Ability of Urografin Density Gradients to Separate Nonculturable Subpopulations of *Escherichia coli*

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**METHODOLOGY**

**Significance**
- Cellular detachment, conducting to bacterial death? (1)
- Response to adverse conditions, similar to spore formation? (2)

**Question**
Can VNBC cells recover culturability? Reactivation? (3)

**Methods**
- Elimination of remaining culturable cells by dilution techniques (6)
- Inhibition of growth of remaining culturable cells (7)
- Separation of nonculturable subpopulations from heterogeneous suspensions (6)

To assess the ability of Urografin to separate nonculturable subpopulations from heterogeneous bacterial suspensions

**Strain:** *E. coli* STCC 416
**Origin of cells:** growth curves (MB, TSB, LB), mixed cultures (stationary phase and aged cultures) or stress exposure (starvation or acid pH)

**External standard of density:** Density Marker Beads (DMBs, American Biosciences)

**Bacterial determinations**
Total number of bacteria (TDC) (8), *bacteria* with intact cytoplasmic membranes (MEMB*) (9), colony-forming units (CFU) on Tryptone Soya Agar plates. Flow cytometry measurements with a FACScAlibur flow cytometer (Becton Dickinson, San Jose, CA, USA).

**Solutions**
- Urografin solution (8)
- Culture solution

**Fraction**
- Culturable (C)
- Nonculturable (NC)
- VIable but nonculturable (VNC)
- Non viable (NV)

**Bases for separation of VNBC cells:** The separation of culturable and nonculturable cells by means of Urografin gradients has been reported (6, 7) to be based on slight difference in cell densities. A greater density is assigned to nonculturable cells, which are thus understood to concentrate in high-density bands.

**Fractionation in Urografin gradients:** very diverse results were obtained when physiologically heterogeneous populations from different culture media, in different phases of growth, and even populations subjected to stress were examined. Formation of low- and high-density bands was observed. The high-density bands (horizon 5 and below) showed a homogeneous composition, mainly of nonculturable cells. In contrast, low-density bands had a heterogeneous composition, being mixtures of culturable and nonculturable cells.

**Remarkable facts**
- Fractionation in Percoll gradients: gets differences with respect to the centrifugation in Urografin gradient were detected. A great heterogeneity was observed in number and distribution of bands regardless of the origin of layered cells. Moreover, there was no correlation between band location and the kind of cells

**Toxicity of fractionation methods based on density gradient:** Urografin has been described as toxic to bacterial cells (11, 12). Centrifugation in density gradients of Urografin may have a harmful effect upon cellular state. This effect depends on time and temperature of centrifugation, as well as on concentration. Our results indicate that Urografin has a toxic effect on cell culturability. The decrease in percentage of culturable cell, and the concomitant increase in the nonculturable fraction, was mainly due to the formation of viable but nonculturable cells rather than the loss of viability.

**Conclusion**
We can thus conclude that Urografin density gradients are a good tool to obtain homogeneous nonculturable subpopulations from a heterogeneous *E. coli* populations. However, it is necessary to pay special attention to working conditions to avoid Urografin toxicity.

**References**
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