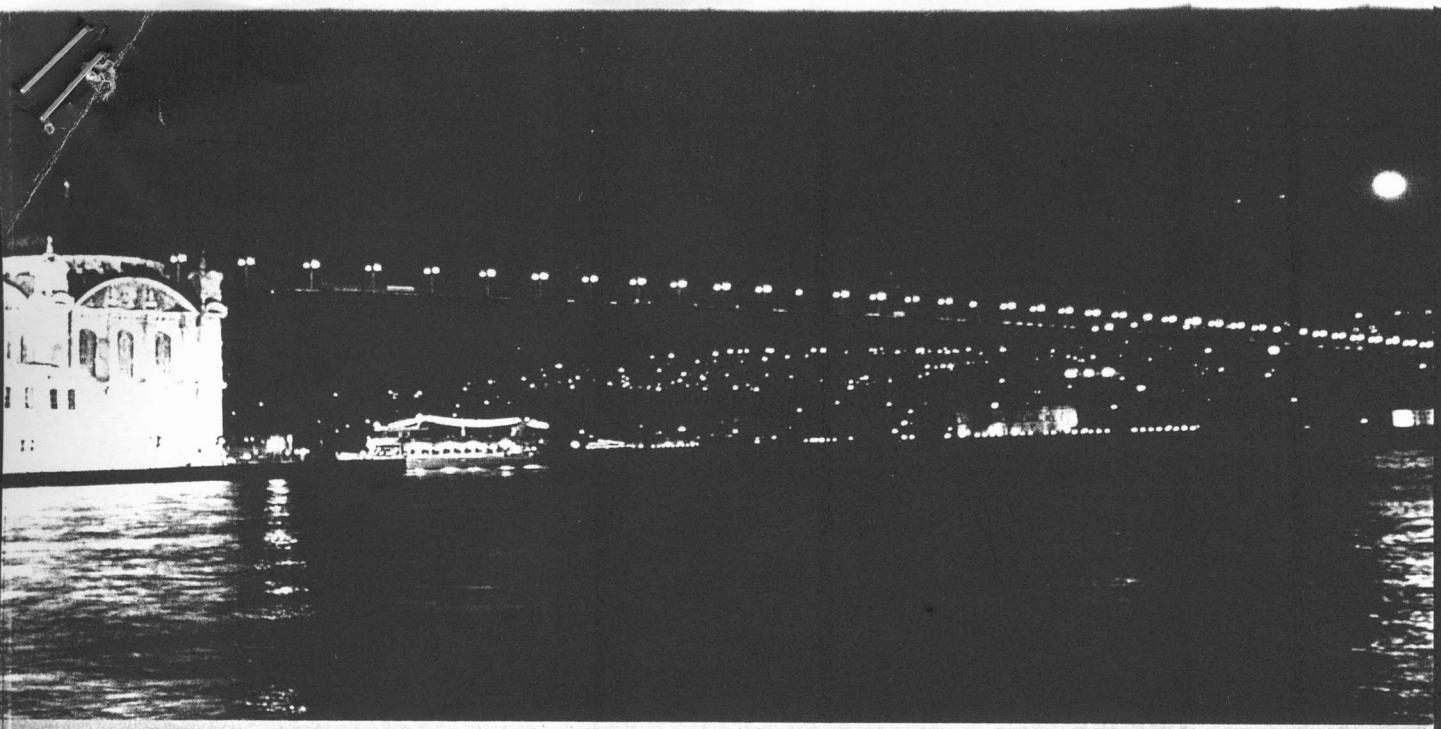

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CLONING OF SACCHAROMYCES CEREVISIAE MnSOD IN *ESCHERICHIA COLI*

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There is increasing interest for enzimic antioxidants for appling in various field expecially in food production. For instance, in fried products the proposed route for acryl amide formation via acrolein formed from lipids should also be considered. The relatively high temperatures combined with low water activity favourabl for the acrylamide formation are also in favour for free radical reactions. Antioxidants and other free radical scavengers or quenchers could act as inhibitors. For a massive application MnSOD should be very interesting due to relative termostability and four subunit composition. In most animal tissues and yeast MnSOD is almost entirely located in mitochondria so that purification and extraction of considable amount for industrial application is very difficult. So we decided to clone Saccharomyces cerevisiae MnSOD in E. coli and examine conditions for production of yeast MnSOD in significant amount.

We isolated DNA from Saccharomyces cerevisiae ("Fermin" – Senta). Then we created primers for both sides of MnSOD gene, and using PCR techniques we multiplied this gene. We ligate gene into the plasmide "pRSet B", which is the plasmide with several different restricted sites and gene for ampiciline insensitivity. Such ligated plasmide we transformed to Escherichia coli. After E. coli growth on LB medium with ampiciline we evidenced the presence of MnSOD gene in the E. coli plasmides by "Mini-prep" techniques and compared the presence of MnSOD protein in E. coli treaded with plasmides and those which are not. In this presentation characteristics of E. coli with expressed yeast MnSOD and capabilities for production of considerable amount of MnSOD are discussed.