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Challenging the concept of bacteria subsisting on antibiotics

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ABSTRACT

Antibiotic resistance concerns have been compounded by a report that soil bacteria can catabolise antibiotics, i.e. break down and use them as a sole carbon source. To date this has not been verified or reproduced, therefore in this study soil bacteria were screened to verify and reproduce this hypothesis. Survival in high concentrations of antibiotics was initially observed; however, on further analysis these bacteria either did not degrade the antibiotics or they used an intrinsic resistance mechanism (β-lactamases) to degrade the β-lactams, as demonstrated by high-performance liquid chromatography. These results did not verify or reproduce the hypothesis that bacteria subsist on antibiotics or catabolise antibiotics as previously reported. This study identified that bacteria with a catabolising phenotype did not degrade streptomycin or trimethoprim and therefore could not utilise the antibiotics as a nutrient source. Therefore, we conclude that soil bacteria do not catabolise antibiotics.

1. Introduction

Antibiotic resistance is emerging as one of the greatest challenges to human health [1]. Within the past 20 years, antibiotic resistance has developed from resistance to single classes of antibiotics to multidrug resistance (MDR) and extensive drug resistance (XDR) [2]. Catabolism, i.e. the capacity of bacteria to not only resist but to subsist on antibiotics, has been presented as a potentially crucial step in the evolution of antibiotic resistance from MDR and XDR to untreatable infections. In 2008, a novel hypothesis was introduced, namely antibiotic catabolism, defined by the identification of soil bacteria from different phyla that were capable of degrading and utilising different classes of antibiotics as a sole carbon source [3]. Bacterial catabolism of antibiotics is a concept that surpasses antibiotic resistance and MDR in terms of complexity in treating and managing bacterial infections. Therefore, it is important for the future successful management of antibiotic-treated bacterial infections that we are able to distinguish between conventional resistance mechanisms and the ability of bacteria to subsist on antibiotics.

Research on antibiotic degradation was reported in the 1970s, but the novel study on multiple antibiotic classes catabolised by soil bacteria exposed the full extent and distribution of antibiotic-degrading genes in the environment [4]. This study has specifically led to the scientific belief that soil bacteria contain previously uncharacterised antibiotic-degrading resistance enzymes and that soil bacteria can subsist on antibiotics, with respect to at least 18 different antibiotics [3]. The only resistance-degrading mechanism described prior to this study was the degradation of β-lactam antibiotics by the β-lactamase resistance mechanisms. The characterised mechanisms of resistance to all other classes of antibiotics do not include degradation. This study revolutionised the current thinking on how bacteria can resist antibiotics whilst also identifying soil bacteria as a potentially large reservoir of novel resistance mechanisms. Antibiotic catabolism would protect the entire bacterial community from the inhibitory effects of antibiotics and concurrently increase the population size as they use the antibiotics as a nutrient source.

Although the hypothesis of antibiotic catabolism by soil bacteria was published 5 years ago, this hypothesis has neither been reproduced nor verified. The aims of the current study were therefore to verify the hypothesis of soil bacteria subsisting on antibiotics.

2. Materials and methods

2.1. Sampling and site descriptions

The soil sampling sites, locations and elevations are described in Table 1. Soil pH was determined by suspending 1 g of soil in 2.5 mL of 0.01 M $CaCl_2$ and measuring the pH using a glass electrode [5]. The pH of each soil was determined three times and the mean ± standard deviation is given in Table 1.

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Table 1
Geographic and descriptive characteristics of the analysed urban, agricultural and pristine soil samples.

Sample ID	Description	Environmental matter	Elevation (m)	Latitude	Longitude	pH ($n = 3$)
FWS1	Wädenswil Apple Orchard	Orchard soil	407	47.2333	8.6667	7.0 ± 0.1
FWS2	Benedictine Abbey	Farm soil	880	47.1167	8.75	7.1 ± 0.1
FWS3	Hospital garden	Lawn soil	667	46.7167	9.4333	7.3 ± 0
FWS4	Lindau Apple Orchard	Orchard soil	485	47.4833	8.2	4.1 ± 0.2
FWS5	Güttingen Apple Orchard	Orchard soil	503	47.6	9.2833	5.3 ± 0.1
FWS6	Rütli meadow	Meadow soil	835	46.9667	8.6	7.1 ± 0.2
FWS7	Area beside Lake Zürich	Lawn soil	408	47.3667	8.55	7.0 ± 0.1
FWS8	Matterhorn mountain trail	Alpine soil	1936	46.0167	7.75	7.2 ± 0.1
FWS9	Farmland treated with pig manure	Manured soil	592	47.1833	8.3167	5.7 ± 0.2
FWS10	Mountain near Zürich	Mountain forest soil	700	47.3496	8.492	7.0 ± 0

2.2. Sampling strategy

For each sample site, eight soil core samples were combined. Soil samples consisted of eight soil cores (10 cm depth) per replicate taken using a stainless steel corer with an internal diameter of 2.5 cm. Soil cores were pooled for each replicate in the field. Pooling of soil cores is standardly applied in order to obtain more representative samples for a certain field plot or a specific experimental treatment [6–8].

