Supplementary data

Supplementary methods

Growth Curve

SM K279a inoculated into 5 mL of LB was grown overnight to stationary phase (14-16 hrs) at 37°C in a shaking incubator. The following day bacteria were washed twice with PBS and re-suspended in tubes containing 25 mL of CA-MHB or RPMI+10%LB to an initial OD_{600} of 0.05. Tubes were then subsequently placed in a shaking incubator at 37°C with re-growth assessed by measuring OD_{600} at selected time intervals up to 24h.

Electron Microscopy

Electron microscopy was performed to assess the effect of SM K279a (extracellularly and intracellularly) in tissue culture media. SM K279a was grown overnight to stationary phase (14-16 hrs) in 5mL of LB at 37°C in a shaking incubator. Bacteria were then washed twice with PBS and re-suspended in RPMI+10% LB prior to being placed in a shaking incubator at 37°C for 2 h. Next cultures were centrifuged at 4000 rpm for 10 min at room temperature. The supernatant was then aspirated and the bacterial pellets re-suspended in modified Karnoversusky's fixative (2.5% glutaraldehyde and 2% paraformaldehyde in 0.15M sodium cacodylate buffer). These samples were then processed and electron microscopy was performed as previously described.[1] Samples were viewed using a Tecnai G2 Spirit BioTWIN transmission electron microscope and photographed with an Eagle 4k HS digital camera. Images were obtained from random fields at 1900X, 9300X and 13000X.

Neutrophil Extracellular Trap & Oxidative Burst Assays

Neutrophil extracellular trap (NET) induction assays and oxidative burst assays were performed as previously described.[2, 3]

References

1. Sato T. A modified method for lead staining of thin sections. J Electron Microsc (Tokyo) 1968; **17**:158-9.

2. Dohrmann S, Anik S, Olson J, et al. Role for streptococcal collagen-like protein 1 in M1T1 group A *Streptococcus* resistance to neutrophil extracellular traps. *Infect Immun* 2014; **82**:4011-20.

3. Okumura CY, Anderson EL, Dohrmann S, et al. IgG protease Mac/IdeS is not essential for phagocyte resistance or mouse virulence of M1T1 group A *Streptococcus*. *mBio* 2013; **4**: e00499-13.

Figure S1. Growth of SM K279a in bacteriologic (CA-MHB) versus supplemented tissue culture (RPMI+10%LB) media. Turbidity as a measure of bacterial growth was determined by OD_{600} at 1, 2, 4, 8 and 24h. Data are plotted as mean ± SEM and represent the combination of 3 experiments. Statistical analysis by unpaired student's t-test revealed no statistical significance (ns).

