USING A SCALED LUNG MODEL TO INVESTIGATE THE DEPOSITION BEHAVIOUR OF AEROSOLS INCLUDING ALLICIN

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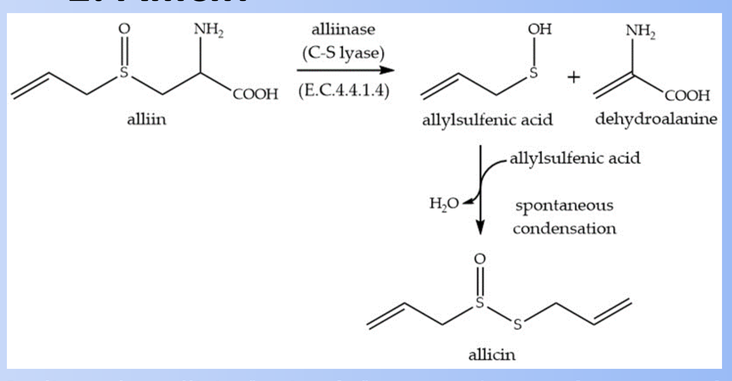
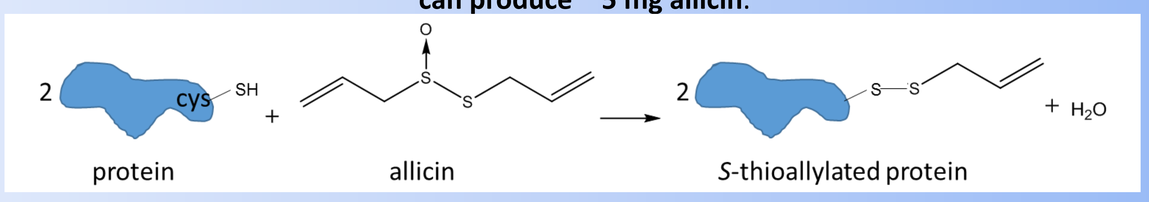
1. Allicin

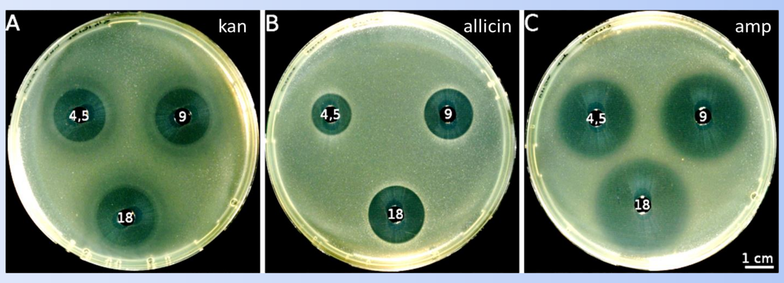
Everyone knows **garlic** as an important ingredient in so many tasty dishes. But we are working on it because it has an **antibiotic effect**. The important component for this effect is Allicin.



Garlic makes allicin for its **defence against pathogens and pests**. Allicin is synthesized when **garlic tissue is damaged**. Only then the non-protein amino acid **alliin** comes into contact with the enzyme **alliinase**. These are in two distinct cell compartments, cytoplasm and vacuole respectively, in healthy garlic cloves. **A 10 g garlic clove can produce ~ 5 mg allicin**.



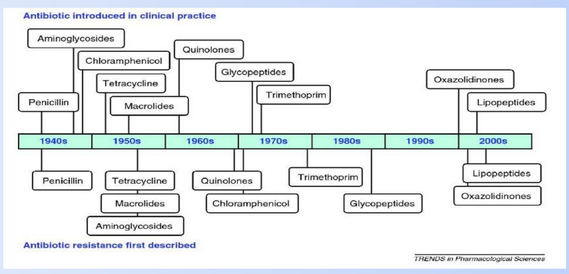
Allicin is a **reactive sulphur species (RSS)** and leads to oxidative stress in cells. Allicin reacts with thiol groups e.g. in glutathione (GSH) or cysteine residues in proteins which can lead to loss of function of **essential enzymes** e.g. DNA gyrase**.** Allicin has a large range of potential cellular targets and GSH acts protectively by titrating it out. **Bacteria, fungi, oomycetes, protozoa and mammalian cells** are inhibited by allicin dose-dependently.



