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Growth Inhibition of Pathogenic Bacteria by Sulfonylurea Herbicides

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Emerging resistance to current antibiotics raises the need for new microbial drug targets. We show that targeting branched-chain amino acid (BCAA) biosynthesis using sulfonylurea herbicides, which inhibit the BCAA biosynthetic enzyme acetohydroxyacid synthase (AHAS), can exert bacteriostatic effects on several pathogenic bacteria, including *Burkholderia pseudomallei*, *Pseudomonas aeruginosa*, and *Acinetobacter baumannii*. Our results suggest that targeting biosynthetic enzymes like AHAS, which are lacking in humans, could represent a promising antimicrobial drug strategy.

As the prevalence of antibiotic resistance continues to rise, new therapeutic targets are urgently needed (1). One strategy for designing new antibiotics is to target essential components that are uniquely present in bacteria and not humans. Bacteria and plants, but not humans, can synthesize branched-chain amino acids (BCAA); thus, enzymes in this pathway are potential targets for antimicrobials and herbicides (2, 3). In addition, BCAA biosynthesis is essential for certain bacterial infections *in vivo*, including *Burkholderia pseudomallei* (4), the causative agent of melioidosis (5), and *Mycobacterium tuberculosis* (6).

Sulfonylurea herbicides (SHs) inhibit acetohydroxyacid synthase (AHAS) (7, 8), the first shared enzyme in the BCAA biosynthetic pathway, from plants and two of the three isozymes in bacteria; type II and type III but not type I are sensitive to SHs (3). Enterobacteria encode a type I isozyme along with a type II and/or type III isozyme and thus are resistant to inhibition by SHs (9, 10). Alternatively, soil bacteria typically contain only a type III AHAS and thus are often sensitive to inhibition by SHs (11–13). The antibacterial activity of SHs on soil microbes has also been noted to explain the potential toxic environmental effects of these compounds (14, 15). The distribution of AHAS isozymes in a variety of pathogenic bacteria is not known.

MATERIALS AND METHODS

Phylogenetic analysis. Bacterial isozymes of AHAS are composed of a catalytic and a regulatory subunit that are encoded by adjacent genes. Catalytic subunits of AHAS were identified by searching the genomes of select organisms using the Enzyme Commission number 2.2.1.6 (2, 16). In most genomes, multiple genes have been annotated as AHAS catalytic subunits. To ensure that only actual catalytic subunits were analyzed, only those genes adjacent to a regulatory subunit were included. Nucleotide sequences of 50 AHAS catalytic subunits from 38 species (Table 1) were translated into protein sequences and aligned (ClustalW2), and gaps were stripped from the alignment (BioEdit). A phylogenetic tree was derived using maximum likelihood methods and subjected to bootstrap analysis 100 times (PHYLIP).

Determination of MICs. All SHs were purchased from Sigma-Aldrich although manufactured by Chem Service, Inc. (analytical standards; purity > 99.0%). SH stocks were prepared in dimethyl sulfoxide (DMSO) (200 μ M), stored at -20°C , and discarded after 2 months. Antibiotics and

other chemicals were also purchased from Sigma-Aldrich unless stated otherwise.

MICs were determined based on the 2-fold microdilution broth technique described in the Clinical and Laboratory Standards Institute guidelines (M7-A6). In brief, exponential-phase starter cultures in LB broth were washed twice in M9 salts (17) before being suspended in growth medium. For experiments with *B. pseudomallei*, *Burkholderia thailandensis*, or *Pseudomonas aeruginosa*, cultures were grown in M9 medium (17) that contained glucose (0.2%) and thiamine (0.5 $\mu\text{g/ml}$). For experiments with *Bacillus subtilis*, cultures were grown in Spizizen's minimal medium (18) containing glucose (1%) and tryptophan (50 $\mu\text{g/ml}$) along with glutamate (2 mg/ml), KNO_3 (0.2%), and trace elements as described by Nakano et al. (19). The MIC values reported for each strain are the median values from three to six independent experiments. For the *P. aeruginosa* clinical isolates, each MIC assay was typically performed once.

