Appendix B: Plasmid-Free Equilibrium and Stability Analysis

B1. Plasmid-Free Equilibrium

The plasmid-free equilibrium corresponds to the absence of plasmids and plasmid-encoded gene expression:

$$P^* = M^* = Q^* = 0$$

At this equilibrium, total efflux activity arises solely from intrinsic host mechanisms:

$$E(Q^*) = E_h$$

The intracellular antimicrobial dynamics reduce to:

$$\frac{dA}{dt} = k_{in}A_{ext} - k_{out}E_hA$$

Setting $dA/dt = 0$ yields the steady-state intracellular antimicrobial concentration:

$$A^* = \frac{k_{in}A_{ext}}{k_{out}E_h}$$

This equilibrium reflects a balance between constant antimicrobial influx and intrinsic host efflux.

B2. Jacobian Matrix Definition

Let the state vector be:

$$x = (A, P, M, Q)^T$$

The Jacobian matrix J is defined component-wise as:

$$J_{i,j} = \frac{\partial \dot{x}_i}{\partial \dot{x}_j}$$

where $\dot{x}_i$ denotes the right-hand side of the differential equation governing x_i.

The Jacobian evaluated at an equilibrium x^*, the Jacobian determines the local linear stability of that equilibrium.

B3. Plasmid Invasion Derivatives

Antimicrobial dynamics

$$\dot{A} = k_{in}A_{ext} - k_{out}(E_h + \beta Q)A$$

The relevant partial derivatives are:

$$\frac{\partial \dot{A}}{\partial A} = -k_{out}(E_h + \beta Q), \qquad \frac{\partial \dot{A}}{\partial Q} = -k_{out}\beta A$$

Evaluated at a plasmid-free equilibrium ($Q = 0, A = A^*$):

$$\frac{\partial \dot{A}}{\partial A^*} = -k_{out}E_h, \qquad \frac{\partial \dot{A}}{\partial Q^*} = -k_{out}\beta A^*$$

All other partial derivatives of $\dot{A}$ vanish.

Plasmid copy-number dynamics

$$\dot{\mathrm{P}} = (r_P G(A) - c_P)P - \gamma P^2$$

The partial derivatives are:

$$\frac{\partial \dot{\mathrm{P}}}{\partial A} = r_P G'(A)P, \qquad \frac{\partial \dot{\mathrm{P}}}{\partial P} = \left(r_p G(A) - c_P\right) - 2\gamma P$$

At $P = 0$:

$$\frac{\partial \dot{\mathrm{P}}}{\partial A^*} = 0, \qquad \frac{\partial \dot{\mathrm{P}}}{\partial P^*} = r_p G(A^*) - c_P$$

Efflux pump mRNA dynamics

$$\dot{\mathrm{M}} = k_m P - \delta_m M$$

The nonzero partial derivatives are:

$$\frac{\partial \dot{\mathrm{M}}}{\partial P} = k_m, \qquad \frac{\partial \dot{\mathrm{M}}}{\partial M} = -\delta_m$$

Efflux pump protein dynamics

$$\dot{Q} = k_q M - \delta_q Q$$

The nonzero partial derivatives are:

$$\frac{\partial \dot{Q}}{\partial M} = k_q, \qquad \frac{\partial \dot{Q}}{\partial Q} = -\delta_q$$

B4. Jacobian Matrix Eigenvalues

Collecting the above terms, the Jacobian evaluated at the plasmid-free equilibrium has the block upper-triangular form:

$$J(x^*) = \begin{pmatrix} -k_{out}E_h & 0 & 0 & -k_{out}\beta A^* \\ 0 & r_P G(A^*) - c_P & 0 & 0 \\ 0 & k_m & -\delta_m & 0 \\ 0 & 0 & k_q & -\delta_q \end{pmatrix}$$

Because the Jacobian is block triangular, its eigenvalues are given by the diagonal elements:

$$\lambda_A = -k_{out}E_h, \qquad \lambda_P = r_P G(A^*) - c_P, \qquad \lambda_M = -\delta_m, \qquad \lambda_Q = -\delta_q$$

The eigenvalue λ_P governs plasmid invasion:

- If $\lambda_P < 0$, the plasmid-free equilibrium is locally stable and plasmids go extinct.
- If $\lambda_P > 0$, the plasmid-free equilibrium is unstable to plasmid invasion.

B5. Plasmid Invasion Thresholds

Plasmid invasion occurs when:

$$r_P G(A^*) > c_P$$

Using the equilibrium intracellular antimicrobial concentration,

$$A^* = \frac{k_{in}A_{ext}}{k_{out}E_h}$$

the growth equilibrium is:

$$G(A^*) = G_{max}\left(1 + \left(\frac{k_{in}A_{ext}}{k_{out}E_h IC_{50}}\right)^h\right)^{-1}$$

Substituting into the invasion condition and solving for intrinsic efflux activity yields the critical threshold:

$$E_h^{crit} = \frac{k_{in}A_{ext}}{k_{out}IC_{50}}\left(\frac{c_P}{r_P G_{max} - c_P}\right)^{1/h}$$

- If $E_h < E_h^{crit}$, intracellular drug accumulation suppresses growth sufficiently to prevent plasmid amplification.
- If $E_h > E_h^{crit}$, intrinsic efflux permits positive plasmid growth.

Alternatively, solving for the critical extracellular antimicrobial concentration gives:

$$A_{ext}^{crit} = \frac{k_{out}E_h IC_{50}}{k_{in}}\left(\frac{r_P G_{max} - c_P}{c_P}\right)^{1/h}$$