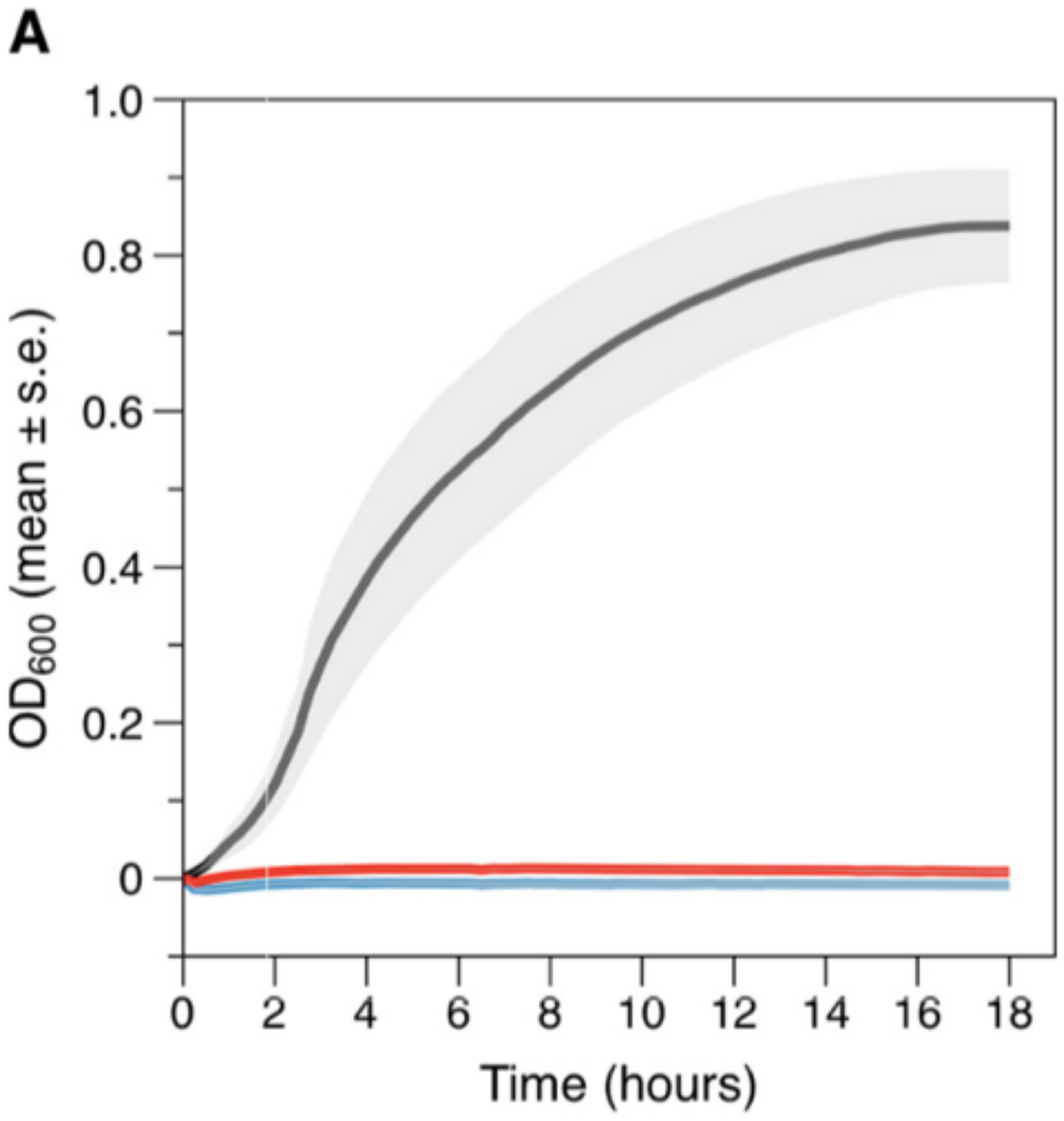

FIG 2 Bald's eyesalve kills *S. aureus* in planktonic culture and in a synthetic wound biofilm model. (A) One hundred microliters of sterile distilled water (black line), the onion variant of the eyesalve (ES-O, red line), or the leek variant of the eyesalve (ES-L, blue line) (both from batch A) was added to a 200-μl mid-log-phase culture (10^4 to 10^5 cells) of *S. aureus* in synthetic wound fluid (SWF), and the optical density of the culture was measured during 18 h of incubation at 37°C. The mean results from four replica populations and the associated standard errors (shaded intervals; too small to see for ES-O and ES-L) are shown. (B) Two hundred microliters of ES-O or ES-L (batch A, filled circles, and batch B, open circles) or of each individual ingredient preparation was added to five 1-day-old cultures of *S. aureus* growing at 37°C in a synthetic wound (400-μl synthetic wound fluid rendered semisolid by adding 2 mg·ml^{-1} collagen). After 24 h of further incubation, the collagen was dissolved to recover cells for agar plate counts. The control treatment was sterile distilled water left to stand for 9 days in the presence of brass, which was also present in all other preparations, to simulate the presence of a copper alloy vessel (see Materials and Methods). Asterisks denote treatments whose results were significantly different from those of the control.