High-resolution growth curves. Glycerol stocks of *Acinetobacter baumannii* were streaked onto LB agar plates and incubated overnight at 37°C . The next day, a single colony was used to inoculate a starter culture of 3 ml of M9 medium (17) containing acetate (0.2%) and thiamine (0.5 $\mu\text{g/ml}$). After 4 h of aerobic growth at 37°C , the starter culture was then diluted to an optical density at 600 nm (OD_{600}) of 0.01 in M9 salts, and 5 μl was used to inoculate M9 medium (250 μl) containing acetate (0.2%) and thiamine (0.5 $\mu\text{g/ml}$). High-resolution growth curves were obtained using a Bioscreen C MBR (Oy Growth Curves Ab Ltd.) integrated incubator and microplate reader. All cultures (250 μl) were grown aerobically at 37°C with DMSO or SHs included as indicated, and OD_{600} readings were collected at regular intervals. The reported OD_{600} readings are the average values across three wells in one experiment, and the data shown are representative of three independent experiments.

Mouse infections. *B. pseudomallei* experiments were performed in accordance with the Animal Care and Use Committee's Guidelines, DSO National Laboratories, Singapore, using protocols approved by the DSO National Laboratories Biosafety Committee. Female 6-week-old BALB/c mice were intranasally challenged as previously described (20) with 10^2

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TABLE 1 Sequences used in the construction of the AHAS phylogeny

Organism (strain)	NCBI GeneID	Isozyme
<i>Acinetobacter baumannii</i> (5377)	4917404	III
<i>Acinetobacter baumannii</i> (AYE)	6003386	III
<i>Actinobacillus pleuropneumoniae</i> (L20)	4848528	II
<i>Actinobacillus pleuropneumoniae</i> (L20)	4848759	III
<i>Arabidopsis thaliana</i> (Columbia)	824015	N/A
<i>Bacillus anthracis</i> (Ames)	1086383	III
<i>Bacillus subtilis</i> (168)	936792	III
<i>Bordetella pertussis</i> (Tohama I)	2664081	III
<i>Brucella suis</i> (1330)	1167071	III
<i>Burkholderia cenocepacia</i> (AU1054)	4093524	III
<i>Burkholderia cenocepacia</i> (J2315)	6933755	III
<i>Burkholderia multivorans</i> (ATCC 17616)	5765837	III
<i>Burkholderia pseudomallei</i> (K96243)	3093947	III
<i>Burkholderia thailandensis</i> (ATCC 700388)	3847786	III
<i>Campylobacter jejuni</i> (NCTC 11168)	904899	III
<i>Caulobacter crescentus</i> (CB15)	943022	III
<i>Enterobacter</i> (638)	5110480	II
<i>Enterobacter</i> (638)	5113032	I
<i>Enterobacter</i> (638)	5113663	III
<i>Escherichia coli</i> (K-12 MG1655)	948182	I
<i>Escherichia coli</i> (K-12 MG1655)	948793	III
<i>Haemophilus influenzae</i> (Rd KW20)	950449	III
<i>Klebsiella pneumoniae</i> (MGH 78578)	5339104	III
<i>Klebsiella pneumoniae</i> (MGH 78578)	5339386	II
<i>Klebsiella pneumoniae</i> (MGH 78578)	5341318	I
<i>Listeria innocua</i> (Clip11262)	1130811	III
<i>Listeria monocytogenes</i> (EGD-e)	984805	III
<i>Mycobacterium bovis</i> (BCG Pasteur 1173P2)	4698974	III
<i>Mycobacterium marinum</i> (M)	6225967	III
<i>Mycobacterium tuberculosis</i> (H37Rv)	887286	III
<i>Neisseria gonorrhoeae</i> (FA 1090)	3282600	III
<i>Neisseria lactamica</i> (020–06)	10007328	III
<i>Neisseria meningitidis</i> (Z2491)	907658	III
<i>Pseudomonas aeruginosa</i> (PA14)	4384635	III
<i>Pseudomonas aeruginosa</i> (PAO1)	881496	III
<i>Ralstonia solanacearum</i> (GMI1000)	1220918	III
<i>Salmonella enterica</i> (Typhimurium LT2)	1251634	III
<i>Salmonella enterica</i> (Typhimurium LT2)	1255320	I
<i>Salmonella enterica</i> (Typhimurium LT2)	1255427	II
<i>Staphylococcus aureus</i> (Mu50)	1122066	III
<i>Staphylococcus epidermidis</i> (ATCC 12228)	1057224	III
<i>Stenotrophomonas maltophilia</i> (K279a)	6394810	II
<i>Streptococcus pneumoniae</i> (TIGR4)	930383	III
<i>Vibrio cholerae</i> (O1 El Tor N16961)	2613025	III
<i>Vibrio cholerae</i> (O1 El Tor N16961)	2614464	II
<i>Vibrio vulnificus</i> (CMCP6)	1177596	III
<i>Vibrio vulnificus</i> (CMCP6)	1177966	II
<i>Yersinia pestis</i> (CO92)	1173384	III
<i>Yersinia pestis</i> (CO92)	1175125	I
<i>Yersinia pestis</i> (CO92)	1176738	II

