brain Heart Infusion broth (BBL) containing 2% rabbit blood was used for *Diplococcus*, *Neisseria*, *Haemophilus*, and *Corynebacterium*; S T S Medium (BBL) was used for *Trichomonas foetus*; and Desulfovibrio medium (Starkey) was used for *Desulfovibrio*. Most of the bacteria and yeasts, as well as the parasite, were incubated for 24 hr at 37 C; *Mycobacterium* was incubated for 5 days; *Desulfovibrio* was incubated for 48 hr under nitrogen purge; and filamentous fungi were incubated for 48 hr at 26 C with mechanical agitation.

The microbicidal effect of DMSO was determined by plating or by diluting out all tubes showing no growth by use of appropriate media. The lowest drug concentration in which growth failed to occur after subculture was considered to be the minimal microbicidal concentration (MMC). Since aqueous solutions of DMSO were known to be alkaline, the pH was carefully monitored in each instance. Under the conditions of these experiments, the mean pH at the microbistatic concentration was  $7.3 \pm 0.29$  for bacteria and  $7.1 \pm 0.39$  for fungi. At the microbicidal concentration, the mean pH after incubation was  $7.8 \pm 0.41$  for bacteria and  $7.7 \pm 0.41$  for fungi.

DMSO exerted a marked inhibitory effect on a wide range of bacteria and fungi, including one parasite, at concentrations likely to be encountered in antimicrobial testing programs in industry. The MMC generally was several-fold higher than the minimal inhibitory concentration, except for certain species which appeared to be ultrasensitive to this agent (*Corynebacterium* sp., *Haemophilus influenzae*, *Pasteurella multocida*, *Herellea* sp., *M. tuberculosis* var. BCG, *Microsporium audouini*). The pH of high concentrations of DMSO in water is likely to contribute to its innate microbicidal effect.

On the basis of unpublished studies performed in our laboratories, it appears that basicity is a greater factor with aqueous mixtures of DMSO than with aqueous mixtures of acetone, the alcohols, or glycols. Microbiologically, DMSO compares favorably with the common solubilizing agents.

## LITERATURE CITED

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TABLE 1. Antimicrobial activity of dimethylsulfoxide

Organism	Culture inoculum		MIC <sup>c</sup>	pH <sup>d</sup>	MMC <sup>e</sup>	pH <sup>d</sup>
	No. <sup>a</sup>	Size <sup>b</sup>				
<i>Bacteria</i>						
<i>Staphylococcus aureus</i>	1,276		8	7.6	30	7.8
<i>S. aureus</i>	1,276	(H)	20	6.8	40	8.2
<i>S. aureus</i>	2,406		30	7.9	40	8.3
<i>S. pyogenes</i>	3,862	(H)	10	7.3	30	7.8
<i>S. faecalis</i>	1,648	(H)	20	7.5	40	8.2
<i>Diplococcus pneumoniae</i>	5,479		20	7.6	40	8.1
<i>Micrococcus lysodeikticus</i>	2,529		10	7.5	30	7.8
<i>Sarcina lutea</i>	8,579	(H)	20	7.5	40	8.2
<i>Corynebacterium</i> sp.	8,621	(H)	8	7.3	10	7.5
<i>Listeria monocytogenes</i>	8,523		20	7.5	30	8.3
<i>Mycobacterium tuberculosis</i> var. BCG	5,516		8		10	
<i>Clostridium pasterianum</i>	1,759		5		10	7.1
<i>Bacillus subtilis</i>	3,777		20	7.6	30	8.7
<i>Desulfovibrio desulfuricans</i>	5,378		10		20	
<i>Neisseria catarrhalis</i>	5,348	(H)	20	7.6	40	8.2
<i>Haemophilus influenzae</i>	8,622		7	7.3	8	7.3
<i>Pasteurella multocida</i>	8,624	(H)	6	7.3	9	7.4
<i>Escherichia coli</i>	2,975	(H)	10	6.8	20	7.5
<i>Aerobacter aerogenes</i>	1,678	(H)	20	7.4	20	7.4
<i>Klebsiella pneumoniae</i>	1,565	(H)	9	7.0	30	7.9
<i>Proteus vulgaris</i>	3,855	(H)	20	7.6	20	7.6
<i>P. mirabilis</i>	3,873	(H)	20	7.5	20	7.5
<i>Salmonella schottmuelleri</i>	3,850	(H)	9	7.1	20	7.6
<i>S. gallinarum</i>	3,030	(H)	10	6.8	30	7.5
<i>S. typhimurium</i>	3,821	(H)	10	7.0	30	7.9
<i>Shigella flexneri</i>	1,461	(H)	10	7.1	30	7.8
<i>Serratia marcescens</i>	1,468	(H)	10	7.2	30	7.8
<i>Herellea</i> sp.	8,334		6	7.0	8	7.0
<i>Pseudomonas aeruginosa</i>	3,840	(H)	8	7.2	20	7.5
<i>Fungi</i>						
<i>Aspergillus niger</i>	2,828		9	7.3	30	8.0
<i>A. fumigatus</i>	2,100		10	7.4	30	7.9
<i>Candida albicans</i>	5,314		8	6.7	30	7.6
<i>C. krusei</i>	2,616		9	7.1	20	7.5
<i>Saccharomyces cerevisiae</i>	1,600		8	7.1	20	7.5
<i>Geotrichum candidum</i>	8,623		8	6.8	20	7.5
<i>Nocardia asteroides</i>	2,626		20	7.6	40	8.1
<i>Cladosporium resinae</i>	5,476		7	6.2	30	6.7
<i>Trichophyton mentagrophytes</i>	2,637		8	7.2	30	8.0
<i>Penicillium notatum</i>	2,122		10	7.2	30	8.1
<i>Fusarium bulbigenum</i>	5,273		20	7.7	30	8.0
<i>Microsporum audouini</i>	5,282		7	7.2	8	7.2
<i>Pullularia pullulans</i>	2,599		9	6.8	20	7.7
<i>Parasite</i>						
<i>Trichomonas vaginalis</i>	8,560		4		NT <sup>f</sup>	

<sup>a</sup> Squibb stock culture number.

<sup>b</sup> (H) is equal to 10<sup>6</sup> cells/ml; for all other bacteria, *Nocardia*, and yeasts, the inoculum was 10<sup>8</sup> cells/ml; for filamentous fungi, the inoculum was 2 ml of a 1:50 dilution of mat or spore suspension derived from a 48-hr growth (25 C) of the organism on 100 ml of Penassay agar in a 250-ml Erlenmeyer flask.

<sup>c</sup> Minimal inhibitory concentration in DMSO (%).

<sup>d</sup> After incubation.

<sup>e</sup> Minimal microbicidal concentration in DMSO (%).

<sup>f</sup> Not tested.