Abstract: Objective: Over the last decade, a dramatic increase in the prevalence of antibiotic resistance in several medically significant bacterial species has been seen. There is an urgent need to develop novel antibacterial agents to eliminate multidrug-resistant bacteria. A very interesting class of novel antibacterials are enzybiotics consisting of lysins, bacteriocins, autolysins, and lysozymes. Bacteriocins are peptides or proteins produced by bacteria to inhibit the growth of other bacteria. The bacteriocin whose antibacterial activity has been studied most thoroughly is Lysostaphin, an endopeptidase encoded by Staphylococcus simulans that specifically cleaves glycyl-glycyl bonds in the interpeptide cross-bridges of the staphylococcal peptidoglycan and is very efficient in lysing S. aureus and can kill practically all strains of this species, including MRSA, NRSA and strains with reduced susceptibility to vancomycin, also it has other medical applications such as: elimination of staphylococci colonizing nasal mucous membrane, prevention of catheter colonization by enzyme molecules coating their surface, and the treatment of staphylococcal infections. Since further evaluation of the anti-staphylococcal potential of lysostaphin as a therapeutic agent depends on the availability of large amounts of a highly purified protein from a safe-nonpathogenic source, we can construct a recombinant plasmid which overproduces mature lysostaphin in the cytoplasm of E.coli cells transformed with the recombinant plasmid. Conclusion: The cloning, expression, and purification procedure of recombinant lysostaphin from a non-pathogenic organism; E.coli; enables preparation of large quantity of r-lysostaphin for structure-function studies and evaluation of its clinical potential in therapy and prophylaxis of staphylococcal infections.