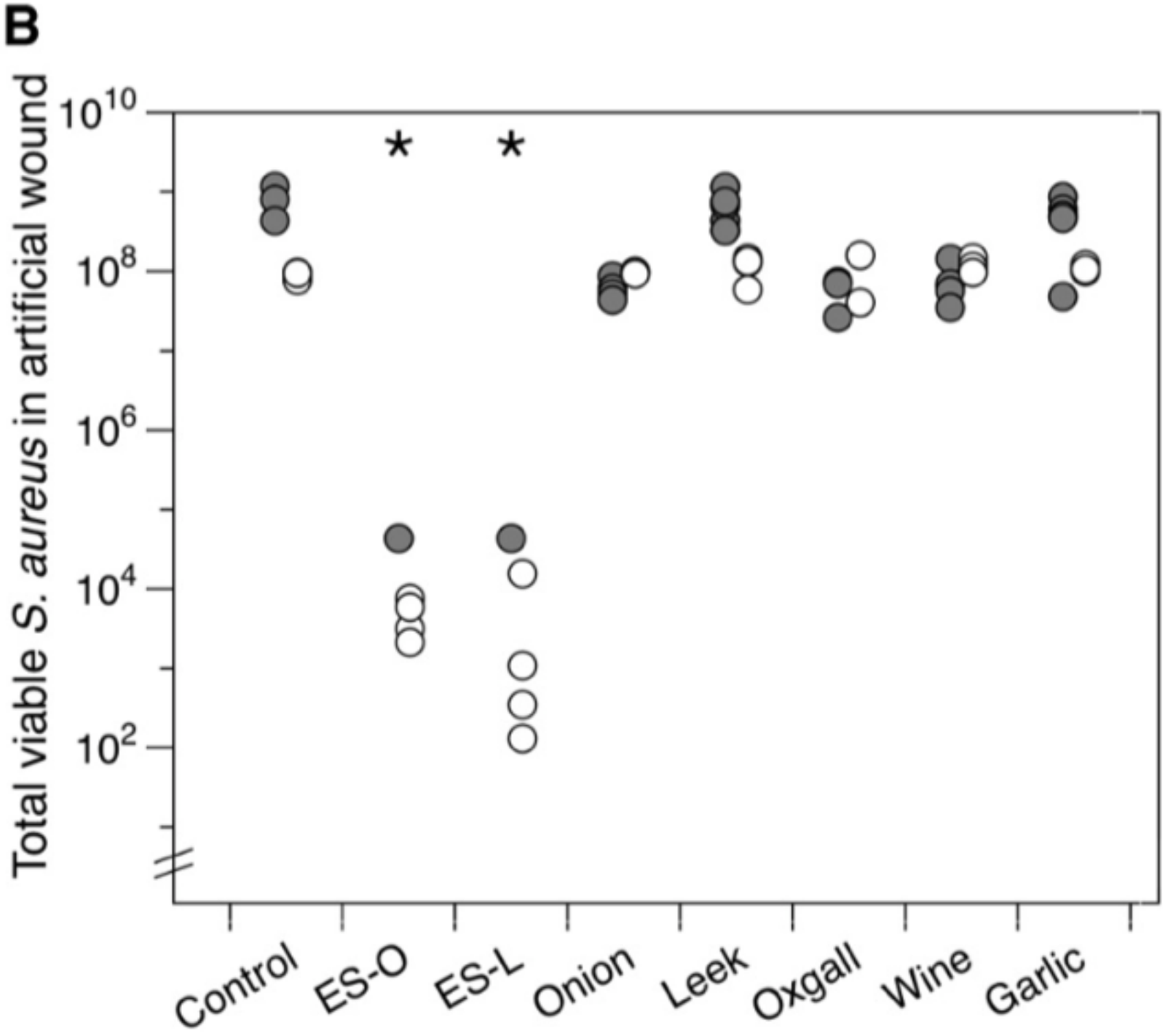

FIG 2 Bald's eyesalve kills *S. aureus* in planktonic culture and in a synthetic wound biofilm model. (A) One hundred microliters of sterile distilled water (black line), the onion variant of the eyesalve (ES-O, red line), or the leek variant of the eyesalve (ES-L, blue line) (both from batch A) was added to a 200-μl mid-log-phase culture (10^4 to 10^5 cells) of *S. aureus* in synthetic wound fluid (SWF), and the optical density of the culture was measured during 18 h of incubation at 37°C. The mean results from four replica populations and the associated standard errors (shaded intervals; too small to see for ES-O and ES-L) are shown. (B) Two hundred microliters of ES-O or ES-L (batch A, filled circles, and batch B, open circles) or of each individual ingredient preparation was added to five 1-day-old cultures