Ami Slic Mod 1.63 [PORTABLE]

to determine if slic is expressed and secreted, we examined wt fa1090 and slic::kan for slic abundance during co-culture with host cells by western blot analysis. for these studies, wt fa1090 and slic::kan were grown in bhi media and pelleted and washed with pbs. the cell pellet was resuspended in pbs and transferred to a 24-well plate containing a confluent monolayer of hela cells (atcc ccl-2). bacterial cell pellets were added to the hela monolayer at a ratio of approximately 50,000 bacteria per hela cell. inocula were allowed to interact for 4 hours. after the interaction period, the plate was centrifuged at 500 xg for 5 min at 4c. the supernatant was collected and the pellet was washed twice with pbs. the supernatant and pellets were separated by sds-page and probed with anti-slic antisera. these studies showed that slic was readily produced and secreted from wt fa1090 (fig 6b). in contrast, slic was not detected in supernatants from slic::kan (fig 6c). this result was confirmed using an anti-daga antiserum (fig 6d). to determine if slic is a surface associated protein, wt fa1090 and slic::kan were grown in bhi media and pelleted at 500 xg for 5 min at 4c. pellets were washed with pbs, resuspended in pbs, and transferred to a 24-well plate (falcon) containing confluent monolayers of hela cells (atcc ccl-2). bacterial cell pellets were added to the hela monolayer at a ratio of approximately 50,000 bacteria per hela cell. following interaction, the 24-well plate was centrifuged at 500 xg for 5 min at 4c. the

supernatant was collected and the pellet was washed twice with pbs. the supernatant and pellets were separated by sds-page and probed with anti-slic antisera. this analysis showed that slic was readily produced and secreted into the medium from fa1090.

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n. gonorrhoeae possesses two lytic transglycosylases, acp and lipolytic transglycosylases, and one lytic transglycosylase, lytic transglycosylases, and one lytic transglycosylases, which are distinguished by their substrate specificity. these enzymes are encoded by the cog1295, ngo1063, cog3895, ngo1054, cog3895, and ngo1054 genes, respectively (table s1). the ngo1063 mutant had a significant decrease in vaginal cfus when assessed in parallel to the wild type strain on days 1, 3, and 5 post-infection in three independent coinfection experiments (fig 7b7d, respectively). the slic mutant was approximately 2-, 58-, and 34-fold lower than the wild type strain on days 1, 3, and 5 post-inoculation, respectively (fig 7b7d). we next examined the impact of mutations in slic on the ability of n. gonorrhoeae to resist lysis by lysozyme. the slic* mutant was notably more sensitive to lysozyme than the wild type or complemented strains (fig 5a). the relative permeability of the slic* mutant was also examined in the presence of heparin, a chemical chaperone which decreases lysozyme activity and renders the bacteria more resistant to lysozyme [4, 5, 10]. n. gonorrhoeae were treated with lysozyme in the presence or absence of heparin. bacteria treated in the absence of heparin were extensively lysed and had a permeability ratio of approximately 20, whereas bacteria treated with heparin alone were significantly more resistant to lysis (fig 5b and 5c). subsequently, n. gonorrhoeae were exposed to lysozyme in the presence or absence of heparin. prior treatment of the bacteria with heparin increased the resistance of the slic* mutant to lysozyme lysis by approximately 6-fold (fig 5b and 5c). this result suggested that slic may be acting as an inhibitor of lysozyme activity. to test this hypothesis, we examined the activity of lysozyme in the presence of slic. we added slic to purified lysozyme and used a fluorogenic substrate that emits at 485 nm upon cleavage. we observed a dose-

dependent inhibition of lysozyme by slic, with half-maximal inhibition at concentrations between 0.5 and 1 μm (fig 5d). the slopes of the dose-response curves were similar between wild type and slic* and the ic50 (concentration of slic required for 50% inhibition of lysozyme activity) for the wild type and mutant proteins were 0.8 and 0.2 μm, respectively. this suggests that slic may act as an inhibitor of lysozyme activity in a concentration-dependent manner. 5ec8ef588b