CFU of *B. pseudomallei* strain 22 (also known as KHW), five times the established 50% lethal dose (LD_{50}) (20). Solutions of metsulfuron methyl were prepared fresh each day by dissolving the powder in DMSO (500 mg/ml) and then diluting into phosphate-buffered saline (PBS), pH 8.0 (5 mg/ml). Groups of 6 mice were fed 200 μ l of either PBS, pH 8.0 (also containing 1% DMSO), or metsulfuron methyl twice daily using a gavage tube. The treatment was initiated 24 h before challenge and continued for 10 days after infection. All experimental procedures were carried out in a biosafety level 3 (BSL3) laboratory. The data shown are representative of three independent experiments.

P. aeruginosa experiments were based on guidelines from the National Advisory Committee for Laboratory Animal Research, Singapore, and were approved by the Institutional Animal Care and Use Committee, A*STAR, Singapore. Intratracheal instillation was carried out using a modification of a previously described method (21). FVB/N mice (8 to 10 weeks old) were anesthetized with a mixture of Hypnorm/Dormicum, after which the trachea was exposed through an anterior midline incision, and the inoculum of *P. aeruginosa* strain PAO1 (3×10^5 CFU in 50 μ l of PBS for survival curves; 2×10^5 CFU in 50 μ l of PBS for lung CFU) was delivered just beneath the cricoid cartilage. Metsulfuron methyl (5 mg/ml) or PBS was injected through the tail vein immediately after intratracheal infection. The survival data are representative of four independent experiments. To determine CFU present in the lungs, lung homogenates were spread on LB agar plates and incubated at 37°C overnight. These experiments were performed first with 12 mice per group and a second time with 10 mice per group. Separately or combined, the results showed a statistically significant difference ($P < 0.01$; Mann-Whitney rank-sum test).

RESULTS AND DISCUSSION

To determine the prevalence of AHAS isozymes across a sample of clinically relevant bacteria, we performed a phylogenetic analysis on the AHAS catalytic subunit, which contains the SH binding site (2, 16). As seen previously (13, 22, 23), type I AHAS was exclusively present in *Enterobacteriaceae* (Fig. 1). Notably, almost all the other bacteria in our analysis, including pathogens such as *Pseudomonas aeruginosa*, *Acinetobacter baumannii*, and *Staphylococcus aureus*, encoded only a type III AHAS (Fig. 1). These findings suggest that BCAA biosynthesis in certain human pathogens may be sensitive to SHs.

MIC assays using SHs were carried out in chemically defined media lacking BCAAs to test *B. pseudomallei* (20, 24), the opportunistic Gram-negative pathogen *P. aeruginosa* (25), and the avirulent soil-resident bacterium *Burkholderia thailandensis* (26). Chlorimuron ethyl and metsulfuron methyl were consistently the most effective SH tested (Table 2). Both SHs were bacteriostatic. The MIC_{50} of both chlorimuron ethyl and metsulfuron methyl on a panel of *P. aeruginosa* clinical isolates was 62.5 μ M (Fig. 2A). The MIC_{90} for chlorimuron ethyl was 125 μ M and 250 μ M for metsulfuron methyl. Addition of BCAAs to the cultures rendered SHs ineffective, consistent with SHs specifically inhibiting AHAS. As it is difficult to compare these MIC values against standard MIC values generated in rich media, chloramphenicol, a clinically effective antibiotic (5), was also tested and found to have similar levels of inhibitory activity (Table 2). Due to the atypical nature of these assay conditions though, it is difficult to predict how SHs would compare against a wider range of antibiotics under more established conditions.

Chlorimuron ethyl and metsulfuron methyl also slowed the growth of *A. baumannii* (Fig. 2B), another opportunistic Gram-negative pathogen and major source of nosocomial infections (27). To study the Gram-positive bacterium *B. subtilis*, MIC assays were performed using Spizizen's minimal medium (18) with additives as described by Nakano et al. (19). SHs again inhibited growth (Table 2), with chlorimuron ethyl being especially potent. Although testing additional bacterial species would be desirable, it is not trivial to determine the proper growth conditions, as chemically defined media lacking in BCAAs are not readily available for most species.

