Meselson-Stahl's Experiment (1958) in *E.coli*

I.Technique used: Density gradient equilibrium centrifugation technique.

II.Requirements:

i)The bacterium *E.coli* as genetic material.

ii)6M CsCl (cesium chloride) solution[which has a close density to that of DNA~1.71gm/cc) to serve as a density gradient solution on ultracentrifugation.

iii)Ultracentrifuge, iv)Bacterial culture media— containing $^{14}NH_4Cl$ medium and medium with $^{15}NH_4Cl$ which contain heavy isotope of nitrogen.

iv)Centrifuge tubes.

v)Bacteria culture chamber, vi)Spectrophotometer ,

vii)Photographic plate etc.

III.Principle:

As DNA has the property of Density gradient equilibrium sedimentation behaviour ,when a mixture of DNA molecules with different densities is present in a density gradient solution they will be separated as bands in their respective areas of the gradient solution where the density of the solution equals the buoyant density of DNA. Since DNA strongly absorbs UV-light at a wavelength of 260 nm the position of the DNA bands along the density gradient can be detected by an optical system which illuminates the centrifuge tube and registers the position of DNA bands on photographic plates.

Based on this principle Meselson and Stahl conducted their experiment.

IV.Experimental procedure:

i)E.coli cells were grown on ^{15}N containing $^{15}NH4Cl$ solution ,to uniformly level them with ^{15}N DNA which were thus heavier than ^{14}N-DNA.

ii)Uniformly ^{15}N labeled E.coli cells were then transferred to unlabeled ^{14}N –medium and allowed to grow for several generation.

iii)DNA was then extracted from a cell-sample of each generation and subjected to ultracentrifugation taking in 6M CsCl solution containing centrifuge tubes for buoyant density.

iv)Heavy^{15}N-and light ^{14}N-DNAs were also extracted from respective cell samples and subjected to ultracentrifugation in CsCl solution for their buoyant densities as reference points.

V. Results :

^{15}N-DNA containing cells growing on unlabeled medium gave the following results in successive generation: -

I} DNA- samples after one generation (G_1)-produced a single band in a position in between ^{15}N-DNA and 14N-DNA i.e.; "hybrid band ($^{14}N/^{15}N$) position.

ii} DNA – samples after 2nd generation (G_2) – produced two bands of equal size-one at hybrid – band position and the other at ^{14}N-band position.

iii} DNA-samples from 3rd generation (G_3) onwards produced two bands in each case with band position similar to 2nd generation, but with a gradual increase in hybrid ^{14}N-band and a gradual decrease in (^{15}N) band as number of generation increases.

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