of *S. aureus* growing at 37°C in a synthetic wound (400-μl synthetic wound fluid rendered semisolid by adding 2 mg·ml^{-1} collagen). After 24 h of further incubation, the collagen was dissolved to recover cells for agar plate counts. The control treatment was sterile distilled water left to stand for 9 days in the presence of brass, which was also present in all other preparations, to simulate the presence of a copper alloy vessel (see Materials and Methods). Asterisks denote treatments whose results were significantly different from those of the control.

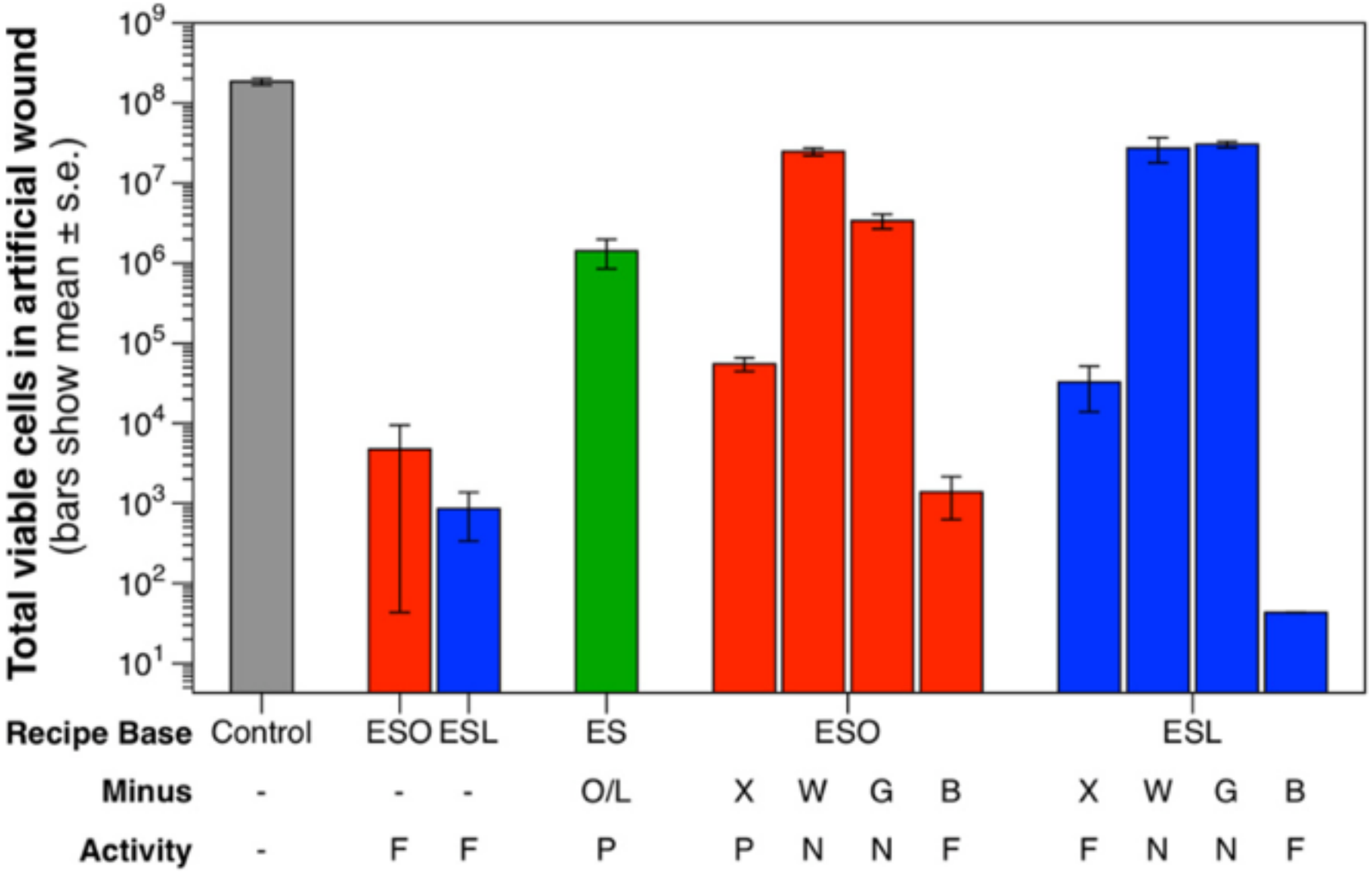

FIG 3 The activity of Bald's eyesalve against *S. aureus* biofilms requires several ingredients. Two hundred microliters of the onion variant of the eyesalve (ES-O), the leek variant of the eyesalve (ES-L) (batch C), or preparations missing a single ingredient