E. coli, 40 µl of 4.5, 9 or 18 mM test solution, 24 h incubation[1]

Allicin's effectivity is similar to conventionally used antibiotics such as e.g. kanamycin and ampicillin, seen here as inhibition zones on Petri dishes containing bacteria-seeded agar.

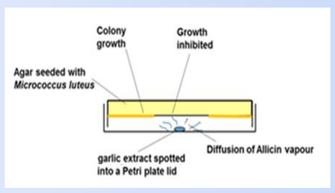
2. Drug Resistant Bacteria



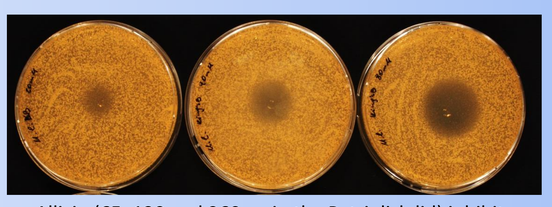
Antibiotic resistance is an ongoing **threat** in modern medicine. Clinically important resistance has usually emerged within two years of the release of a novel antibiotic. Moreover, since the early seventies, development of new antibiotics has been slow [2].

Multiple drug resistant (MDR) bacteria, which are resistant against at least three classes of antibiotics, pose a special problem. There is a desperate **need for new products and new classes of antibiotics**. Allicin has been shown to be effective against lung pathogenic MDR *Streptococcus pneumoniae* and methicillin resistant *Staphylococcus aureus* (MRSA) isolates[3].

3. Allicin shows antibiotic activity via gas-phase



Inhibition zone test via the gas phase [4]

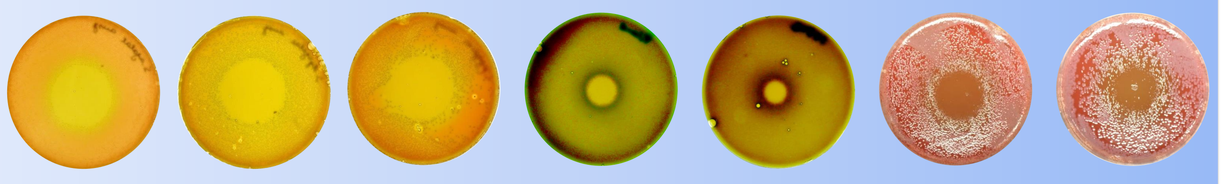


Allicin (65, 130 and 260 µg in the Petri dish lid) inhibits *M. luteus* growth via the vapour phase.

Mask for tuberculosis treatment [5]

Garlic has a strong smell, and allicin is actually responsible for that. So we tested whether allicin can also be used via the gas phase. If a **drop of allicin is placed on the lid** of an inverted petri-dish and **bacteria-seeded-agar** placed above it, after one day it can be seen that **no bacteria grow** in the spot above the drop.

Lung-pathogenic bacteria like *Streptococcus pneumonia* and *Pseudomonas aeruginosa* (and *Streptococcus agalactiae, dysgalactiae* and *pyogenes* colonialize the nasopharyngeal space), including multi-drug-resistant **(MDR) strains are susceptible to allicin via the gas-phase[3]**.



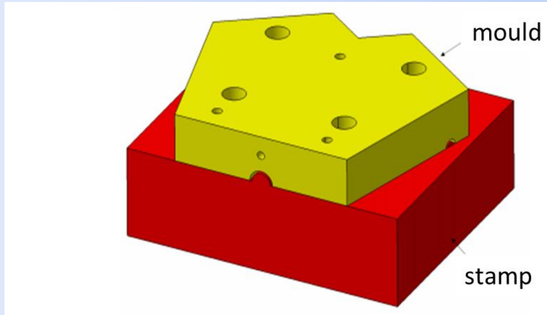
S. agalactiae | *S. dysgalactiae* | *S. pyogenes* | *S. pneumoniae* Poland ^{23F}-16 | *S. pneumoniae* PS26847 | *P. aeruginosa* PAO 25 | *P. aeruginosa* PAO 1