Two mouse infection studies were performed. Administration of metsulfuron methyl (50 mg/kg) orally twice a day to

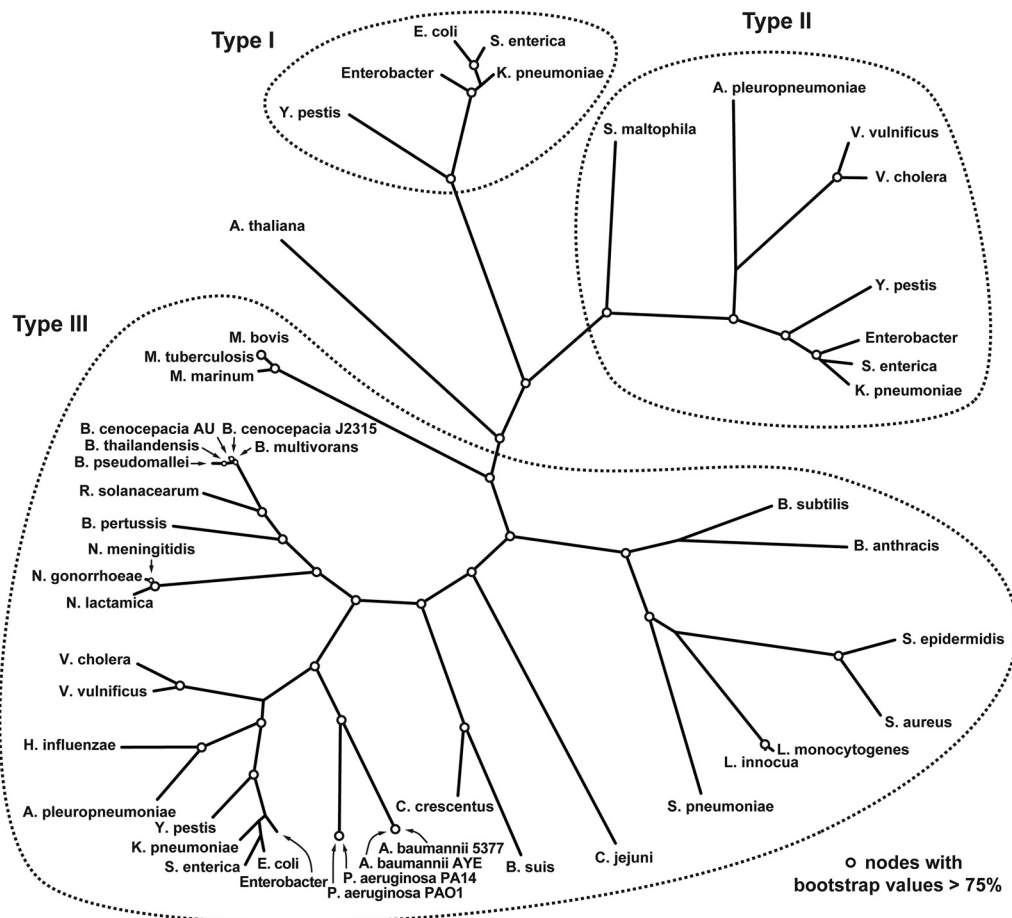


FIG 1 Type II and III AHAS are the sole AHAS isozymes present in many pathogenic bacteria. A phylogenetic tree of the catalytic subunit of AHAS was constructed using maximal likelihood methods. Bootstrap analysis was performed on 100 trees, and nodes appearing more than 75 times are indicated with a circle. The clusters formed by each isozyme are circled.

mice infected intranasally with *B. pseudomallei* led to a statistically significant increase in survival (Fig. 3A; $P < 0.01$; log rank test, Yates corrected). Upon cessation of treatment, all of the metsulfuron methyl-treated mice remained alive for the extent of follow-up, an additional 10 days. Mice infected intratracheally with *P. aeruginosa* and then given a single tail vein injection of metsulfuron methyl (200 mg/kg) survived more readily than mice injected with PBS (Fig. 3B; $P < 0.01$; log rank test, Yates corrected). Lungs harvested from the metsulfuron

methyl-treated mice 24 h after infection had a reduced bacterial load (Fig. 3C; $P < 0.01$; Mann-Whitney rank-sum test). In this acute infection model, all of the mice alive on day 6 had cleared the infection, and no additional mortality was observed.

