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# Correspondence

## Aromatic alcohols and their effect on Gram-negative bacteria, cocci and mycobacteria

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Sir,

Phenethyl alcohol (2-phenylethanol; PEA, mol. wt 122.17) inhibits a range of Gram-negative bacteria, but not *Pseudomonas fluorescens*, at a concentration of 0.2% (2000 mg/L), whereas Gram-positive cocci, such as *Staphylococcus aureus* are inhibited at 0.5% w/v (5000 mg/L), with *Enterococcus faecalis* requiring still higher concentrations.[1] PEA-containing media had earlier been suggested as a means of selecting for Gram-positive bacteria in mixed flora.[2] Interestingly, *Mycobacterium smegmatis* and *Mycobacterium phlei* were also inhibited at 0.2%, suggesting that the solubility of PEA in lipids could play a major role in its selective action.[1] However, Wilson *et al*.[3] reported that PEA was only slightly less inhibitory to *Staphylococcus aureus* than *Escherichia coli*, and that the MIC for *Pseudomonas aeruginosa* was 0.46% (4600 mg/L).

As part of comprehensive studies of the responses to biocides of mycobacteria, other Gram-positive bacteria and Gram-negative organisms, it was decided to investigate the effects of PEA and of another, significantly more hydrophobic, aromatic alcohol (5-phenyl-1-pentanol; 5-PP, mol. wt 164.25) on some strains of *Mycobacterium chelonae*, *M. smegmatis* and *Burkholderia cepacia*. *B. cepacia* K56-2 triclosan- and chlorhexidine-susceptible mutants were isolated using a Tn*5*-pOT182 random mutagenesis system. Strain K56-2 is a genetically amenable clone of the genome sequencing strain J2315. A single strain used in disinfectant testing [EN 1276 (1997)] of each of *E. coli*, *P. aeruginosa* and *S. aureus* was included for comparative purposes. *M. chelonae* NCTC 946 is a glutaraldehyde-susceptible control, whereas *M. chelonae* strains Epping and Harefield are glutaraldehyde resistant but *ortho*-phthalaldehyde susceptible.[4] *M. smegmatis* LIMP7 is a derived mutant of *M. smegmatis* mc$^2$155 with an altered cell envelope permeability. This mutant has a defective *impA1* (inositol monophosphate phosphatase) gene, leading to a dramatic decrease in lipoarabinomannan synthesis.

MIC determinations were undertaken in triplicate by means of the Denley multipoint inoculator with an inoculum size of ~$10^5$ cfu in 1 μL delivered. Incubation temperature was at 30°C (*M. chelonae* strains) for 3–4 days, 37°C (*M. smegmatis* mc$^2$155 for 3–4 days, *S. aureus* and Gram-negative bacteria for 2 days) and 42°C (LIMP7 for 3–4 days). At 37°C, the LIMP7 mutant reverts to the parental strain due to the genomic insertion of a temperature-sensitive transposon.

MICs of PEA and 5-PP for Gram-positive and -negative bacteria are presented in Tables 1 and 2, respectively. As noted previously, PEA was less active against *S. aureus* than against *E. coli* or mycobacteria, and its MIC for *P. aeruginosa* was also high. Glutaraldehyde-resistant mycobacteria (strains Epping and Harefield) had the same response to both PEA and 5-PP as the susceptible strain (NCTC 946). This is an interesting observation, because some *M. chelonae* strains trained in the laboratory to high glutaraldehyde resistance are believed to show alterations in the mycoylarabinogalactan complex of the cell wall.[5] All of the *B. cepacia* strains showed the same order of response to PEA, whereas two strains (ER10-11 and ER17-15, especially the former) were more susceptible than the other strains to 5-PP. The LIMP7 mutant

**Table 1.** MIC ranges of PEA and 5-PP for Gram-positive bacteria

| Organism | MIC range (mg/L) | |
|---|---|---|
| | PEA | 5-PP |
| *S. aureus* ATCC 6538[a] | 4000–4050 | 450–475 |
| *M. chelonae* | | |
| NCTC 946 | 2250–2300 | 250–275 |
| Epping | 2250–2300 | 250–275 |
| Harefield | 2250–2300 | 250–275 |
| *M. smegmatis* | | |
| mc$^2$155 | 2650–2700 | 325–350 |
| LIMP7 | 1500–1550 | 275–300 |

[a]EN 1276 (1997) test strain.

**Table 2.** MIC ranges of PEA and 5-PP for Gram-negative bacteria

| Organism | MIC range (mg/L) | |
|---|---|---|
| | PEA | 5-PP |
| *E. coli* ATCC 10536[a] | 2300–2350 | 425–450 |
| *P. aeruginosa* ATCC 15442[a] | 4050–4100 | >1700[b] |
| *B. cepacia* | | |
| K56-2 (wild-type) | 1100–1200 | 340–360 |
| ER10-11 (triclosan susceptible) | 1000–1100 | 100–140 |
| ER17-15 (triclosan susceptible) | 1100–1200 | 250–300 |
| R3-33 (CHX susceptible) | 1100–1200 | 340–360 |
| R14-37 (CHX susceptible) | 1100–1200 | 340–360 |
| R11-27 (CHX susceptible) | 900–1000 | 340–360 |
| Jz-1 (Tn*5* control) | 1100–1200 | 340–360 |
| J2315 (sequencing strain)[c] | 1100–1200 | 380–390 |

CHX, chlorhexidine.
[a]EN 1276 (1997) test strain.
[b]No activity at maximum solubility.
[c]This strain has been the subject of a genome sequence initiative (http://www.sanger.ac.uk/Projects/B_cepacia/) and will become the Type strain for the new species *Burkholderia cenocepacia* (E. Mahenthiralingam, unpublished data).

strain was more susceptible than the wild-type to PEA, but this could be due, at least partly, to the increase in incubation temperature potentiating the activity of this alcohol. 5-PP was considerably more effective than PEA against any strain, although with *P. aeruginosa* no inhibition was achieved at its maximum solubility. The log (p) [*n*-octanol/water] value (Crippen's fragmentation) of PEA is +1.74 and of 5-PP is +2.99.

In conclusion: (i) apart from *P. aeruginosa*, PEA shows greater activity against Gram-negative bacteria and mycobacteria than against the *S. aureus* strain; (ii) at its maximum solubility, 5-PP does not inhibit the growth of *P. aeruginosa*; (iii) inhibitory concentrations of PEA and 5-PP are ~1.5–2.6 and 1.4–1.75 times higher, respectively, against *S. aureus* than against mycobacteria (Table 1); (iv) on a molar basis, inhibitory concentrations of PEA (Table 2) are ~7.2 times higher than 5-PP against *E. coli* and ~3.7–4.2 times greater against *B. cepacia* strains, except for ER10-11 (11.8 times) and ER17-15 (5.6 times); and (v) 5-PP appears to be taken up to a greater extent than PEA against all bacteria (except *P. aeruginosa*, for which no comparison can be made), probably because of its greater lipophilicity and damage to the cytoplasmic membrane.

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