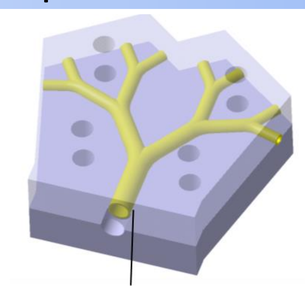
In 1927, W. C. Minchin reported success **treating tuberculosis patients with** garlic fumes[5]. He used a **mask** filled **with garlic juice** (see above). **We are investigating the possibility of developing allicin treatments for lung diseases.**

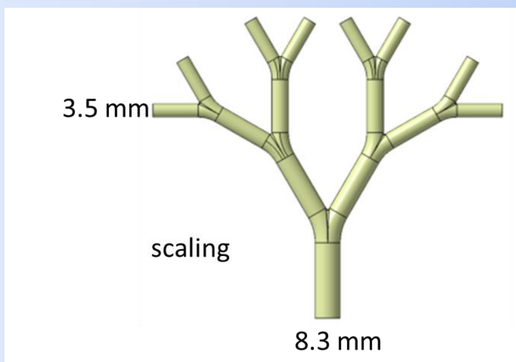
4. A lung model to test the effectivity of allicin

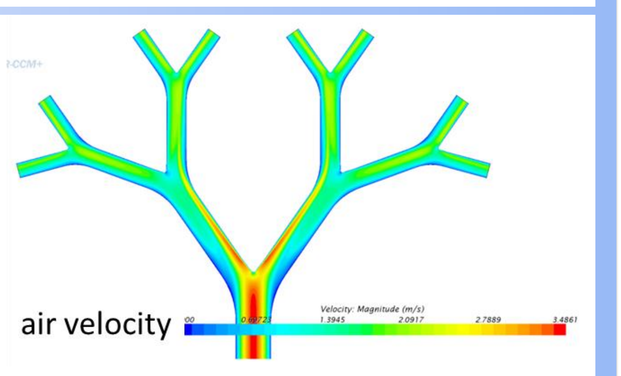
We have designed and built a **simulated lung air-flow device** which allowed us to model accurately the exposure of the lung air-passage surfaces to allicin (and other antibiotics) as a feasibility study for the use of allicin to combat lung infections by direct inhalation of allicin preparations, either alone or in combination with other antibiotics. Using **this model avoids animal sacrifice** for preliminary testing of new antibiotics to combat lung-pathogens by inhalation.

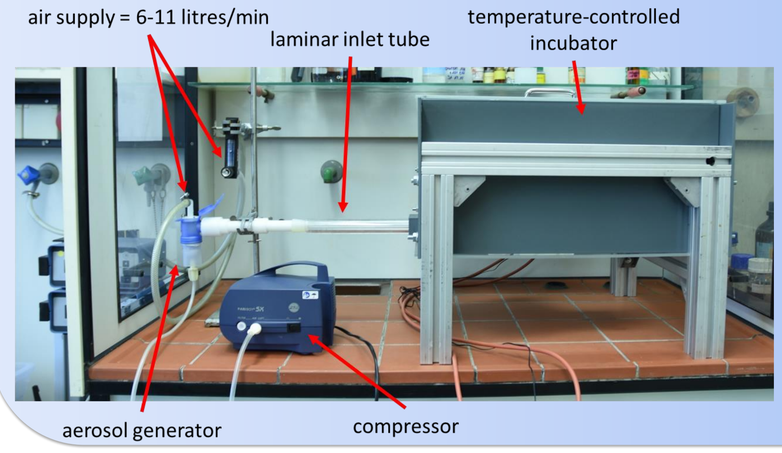
The aerodynamic flow of ventilated air through three bifurcations of a lung was modelled theoretically and a prototype built which could be coated internally with a thin film of agar medium to support bacterial or fungal growth. The apparatus is sterilisable, temperature-controlled and the air flow rate is adjustable. The deposition of antimicrobial aerosols on the bronchial surfaces was followed in preliminary tests without the need for animal experiments. **The theoretical air-flow prediction, reflecting aerosol droplet deposition, correlated with the inhibition of bacterial growth, showing that the model has predictive value.**