Based on this, we propose that developing inhibitors of bacterial type II and III AHAS may represent a promising strategy for new antibiotics. Just as SHs have been designed for targeted agricultural applications (2), medicinal chemists could modify

TABLE 2 MICs in minimal medium of SHs and chloramphenicol

Gram stain result	Organism	Strain	MIC (μM) ^a					
			Ce	Me	Bm	Rim	Sm	Cm
Gram-negative human pathogen	<i>B. pseudomallei</i>	K96423	31.2	31.2	125	125	250	40
	<i>B. pseudomallei</i>	22	31.2	62.5	62.5	125	250	80
	<i>P. aeruginosa</i>	PAO1	62.5	62.5	62.5	62.5	250	40
	<i>P. aeruginosa</i>	PA14	15.6	31.2	31.2	31.2	62.5	20
Gram negative	<i>B. thailandensis</i>	ATCC 700388	62.5	125	125	250	>500	40
Gram positive	<i>B. subtilis</i>	168	3.12	31.2	62.5	NT	NT	5

^a Assays were repeated multiple times for each strain, and the median value is reported. Ce, chlorimuron ethyl; Me, metsulfuron methyl; Bm, bensulfuron methyl; Rim, rimsulfuron; Sm, sulfometuron methyl; Cm, chloramphenicol; NT, not tested.

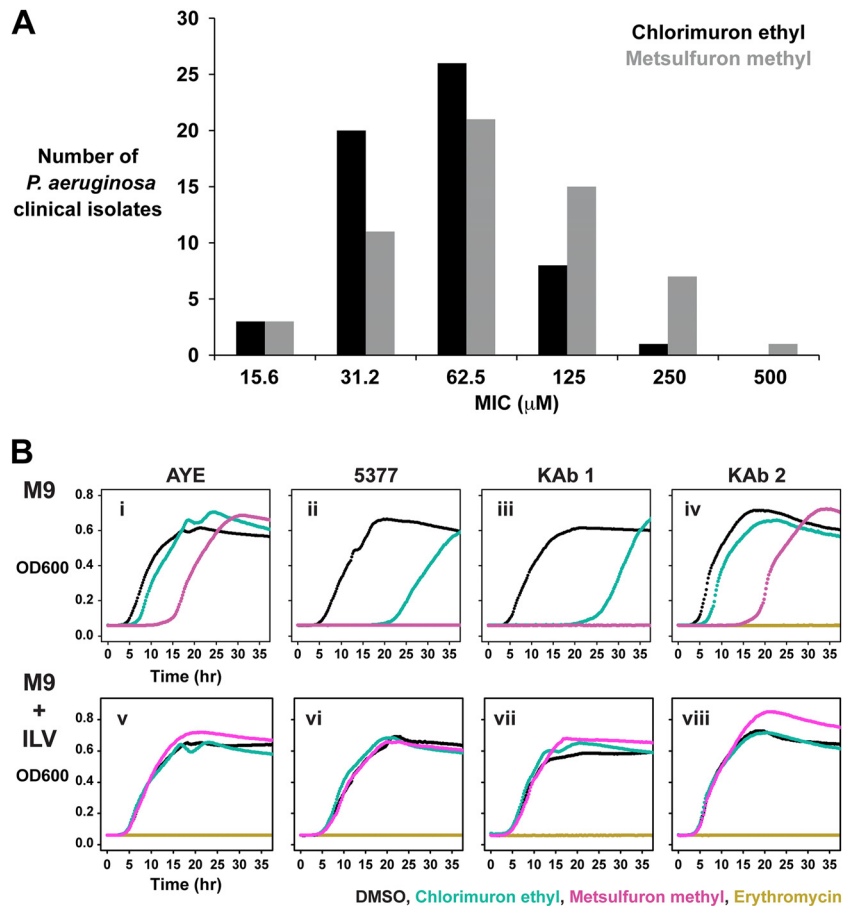


FIG 2 Clinical isolates of *P. aeruginosa* and *A. baumannii* are sensitive to SHs. (A) MIC values were determined for both chlorimuron ethyl and metsulfuron methyl against a panel of 58 local *P. aeruginosa* clinical isolates. (B) Two reference strains, AYE (i, v) (37) and 5377 (ii, vi) (38), and two local clinical isolates, KAB 1 (iii, vii) and KAB 2 (iv, viii), of *A. baumannii* were treated with DMSO (1%), chlorimuron ethyl (2 mM), metsulfuron methyl (2 mM), or erythromycin (20 μM), and high-resolution growth curves were obtained with OD₆₀₀ readings collected every 12 min. In panels i to iv, no BCAAs were added to the M9 medium, whereas in panels v to viii, the cultures contained leucine, isoleucine, and valine (each 75 $\mu\text{g}/\text{ml}$). AYE and KAB 2 are multidrug-resistant strains.

