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# Preserving Viability and Stabilizing Properties of Rhodococcus Biodegraders of Pharma Pollutants

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Methods of short- and long-term storage of Rhodococcus biodegraders of NSAID-based pharma pollutants (diclofenac, drotaverine, ibuprofen, ketoprofen, meloxicam, and naproxen) maintained in the Regional Specialized Collection of Alkanotrophic Microorganisms (acronym IEGM, WFCC # 285, http://www.iegmcol.ru) were developed. For preserving live cultures, subculturing from minimal agar, water and 0.5% NaCl was most appropriate and guaranteed 30–74% survival rates and maintenance of cell metabolic activities for 2–10 years. Preservation of non-dividing vegetative Rhodococcus cells on membrane filters applied to the surface of nutrient agar or mineral agar with n-hexadecane and followed by removal of filters with grown cells and storage in sealed sterile test tubes at 4 °C guaranteed survival and unchanged phenotypic properties of strains within 2–3 years. The most reliable methods of long-term storage were lyophilization and low temperature freezing. To increase viability of cells during storage and at the rehydration-reactivation step, rhodococci at the concentration of 10^8–10^9 cells/mL with induced alkanotrophic metabolism and upon transition to the stationary phase were used. Transiting cells realized “self-preservation” processes, such as the formation of capsule-like structures, cyst-like cells and carotenoid pigments, synthesis of protective compounds (trehalose, biosurfactants, and amino acids), cell aggregation and immobilization. A protective mechanism of alkanotrophy was related with endogenous respiration, accumulation of poly-β-oxybutyrate granules, increased amounts of odd-numbered fatty acids, and enhanced synthesis of biosurfactants and amino acids. It was advisable to pre-grow rhodococci on liquid n-alkanes rather than gaseous ones for a higher proportion of unsaturated fatty acids. To prevent their oxidation, 1 mM α-tocopherol acetate was added to the cell suspension before storage. To store frozen Rhodococcus cells, they were immobilized in the growth medium on paper disks, dried at 28 °C, frozen and stored at -85 °C. To cryopreserve Rhodococcus cell suspensions at lower (4.3×10^7 cells/mL) concentration, 5% dimethyl sulfoxide and 10% glycerol were added. However, for certain strains, cryopreservation without a protective agent was more effective. The approximate time for Rhodococcus spp. to preserve viability in the lyophilized state was estimated at 5.9−43.9 years. According to the control testing the 64 lyophilized Rhodococcus cultures stored for 15 years, 73% were successfully recovered and retained cell integrity, main morphological and cultural properties. Low temperature freezing provided 45−94% viability of rhodococci by the end of the first storage year and resulted in the average survival rate of 77%. The study was fulfilled under the State Assignment (AAAA-A19-119112290008-4) and the Russian Science Foundation grant (21-14-00132).