Members of phylum actinomycetes, widely distributed in diverse habitats, are the promising source of antibiotics. In the present study, 102 actinomycete strains were isolated from 20 soil samples which had been collected from Skardu, Kachura, Deosai, Sialkot, Sahiwal, Shiekhupura, Lahore and Gojra, Pakistan. Fifty-three of these isolates, having antimicrobial potential, were subjected to secondary screening against *Bacillus subtilis* BO1, *Escherichia coli* and *Aspergillus japonicas*. Microscopic morphology of selected actinomycete strain was studied using inclined cover slip, slide culture method and gram staining. Optimum temperature, pH and salt tolerance were studied for physiological studies. In addition, biochemical characterization was carried out by Acid-fast staining, Catalase test, Oxygen requirement test, Motility test, carbon utilization test, Urease test, citrate utilization test, Starch hydrolysis test and Lipolytic test. Yield improvement was carried out by optimizing fermentation conditions which included incubation time, fermentation media, incubation temperature, initial pH and inoculum size. Secondary screening results revealed that 22 actinomycete isolates showed zone of inhibition against *Aspergillus japonicas* and 25 actinomycete strains showed antibacterial activity against *Bacillus subtilis* BO1 including 6 strains which were found active against both. In addition, 9 actinomycete strains exhibited zone of inhibition against both gram positive (*Bacillus subtilis BO1*) and gram negative (*Escherichia coli*) test bacterial strains. Actinomycete strain A22 was selected for its significant antibacterial activity i.e. 12mm ± 0.1 against *Bacillus subtilis BO1* and 8mm ± 0.2 against *Escherichia coli*. Optimization studies increased antibacterial activity of actinomycete strain A22 to 30mm ± 0.3 against *Bacillus subtilis* BO1 and 20mm ± 0.3 against *Escherichia coli*. Dry cell weight of A22 actinomycete strain was increased to 58mg ± 0.4 per ml, as a result of optimization studies. Actinomycete strain A22 had shown inhibitory activity against antibiotic sensitive pathogenic strains i.e. 10mm ± 0.2 zone of inhibition against *Staphylococcus aureus*, 8mm ± 0.3 against *Escherichia coli* and 6mm ± 0.3 against *Pseudomonas aeruginosa*. It was concluded from the present study that Pakistani soil had diverse actinomycete isolates possessing bioactive metabolite production potential with strong antimicrobial activity.