were added to three 1-day-old cultures of *S. aureus* in synthetic wounds, and bactericidal activity quantified as described in Fig. 2. Red bars show the results for the full or reduced versions of ES-O, blue bars show the results for the full or reduced versions of ES-L, and the green bar shows the result for a variant of the eyesalve with neither onion nor leek (ES). The control treatment (grey bar) was sterile distilled water. Individual ingredients dropped out of the recipe (Minus) are coded as follows: O, onion; L, leek; X, oxgall; W, wine; G, garlic; B, brass. Reduced recipes were assessed as having full (F), partial (P), or no (N) activity in comparison with the results for the control treatment and the appropriate complete recipe.

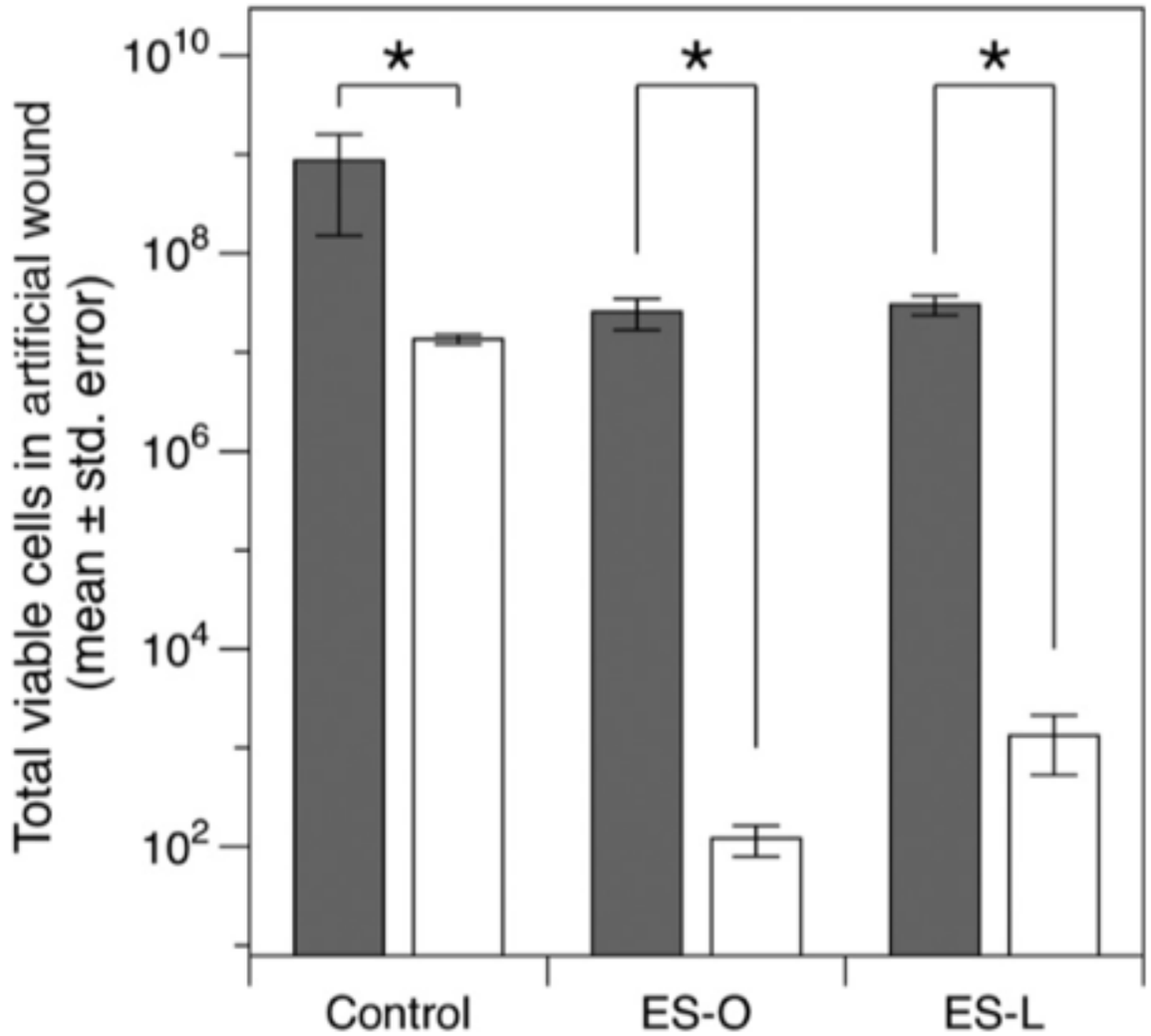

FIG 4 The activity of Bald's eyesalve against *S. aureus* biofilms requires the 9-day waiting period specified by the recipe. Immediately after preparation, 200 μl of the onion variant of the eyesalve (ES-O), the leek variant of the eyesalve (ES-L) (batch D), or sterile distilled water was added to five 1-day-old cultures of *S. aureus* growing at 37°C in synthetic wounds, and the bactericidal activity quantified as previously described (filled bars); the experiment was repeated using ES-O and ES-L (batch D) after the 9-day waiting period specified by Bald (open bars). Asterisks denote treatments whose results were significantly different from those of the control. While the control cultures for the 9-day experiment grew to slightly lower densities than the control for the fresh eyesalve ($P = 0.005$), this difference was small compared with the differences observed for ES-O and ES-L (1 to 2 log versus 4 to 5 log difference).