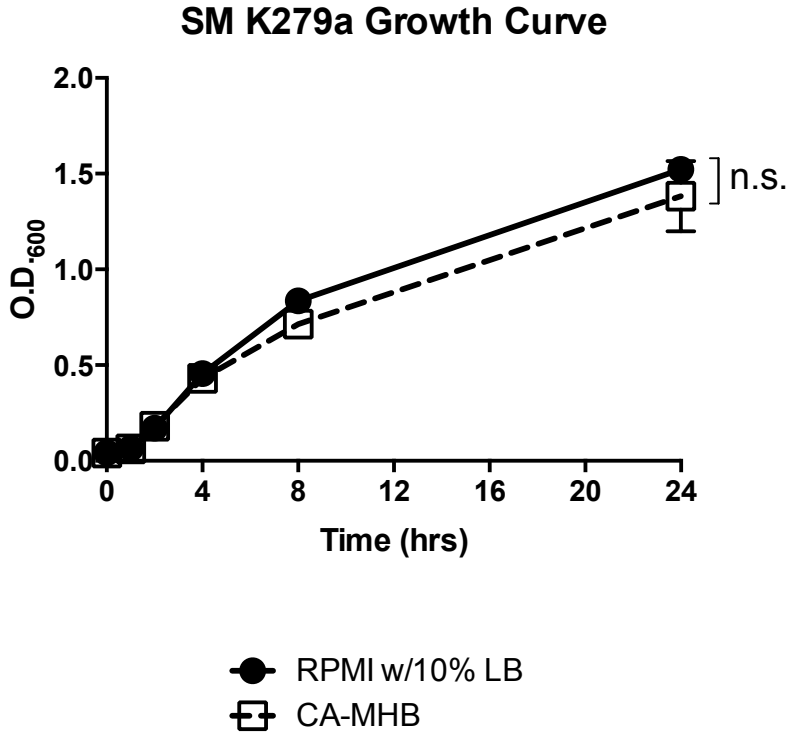

Figure S2. Transmission electron microscopy images (1900X, 9300X and 13000X) of stationary phase SM K279a treated for 2h with AZM 0.25 mg/L. Images reveal ribosomal clustering (indicated by arrow) in bacteria treated with AZM, a protein synthesis inhibitor known to inhibit the translation of mRNA by binding to the 50S subunit of the bacterial ribosome. Images were taken from multiple random fields (>5) and analyzed in a blinded fashion.

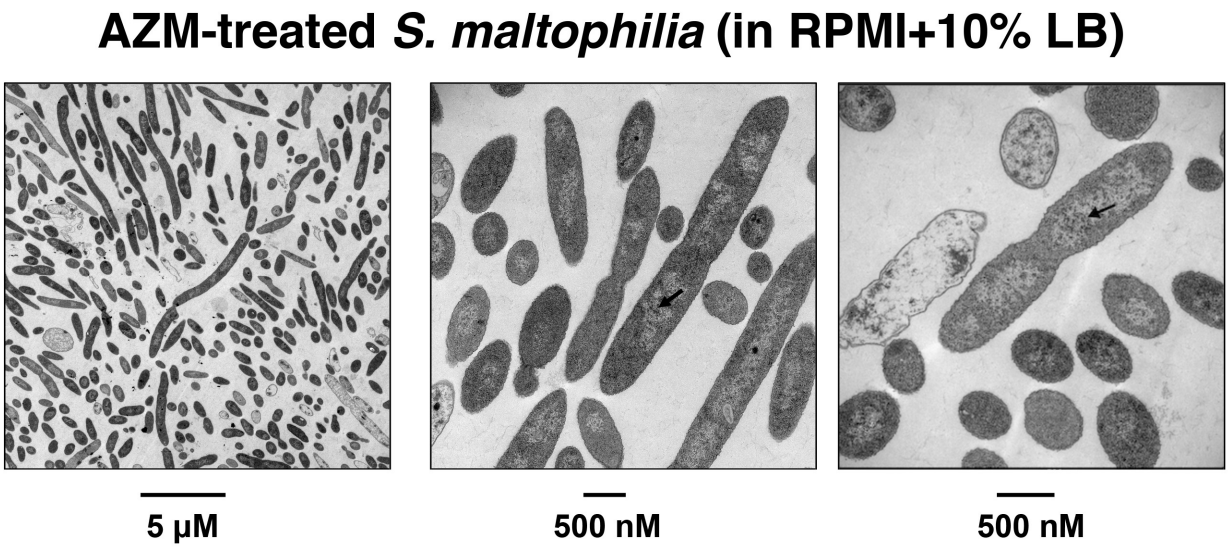

Figure S3. Neutrophil production of reactive oxygen species (ROS) and extracellular traps (NETs) in response to SM. *A,* ROS production induced by SM K279a at an MOI = 10 in comparison to the potent neutrophil agonist PMA. *B,* SM K279a induces NET formation from human neutrophils at an MOI = 10. Data represents the mean ± SEM of 3 experiments performed in triplicate. **P* < 0.05, ***P* < 0.01, *** *P* < 0.001 by two-way ANOVA.

