

## **A New Tool for Isolating Novel Uncultured Microorganisms: Adding the Various Cell-free Extracts to Culture Media**

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Easily isolated microorganisms by virtue of their ability to grow rapidly into colonies on high-nutrient artificial growth media, typically under aerobic conditions at moderate temperatures are the "weeds" of the microbial world and are estimated to constitute less than 1% of all microbial species. Recently, we have found the presence and abundance of thermophilic symbiotic microorganisms that grow slowly in nitrate-reducing conditions. Based on this finding, we have tried to invent a new method for isolating novel uncultured microorganisms by adding the cell extract to culture media. We employed the nitrate-reducing and thermophilic (60 °C) condition as a selective pressure. Several micro-colonial (20-50 mm in diameter) thermophiles were isolated from the compost and soil after 2-4 days incubation by adding the cell-free extract of *Geobacillus*. The isolated micro-colonial thermophilic microorganisms were represented as novel strains from the phylogenetic analysis with 16S rDNA sequence. If the cell-free extract treated with proteinase K, was added to media, the isolated strains could not be grown, indicating that certain of proteins in the extract played an important role as symbiotic factors. As a conclusion, the screening strategy by adding the various cell-free extracts to culture medium was thus demonstrated to be effective for the isolation of novel thermophilic microorganisms. [This work was supported by National Research Laboratory (NRL) program grant NLM002000123 from the Ministry of Science and Technology of Korea.]