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菠菜叶绿体DNA的分离与鉴定

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摘要 高等植物中，经常遇到的母本遗传现象，有一部分是与叶绿体相联系的，这种联系表现在两个方面：或则为叶绿体DNA所编码，或则为核DNA编码，但是通过核质之间的代谢，从而影响到叶绿体DNA的变化。因此，分离与制备叶绿体DNA，是研究叶绿体遗传的第一步。

关键词

分类号

Isolation and Identification of Chloroplast DNA from Spinach

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Abstract

Our studies began from 1980.Spinach used in this experiment were purchased in market together with a small portion grown in our experimental station.Procedure of isolation was the same as described by Kolodner et al.only with some modification.

Fresh leaves of spinach (200—250g) were washed with distilled water and homogenized with 100ml buffer A.The homogemate was filtered through 10 layered cheesecloth and centrifuged at 1020×g for 15min .Crude chloroplasts were treated with DNase 1 and washed three times with buffer B.Finally,the chloroplast pellet was resuspended in buffer C.

For the extraction of chloroplast DNA saturated phenol was used.Procedure of extraction was repeated three times.The DNA aqueous phase obtained were mixed with two volume of cold 95% ethanol and placed in a refrigerator over night.

For the purification of chloroplast DNA one of the following three methods was used. a) CsCl-density gradient centrifugation

DNA was collected by centrifugation at 12000×g for 15 min and dissolved in 10ml buffer C.The DNA solution was adjusted with CsCl to ρ=1.58,supplemented with ethidium bromide (10mg/ml) 0.15 and centrifuged with MSE 75 at 40000 rpm/min for 44h. DNA band was clearly observed and collected under an ultraviolet lamp.Ethidium bromide was removed by adding an equal volume of isopropanol and discarding the coloured upper layer.Then the colourless DNA was dialyzed against dialysate.

b) Agarose gel electrophoresis

Crude DNA was dissolved in 10ml SSC,supplemented with 10% sucrose,ethidium bromide and bromophenol blue.This mixture was used as sample and loaded on 0.7% agarose gel column.Buffer used for electrophoresis and electrode contained 0.089 M Tris,0.089 M boric acid and 2.5mM EDTA-Na2 pH 8.5.Samples run under 70V for 12h.After electrophoresis two DNA band were observed.The slowly migrating band,as a sample,run horizontally again from solid phase to aqueous phase.Then DNA was collected and dialyzed.

c) Chromotography on Sepharose 2B column

Crude DNA extracted was dissolvd in 2M NaCl.The Sepharose 2B (or 4B) column was equlibrated with 2M BaCl previously.DNA sample was loaded on the column and eluted with 2M NaCl.Elutes were determined by LKB automatic analyzer through 260nm and 280nm.The first peak was collected and DNA was dialyzed.

Electron microscopy of chloroplast DNA

Method of Ferguson et al.was used.Spreading solution contained DNA 0.5μg/ml,cytochrome C 0.1mg/ml,ammonium acetate 0.5M EDTA-Na2 ImM.Hypophase contained only ammonium acetate 0.25M.After the spreading solution was spread on the hypophase,the monomolecular layer of DNA was picked

on the 250 mesh copper grides.Then,the copper grides were sprayed with 60% platinum and 40% iridium wire in rotary shadowed and observed under the electron microscopy Type Hu-11 and Dx-2.
Results obtained by observation under electrom microscopy showed that from those three methods of purification as mentioned above a number of circular DNA molecules may be divided into three types: closed circular DNA molecules,open circular DNA molecules and linear DNA fragments.However,in view of the amount and purity of the obtained circular DNA molecules method of CsCl density gradient centrifugation is the best one.

physical propertiesof the closed circular DNA molecules were calculated as follows: contour length of the closed circular DNA molecules is 41μ.Molecular weight of them is 84×10 6 daltons,Sedimentation coefficient (S) is 31.All of these values are agreeable with others.

Key words

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