SHs to potentially increase potency, bioavailability, or specificity.

Some toxicity information on SHs is publically available (28), including a technical bulletin issued by DuPont on metsulfuron methyl (Escort XP Technical Bulletin, K-14796, 2007), which reports that acute oral toxicity in rats is $>5,000$ mg/kg and that in an 18-month dietary study in mice, no observed adverse effects were seen at 666 mg/kg/day, the highest dose tested. Uninfected mice

treated with metsulfuron methyl here showed no indications of adverse effects. However, detailed safety data for intravenous administration of SHs is currently unknown.

As with all antimicrobials, resistance is of great concern. Not surprisingly, the emergence of SH-resistant weeds is a significant problem in the agricultural community (29). In *P. aeruginosa*, *B. thailandensis*, and *B. subtilis*, we found that spontaneous mutants resistant to chlorimuron ethyl occurred at a frequency of approx-

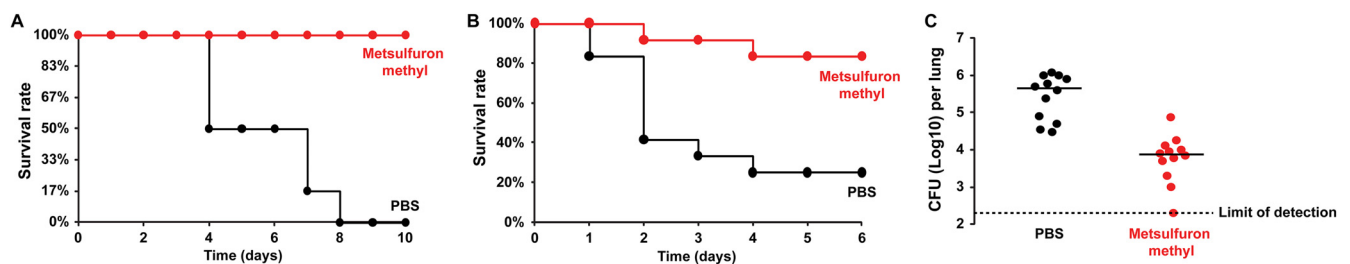


FIG 3 SHs increase mouse survival in response to an otherwise lethal challenge by *B. pseudomallei* or *P. aeruginosa*. (A) The survival curves of BALB/c mice ($n = 6$ per group) inoculated through the intranasal route with *B. pseudomallei* strain 22 (20) and orally treated twice daily with either PBS or metsulfuron methyl (50 mg/kg). Survival curves (B) or CFU (C) present in the lungs of FVB/N mice ($n = 12$ per group in both experiments) inoculated intratracheally with *P. aeruginosa* strain PAO1 and given a single tail vein injection with PBS or metsulfuron methyl (200 mg/kg).

imately 10^{-8} . Similar to plants (2, 29), a single mutation in the catalytic subunit of AHAS was sufficient to confer resistance to chlorimuron ethyl and other SHs. To counter the rapid emergence of single-step, high-level SH-resistant mutants, AHAS inhibitors could be used in combination with other antibiotics (30) or inhibitors of BCAA biosynthesis (31–33).

There are approximately 30 to 40 biosynthetic enzymes present in microbes but absent in humans that are responsible for the production of essential amino acids. Auxotrophs for some of these are attenuated (34–36), suggesting that these pathways might be fruitful targets for future small-molecule discovery.

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J.F.K. and P.T. have applied for a patent covering the medical use of sulfonylurea herbicides (Singapore patent application 201008793-0).

