E325 A Convenient Way of Fractionation of Mitochondria from the Somatic Hyphae of a Basidiomycete Fungus

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We developed a simpler way of fractionating mitochondria from the somatic hyphae of *Pleurotus ostreatus*, a basidiomycete fungus. Shaken cultured hyphae were broken with a bead-beater in ice cold 1M sorbitol in Tris-Mes buffer (pH 7.2). Homogenate was centrifuged briefly at the lower speed (x600g) to remove bulky materials, then filtered through a couple of layers of glass fiber followed by the glass filters to remove unbroken hyphae, centrifuged down at xxxg for 15 mins to obtain a pellet containing most mitochondria. The pellet was resuspended in 1M sorbitol and applied to the top of the discontinuous sucrose gradient in order to retrieve these organelles in a pure fraction. Sucrose gradient centrifugation results in two distinct bands: one at the interphase of 1.2 and 1.6M, another at the interphase of 1.6M and 2.0M. Measurement of the carbamyl phosphate transferase activity of these separate bands was conducted to identify the fraction containing mitochondria. Results of measurement showed that the band positioned at the interphase of 1.6 and 2.0M sucrose contains the most activity of carbamyl phosphate transferase indicating that this band is the major mitochondrial fraction. Along with these results, the details of experimental procedures described.

E326 FMN AND ITS ANALOGS INHIBIT THE SPLICING OF T4 PHAGE THYMIDYLATE SYNTHASE INTRON RNA

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Effects of FMN and its analogs on the self-splicing of phage T4 thymidylate synthase intron RNA have been examined. FMN and its analogs such as FAD, lumichrome, lumiflavin-3-acetic acid, alloxazine and lumazine exerted inhibitory action on the self-splicing of T4 td intron RNA at a concentration ranging from 0.2 to 5 mM whereas riboflavin and lumiflavin had no effect up to a concentration of 4 and 10 mM, respectively. Kinetic analysis demonstrated that FMN acts as a competitive inhibitor on the self-splicing of T4 td intron RNA with Ki of 1.3 mM. From these results, we would suggest that common structural features required for inhibition of splicing may be pyrimidine ring in these compounds, whose 7,8-dimethyl group and 10 position nitrogen which has no additional functional group may play crucial role in an acceleration of inhibition of self-splicing of T4 td intron RNA.