2.3. Isolation of antibiotic-catabolising bacteria from Swiss soil

Soil bacteria with an antibiotic-catabolising phenotype were isolated and cultured using methods described previously from ten soils under a variety of anthropogenic influences from urban, pristine and farmland soils (Table 1) [3]. Minimal medium [single carbon source (SCS)] broth cultures of putative catabolising bacteria were serially diluted and spread-plated onto SCS agar supplemented with the appropriate corresponding antibiotic [3]. Negative controls comprised SCS agar without antibiotic, inoculated in the same manner as described by Dantas et al. [3]. The antibiotics tested comprised penicillin, dicloxacillin, amikacin, cefalexin, kanamycin, gentamicin, sisomicin, streptomycin, vancomycin, levofloxacin, ciprofloxacin, sulfamethizole, nalidixic acid, chloramphenicol, d-cycloserine, cefotaxime, novobiocin, trimethoprim, sulfisoxazole, tetracycline, erythromycin, colistin and rifampicin. All antibiotics and chemicals, except kanamycin and vancomycin (Carl Roth GmbH and Co. KG, Karlsruhe, Germany), were obtained from Sigma-Aldrich Chemie GmbH (Buchs, Switzerland).

Two bacteria described as having the capacity to catabolise carbenicillin and penicillin were obtained from the Dantas group: CA-S3F-1, *Burkholderia* sp.; and PE-S2R-1, *Burkholderia* sp. Due to unforeseen circumstances in the Dantas laboratory, further isolates catabolising non-β-lactam antibiotics could not be provided.

The abilities of the antibiotic-catabolising bacteria to grow or survive in sterile-filtered SCS medium without added antibiotics were investigated. Assimilable organic carbon-free glassware was prepared as previously described [9]. Bacteria were inoculated into SCS medium and grown at 22 °C for 3 days. Then, 10 μL was removed and added to SCS medium with and without antibiotic at a final concentration of 1 g/L. Total bacterial counts were performed on Days 0, 5, 7, 14, 21 and 28 by plate counts on Luria–Bertani (LB) agar. The total organic carbon and dissolved organic carbon (DOC) concentrations were determined as previously described [10].

2.4. Resistance profiling of antibiotic-catabolising bacteria

The resistance profile of each isolate was determined as previously described using 1 mg/mL of each antibiotic [11]. Antibiotic susceptibility tests were performed in duplicate. Where there was a discrepancy between the two results, susceptibility testing was performed a third time [12,13]. Two consistent susceptibility results were taken as the final result.

2.5. Bacterial phylogenetic profiling

The phylogenetic profiles of the bacteria were determined as previously described [3]. Briefly, the variable regions (nucleotides 63–1389) of the 16S rRNA genes were amplified by PCR and sequenced. The phylogeny of the bacteria based on sequence variation of the 16S rRNA genes was identified using BLASTn and the Greengenes 2011 database. A phylogenetic tree of the bacterial species based on the 16S rRNA sequences was constructed using the neighbour-joining algorithm in ARB [14].

2.6. High-performance liquid chromatography (HPLC) investigation of bacterial antibiotic degradation

Degradation of carbenicillin (bacteria CA-S3F-1, *Burkholderia* sp.), penicillin (bacteria PE-C-1, *Pseudomonas* sp. and bacteria PE-S2R-1, *Burkholderia* sp.), streptomycin (bacteria ST-C-1, *Achromobacter* sp.) and trimethoprim (bacteria TR-C-1, *Pseudomonas* sp.) was investigated by HPLC [1] performed using a Dionex PDA-100 photodiode array detector, Chromeleon v.6.80 software (Dionex, Idstein, Germany) and the conditions described in Table 2. Aliquots (80 μL) of cultures grown in SCS medium containing 1 mg/mL of antibiotic were inoculated into 20 mL of SCS medium containing 1 mg/mL of appropriate antibiotic and incubated at 22 °C for 28 days. SCS–antibiotic medium containing no bacteria was also incubated at 22 °C. Then, 1 mL was removed at Days 0, 2, 4, 7, 14, 21 and 28 and was analysed in duplicate using HPLC. Following the initial results of the HPLC experiments, all bacterial isolates were also inoculated into SCS medium containing no antibiotic as negative controls at a final concentration of 10^3 or 10^4 CFU/mL and incubated at 22 °C for 28 days.

2.7. Biological activity of the antibiotic solutions following incubation with the catabolising bacteria

The biological activities of the trimethoprim and streptomycin HPLC solutions containing the respective catabolising bacteria from Days 0, 14 and 28 were investigated using an adaptation of the antibiotic disk diffusion assay [15]. *Staphylococcus aureus* ATCC

Table 2
Description of the high-performance liquid chromatography (HPLC) experimental conditions and columns.

Antibiotic	Wavelength (nm)	Flow rate (mL/min)	Column
Penicillin	220	0.7	Nucleosil 100-5, C18
Carbenicillin	220	0.7	Nucleosil 100-5, C18
Streptomycin	195	0.7	Nucleosil 100-5, C18
Trimethoprim	250	0.7	Nucleosil 100-5, C18