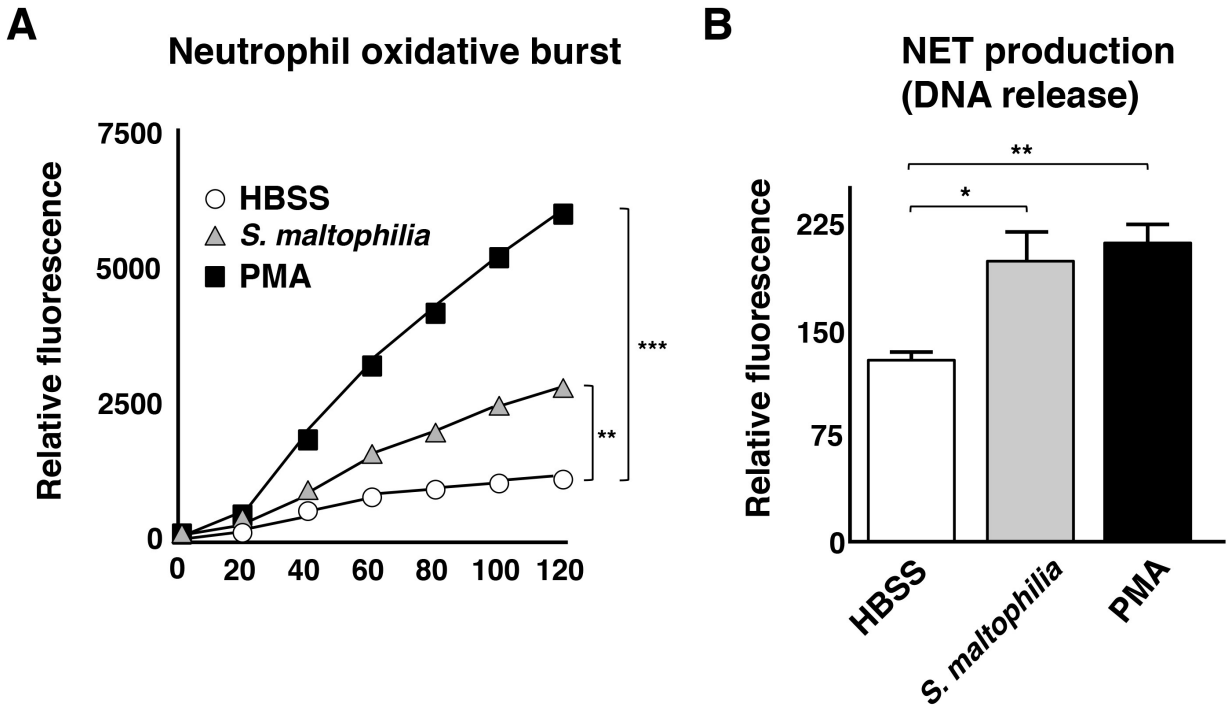

Figure S4. Murine lung infection model. *A*, Histological grading. A/J mice were infected with 2x107 cfu of SM K279a or PBS intratracheally and treated with AZM (50 mg/kg) or PBS daily for 48h prior to harvesting lungs; n = 4 for control (PBS/PBS), PBS (SM K279a/PBS) and AZM (SM K279a/AZM). *B*, ELISA detection of inflammatory cytokines (IL-1β and MIP-2) from BALF; n = 8 for control (PBS/PBS), PBS (SM K279a/PBS) and AZM (SM K279a/AZM). Results expressed as mean ± SEM. Statistical analysis by unpaired student's t-test revealed no statistical significance (ns).

A

Murine lung infection model
***S. maltophilia* (2 x 10^7 CFU), 48 h**

Group	Intra-alveolar edema/debris	Intra-alveolar macrophages	Perivascular/ peribronchial inflammation	Intra-alveolar inflammation	Overall % airspace involvement
Uninfected	1.00 ± 0.00	1.25 ± 0.25	0.25 ± 0.25	1.00 ± 0.00	1.00 ± 0.00
PBS	1.75 ± 0.25	2.75 ± 0.25	2.25 ± 0.25	3.00 ± 0.00	22.50 ± 0.00
AZM	2.50 ± 0.30	2.75 ± 0.25	2.50 ± 0.30	3.00 ± 0.00	17.50 ± 2.50

B