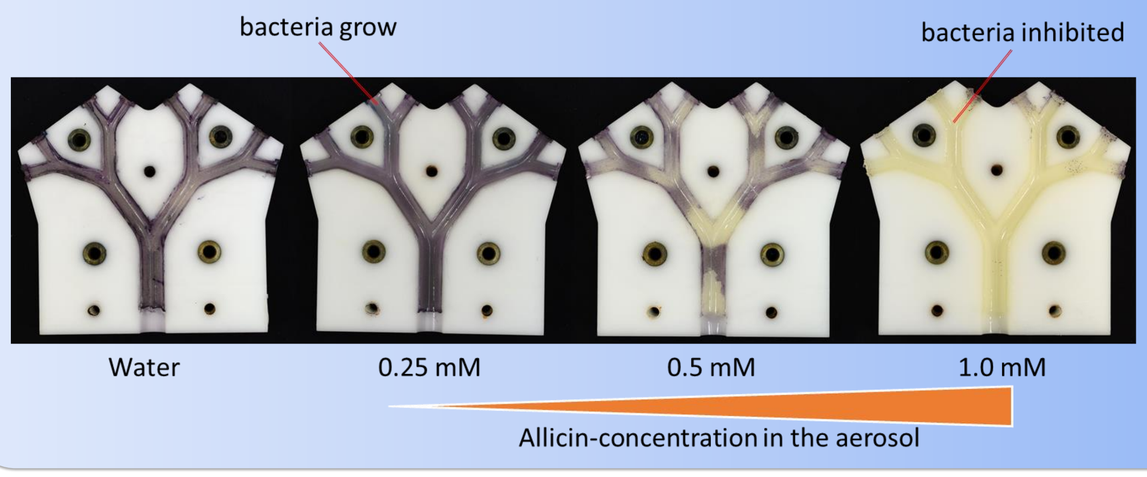




The agar-coated artificial lung segment has the active substance flow over it either as aerosol droplets or as a vapour (gas phase). The bacteria-seeded agar was sprayed with MTT 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide and incubated. **Where allicin was deposited and bacterial growth was inhibited, MTT remained colourless**.

5. Allicin as aerosol droplets

Air-flow rate 11 litre/min, flow time 20 min.



6. Allicin vapour – synergistic effect of the solvent

Conclusions:

The artificial lung model allows the testing of antibiotic deposition/distribution in the lung to be followed without the need for animal testing. Dosage rates can be modelled and the combination of allicin with other test substances can be investigated. Preliminary optimization can thus be achieved before animal testing becomes necessary. This will lead to a reduction in animal sacrifice.

[1] Borlinghaus, J., Albrecht, F., Gruhlke, M. C., Nwachukwu, I. D., & Slusarenko, A. J. (2014). Allicin: chemistry and biological properties. *Molecules*, *19*(8), 12591-12618.
[2] Högberg, L. D., Heddini, A., & Cars, O. (2010). The global need for effective antibiotics: challenges and recent advances. *Trends in pharmacological sciences*, *31*(11), 509-515.
[3] Jana Reiter, Natalja Levina, Mark van der Linden, Martin Gruhlke, Christian Martin and Alan J. Slusarenko (2017) Diallylthiosulfinate (Allicin), a Volatile Antimicrobial from Garlic (*Allium sativum*), Kills Human Lung Pathogenic Bacteria, Including MDR Strains, as a VaporMolecules **2017**, 22, 1711; doi:10.3390/molecules22101711
[4] Curtis, H., Noll, U., Störmann, J., & Slusarenko, A. J. (2004). Broad-spectrum activity of the volatile phytoanticipin allicin in extracts of garlic (*Allium sativum* L.) against plant pathogenic bacteria, fungi and Oomycetes. *Physiological and Molecular Plant Pathology*, *65*(2), 79-89.
[5] Minchin, W. C. (1927). A study in tubercle virus, polymorphism and the treatment of tuberculosis and lupus with *Oleum alii*. Bailliere, Tindall & Cox, London, UK.