REFERENCES

- Boucher HW, Talbot GH, Bradley JS, Edwards JE, Gilbert D, Rice LB, Scheld M, Spellberg B, Bartlett J. 2009. Bad bugs, no drugs: no ESCAPE! An update from the Infectious Diseases Society of America. *Clin. Infect. Dis.* 48:1–12.
- Duggleby RG, McCourt JA, Guddat LW. 2008. Structure and mechanism of inhibition of plant acetohydroxyacid synthase. *Plant Physiol. Biochem.* 46:309–324.
- McCourt JA, Duggleby RG. 2006. Acetohydroxyacid synthase and its role in the biosynthetic pathway for branched-chain amino acids. *Amino Acids* 31:173–210.
- Atkins T, Prior RG, Mack K, Russell P, Nelson M, Oyston PC, Dougan G, Titball RW. 2002. A mutant of *Burkholderia pseudomallei*, auxotrophic in the branched chain amino acid biosynthetic pathway, is attenuated and protective in a murine model of melioidosis. *Infect. Immun.* 70:5290–5294.
- Cheng AC, Currie BJ. 2005. Melioidosis: epidemiology, pathophysiology, and management. *Clin. Microbiol. Rev.* 18:383–416.
- Hondalus MK, Bardarov S, Russell R, Chan J, Jacobs WR, Jr, Bloom BR. 2000. Attenuation of and protection induced by a leucine auxotroph of *Mycobacterium tuberculosis*. *Infect. Immun.* 68:2888–2898.
- Chaleff RS, Mauvais CJ. 1984. Acetolactate synthase is the site of action of two sulfonylurea herbicides in higher plants. *Science* 224:1443–1445.
- Ray TB. 1984. Site of action of chlorsulfuron: inhibition of valine and isoleucine biosynthesis in plants. *Plant Physiol.* 75:827–831.
- LaRossa RA, Schloss JV. 1984. The sulfonylurea herbicide sulfometuron methyl is an extremely potent and selective inhibitor of acetolactate synthase in *Salmonella Typhimurium*. *J. Biol. Chem.* 259:8753–8757.
- LaRossa RA, Smulski DR. 1984. *ilvB*-encoded acetolactate synthase is resistant to the herbicide sulfometuron methyl. *J. Bacteriol.* 160:391–394.
- Boldt TS, Jacobsen CS. 1998. Different toxic effects of the sulfonylurea herbicides metsulfuron methyl, chlorsulfuron and thifensulfuron methyl on fluorescent pseudomonads isolated from an agricultural soil. *FEMS Microbiol. Lett.* 161:29–35.
- Burnet M, Hodgson B. 1991. Differential effects of the sulfonylurea herbicides chlorsulfuron and sulfometuron methyl on microorganisms. *Arch. Microbiol.* 155:521–525.
- Nelson DR, Duxbury T. 2008. The distribution of acetohydroxyacid synthase in soil bacteria. *Antonie Van Leeuwenhoek* 93:123–132.
- El-Ghamry AM, Xu JM, Huang CY, Gan J. 2002. Microbial response to bensulfuron-methyl treatment in soil. *J. Agric. Food Chem.* 50:136–139.
- Gigliotti C, Allievi L. 2001. Differential effects of the herbicides bensulfuron and cinosulfuron on soil microorganisms. *J. Environ. Sci. Health B* 36:775–782.
- McCourt JA, Pang SS, King-Scott J, Guddat LW, Duggleby RG. 2006. Herbicide-binding sites revealed in the structure of plant acetohydroxyacid synthase. *Proc. Natl. Acad. Sci. U. S. A.* 103:569–573.
- Elbing K, Brent R. 2002. Media preparation and bacteriological tools. *Curr. Protoc. Mol. Biol.* doi:10.1002/0471142727.mb0101s59.
- Spizizen J. 1958. Transformation of biochemically deficient strains of *Bacillus subtilis* by deoxyribonucleate. *Proc. Natl. Acad. Sci. U. S. A.* 44:1072–1078.
- Nakano MM, Dailly YP, Zuber P, Clark DP. 1997. Characterization of anaerobic fermentative growth of *Bacillus subtilis*: identification of fermentation end products and genes required for growth. *J. Bacteriol.* 179:6749–6755.
- Tan GY, Liu Y, Sivalingam SP, Sim SH, Wang D, Paucod JC, Gauthier Y, Ooi EE. 2008. *Burkholderia pseudomallei* aerosol infection results in differential inflammatory responses in BALB/c and C57Bl/6 mice. *J. Med. Microbiol.* 57:508–515.
