Sphingolipids are essential components produced by most eukaryotes, playing significant roles in cellular process such as cell growth, programmed cell death, angiogenesis, and inflammation. Despite the previous belief that sphingolipids were uncommon in bacteria, recent bioinformatic analysis of identified bacterial synthesis genes suggest a broader production among microbial species. The sphingolipid synthesis pathway involves three key enzymes: serine palmitoyl transferase, which catalyzes the condensation of serine with palmitoyl-CoA; ceramide synthase, responsible for adding the second acyl chain; and a reductase, which reduces the ketone on the long-chain base. While the identity of these bacterial enzymes is generally agreed upon, the precise mechanism and order of chemical reactions for microbial sphingolipid synthesis remains quite unclear, two proposed mechanisms include following the well-characterized eukaryotic pathway, where the long-chain base is reduced before the addition of the second acyl chain, or an alternative model where the second acyl chain is added before the reduction of the long-chain base. To distinguish between these models, we investigated the subcellular localization of the three key enzymes. Our findings reveal that serine palmitoyl transferase and ceramide synthase are located in the cytoplasm, while ceramide reductase is found in the periplasmic space. This supports our previous model, suggesting that the second acyl chain is added in the cytoplasm before export to the periplasm, where the lipid molecule undergoes reduction.