Allicin as an addition to conventional antibiotics? Jana Reiter <sup>1</sup>, Mark van der Linden<sup>2</sup>, Natalia Levina<sup>2</sup>, Alan Slusarenko<sup>1</sup>

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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.



Allicin is synthesized when garlic **tissue is damaged**. Only then the non-protein amino acid **alliin** comes into contact with the enzyme **alliinase**. These two are in two distinct cell compartments, cytoplasm and vacuole, in healthy garlic cloves.



MIC: Minimum Inhibitory ConcentrationMBC: Minimum Bactericidal Concentration

	Organism	MIC (mM)	MBC (mM)
Pseudomonas	Pseudononas aeruginosa PAO1 SBUG8	0.39	0.79
	Pseudononas aeruginosa PAO25	0.39	1.58
aeruginosa	Pseudononas aeruginosa DSM 2659	3.16	0.31
Streptococci	Streptococcus dysgactiae S No 67799	0.39	6.31
	Streptococcus agalactiae S No 67764	0.39	0.79
MDR-	<i>Streptococcus pneumoniae</i> Spain <sup>23</sup> F-1	0.39	0.79
	<i>Streptococcus pneumoniae</i> CSR <sup>14</sup> -10	0.39	0.79
Pneumococci	<i>Streptococcus pneumoniae</i> S.Africa <sup>19</sup> A-13	0.39	0.79
	<i>Streptococcus pneumoniae</i> Poland <sup>23</sup> F-16	0.39	0.39

We tested the susceptibility of lung-pathogenic bacteria to allicin with the standardized resistance test following the guidelines of the European committee on antimicrobial susceptibility testing (EUCAST). Two values are distinguished: minimum inhibitory The (MIC), concentration that inhibits bacterial growth, and bactericidal the minimum





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 thiols in proteins. This can lead to loss of function of essential enzymes. Thus, allicin has a large range of potential cellular targets and GSH can protect cells against it. Allicin is a concentration-dependent biocide and effectively kills bacteria, fungi, oomycetes and protozoa.



Allicin has a **strong antibiotic effect**, similar to conventionally used antibiotics such as e.g. kanamycin and ampicillin.

*E. coli*, 40  $\mu$ l of 4.5, 9 or 15 mM allicin solution, 24 h<sup>1</sup>

## **Drug Resistant Bacteria**



## Allicin MIC vs. conventional antibiotics

Susceptibility testing was done at the National Reference Laboratory (NRZ) on Streptococcal Diseases based on µg ml<sup>-1</sup> concentrations (Uniklinik RWTH Aachen). Most antibiotics work at lower concentrations than allicin. But, when looking on the MDR strains, allicin can inhibit bacterial growth in contrast to erythromycin and clindamycin.

	Organismus	Allicin	Pen	Ctx	Ery	Clin	Tet	Chl	Sxt	
ococci	<i>Streptococcus pyogenes</i> SNo 67467	32	0.015	0.015	256	64	64	2	0.25	
rept	<i>Streptococcus dysgactiae</i> S No 67799	64	0.015	0.015	0.12	0.12	16	2	0.25	Pen = Penicillin
St	<i>Streptococcus agalactiae</i> S No 67764	64	0.030	0.060	0.12	0.12	64		0.25	Ctx = Cefotaxime
occi	Strentococcus nneumoniae									Ery = Erythromycin
noc	Spain 23F-1	64	1	1.5	0.025	0.19	64	16	2	
neun	<i>Streptococcus pneumoniae</i> CSR 14-10	64	8	1	256	256	48	32	0.25	Clin = Clindamycin
R-PI	<i>Streptococcus pneumoniae</i> S. Africa 19A-13	64	1.5	0.5	256	256	48	24	4	Tet = Tetracycline
MD	<i>Streptococcus pneumoniae</i> Poland 23F-16	64	8	4	256	256	64	16	1.5	Chl = Chloramphenico
occi										Sxt = Trimethoprim/
mocc	<i>Streptococcus pneumoniae</i> PS26847	32	0.015	0.015	0.12	0.12	0.25	2	0.25	Sulfamethoxazole



Antibiotic resistance is an ongoing threat in modern medicine. Clinically importance has usually emerged within two years of the release of a novel antibiotic. Also, since the early seventies, development of new products has been slow <sup>2</sup>.

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 shows antibiotic activity via gas-phase

Agar seeded with Micrococcus luteus garlic extract spotted into a Petri plate lid



Inhibition Zone Test via gas –phase <sup>3</sup>

Allicin (65, 130 and 260 µg) inhibits *M. luteus* growth Mask for tu

Mask for tuberculosis treatment <sup>4</sup>

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 you place a **drop of allicin on the lid** of an inverted petri-dish and have **bacteria-seeded-agar** above it, after one day you can see that **no bacteria grow** in the spot above the drop of allicin.



# Allicin decreased bacterial titre in lung epithelial cells

P. aeruginosa PAO1 and lung epithelia cell line A549

Lung pathogenic bacteria can attach to the cell surface of lung epithelia or go inside the cell. In control this experiment the cell culture incubated with diluted was bacterial suspension and 100 μM sublethal allicin concentrations allicin for one hour. Allicin treatment do not affect the total number of bacteria, but the number of 200 µM allicin intracellular bacteria was reduced by allicin treatment.



## Effect of allicin on mammalian cells in culture

The next important question: What allicin concentrations can our **lung cells survive**? Cell cultures, with or without **1 mM** 



Lung-pathogenic bacteria like *Streptococcus pneumonia* and *Pseudomonas aeruginosa* (and *Streptococcus agalactiae, dysgalactiae* and *pyogenes* colonialize the nasopharyngeal space) are susceptible to allicin via gas-phase.



S. agalactiae S. dysgalactiae S. pyogenes S. pneumoniae S. pneumoniae P. aeruginosa P. aeruginosa Poland <sup>23F</sup>-16 PS26847 PAO 25 PAO 1 In 1927, W. C. Minchin reported success **treating tuberculosis patients with** garlic fumes. He used a **mask** filled **with garlic juice** (see above). We are investigating the possibility of developing allicin treatments for lung diseases. glutathione (GSH), were stressed for one hour with allicin and a vitality test was performed with MTT (3-(4,5-dimethylthiazol-2-yl)-2,5diphenyltetrazolium bromide). [GSH] in blood is ~1 mM. We see that cell resistance is increased with added glutathione. The half maximal effective allicin concentration (EC<sub>50</sub>, black dotted line) with glutathione is about 500  $\mu$ M.

While lung epithelial cells are well supplied with blood, bacteria are colonizing the air-filled alveoli. Since the MIC of most tested bacteria was 390  $\mu$ M (green dotted line) it may be possible inhibit bacterial growth without killing lung epithelial cells. Animal testing and synergism tests with other antibiotics must be carried out.

#### Literatur

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