- Guilbault C, Martin P, Houle D, Boghdady ML, Guiot MC, Marion D, Radzioch D. 2005. Cystic fibrosis lung disease following infection with *Pseudomonas aeruginosa* in Cftr knockout mice using novel non-invasive direct pulmonary infection technique. *Lab. Anim.* 39:336–352.
- Bowen TL, Union J, Tumbula DL, Whitman WB. 1997. Cloning and phylogenetic analysis of the genes encoding acetohydroxyacid synthase from the archaeon *Methanococcus aeolicus*. *Gene* 188:77–84.
- Gollop N, Damri B, Chipman DM, Barak Z. 1990. Physiological implications of the substrate specificities of acetohydroxy acid synthases from varied organisms. *J. Bacteriol.* 172:3444–3449.
- Holden MT, Titball RW, Peacock SJ, Cerdeno-Tarraga AM, Atkins T, Crossman LC, Pitt T, Churcher C, Mungall K, Bentley SD, Sebahia M, Thomson NR, Bason N, Beacham IR, Brooks K, Brown KA, Brown NF, Challis GL, Cherevach I, Chillingworth T, Cronin A, Crossett B, Davis P, DeShazer D, Feltwell T, Fraser A, Hance Z, Hauser H, Holroyd S, Jagels K, Keith KE, Maddison M, Moule S, Price C, Quail MA, Rabbittowitsch E, Rutherford K, Sanders M, Simmonds M, Songsivilai S, Stevens K, Tumapa S, Vesaratchavest M, Whitehead S, Yeats C, Barrell BG, Oyston PC, Parkhill J. 2004. Genomic plasticity of the causative agent of melioidosis, *Burkholderia pseudomallei*. *Proc. Natl. Acad. Sci. U. S. A.* 101:14240–14245.
- Page MG, Heim J. 2009. Prospects for the next anti-*Pseudomonas* drug. *Curr. Opin. Pharmacol.* 9:558–565.
- Galyov EE, Brett PJ, DeShazer D. 2010. Molecular insights into *Burkholderia pseudomallei* and *Burkholderia mallei* pathogenesis. *Annu. Rev. Microbiol.* 64:495–517.
- Dijkshoorn L, Nemeč A, Seifert H. 2007. An increasing threat in hospitals: multidrug-resistant *Acinetobacter baumannii*. *Nat. Rev. Microbiol.* 5:939–951.
- Healy CE, Heydens WF, Naylor MW. 2004. Mammalian toxicology overview and human risk assessment for sulfosulfuron. *Regul. Toxicol. Pharmacol.* 39:310–324.
- Tranel PJ, Wright TR. 2002. Resistance of weeds to ALS-inhibiting herbicides: what have we learned? *Weed Sci.* 50:700–712.
- Fischbach MA. Combination therapies for combating antimicrobial resistance. *Curr. Opin. Microbiol.* 14:519–523.
- Epelbaum S, Chipman DM, Barak Z. 1996. Metabolic effects of inhibitors of two enzymes of the branched-chain amino acid pathway in *Salmonella Typhimurium*. *J. Bacteriol.* 178:1187–1196.
- Grandoni JA, Marta PT, Schloss JV. 1998. Inhibitors of branched-chain amino acid biosynthesis as potential antituberculosis agents. *J. Antimicrob. Chemother.* 42:475–482.
- Lee YT, Ta HT, Duggleby RG. 2005. Cyclopropane-1,1-dicarboxylate is a slow-, tight-binding inhibitor of rice ketol-acid reductoisomerase. *Plant Sci.* 168:1035–1040.
- Cuccui J, Easton A, Chu KK, Bancroft GJ, Oyston PC, Titball RW, Wren BW. 2007. Development of signature-tagged mutagenesis in *Burkholderia pseudomallei* to identify genes important in survival and pathogenesis. *Infect. Immun.* 75:1186–1195.
- Hoiseith SK, Stocker BA. 1981. Aromatic-dependent *Salmonella Typhimurium* are non-virulent and effective as live vaccines. *Nature* 291:238–239.
- Pavelka MS, Jr, Chen B, Kelley CL, Collins FM, Jacobs WR, Jr. 2003. Vaccine efficacy of a lysine auxotroph of *Mycobacterium tuberculosis*. *Infect. Immun.* 71:4190–4192.
- Fournier PE, Vallenet D, Barbe V, Audic S, Ogata H, Poirel L, Richet H, Robert C, Mangenot S, Aberger C, Nordmann P, Weissenbach J, Raoult D, Claverie JM. 2006. Comparative genomics of multidrug resistance in *Acinetobacter baumannii*. *PLoS Genet.* 2:e7. doi:10.1371/journal.pgen.0020007.
- Smith MG, Gianoulis TA, Pukatzki S, Mekalanos JJ, Ornston LN, Gerstein M, Snyder M. 2007. New insights into *Acinetobacter baumannii* pathogenesis revealed by high-density pyrosequencing and transposon mutagenesis. *Genes Dev.* 21:601–614.