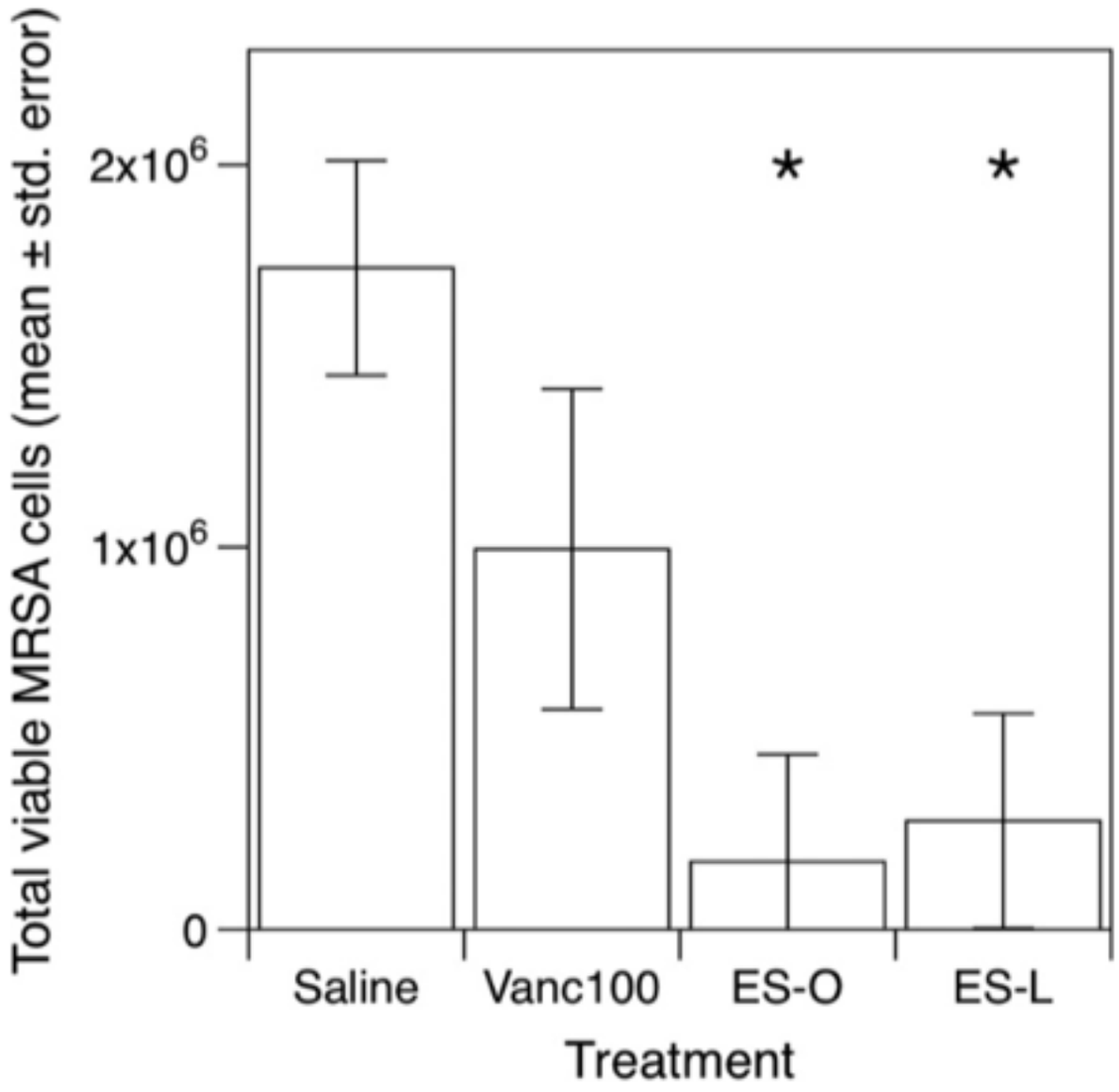

FIG 5 Bactericidal activity of Bald's eyesalve in a mouse chronic wound model of MRSA infection. Six adult female Swiss-Webster mice were administered wounds and infected with ca. 10^5 CFU *S. aureus* Mu50. Four days postinfection, mice were euthanized. Wound tissue was excised and cut into either three ($n = 3$ mice) or four ($n = 3$ mice) equal pieces, which were weighed, submerged in 300 μl sterile saline (one replicate from $n = 6$ mice), 100 $\mu g \cdot ml^{-1}$ vancomycin (one replicate from $n = 3$ mice), the onion variant of the eyesalve (ES-O; one replicate from $n = 6$ mice), or the leek variant of the eyesalve (ES-L; one replicate from $n = 6$ mice) (both ES-O and ES-L were from batch B) for 4 h, and then rinsed in sterile saline and homogenized. Viable bacteria were enumerated, and the counts standardized per gram of tissue. Asterisks denote treatments whose results were significantly different from those of the control.