Appendix B: Plasmid-Free Equilibria and Jacobian Eigenvalues

B1. Plasmid-Free Equilibrium

The plasmid-free equilibrium is defined by the absence of plasmids and plasmid-encoded gene expression:

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

At this state, efflux activity is purely intrinsic:

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

The intracellular antimicrobial dynamics are given by:

$$\frac{dA}{dt} = k_{in}A_{ext} - k_{out}E(Q)A$$

Substituting $Q = Q^*$ and setting $dA/dt = 0$ yields:

$$0 = k_{in}A_{ext} - k_{out}E_hA^*$$

Solving for the steady-state intracellular antimicrobial concentration gives:

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

This equilibrium value reflects the balance between 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 as:

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

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

Evaluated at an equilibrium x^*, the Jacobian determines the local stability of the system.

B3. Plasmid Invasion Derivatives

Derivatives of $\dot{A}$

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

With respect to A

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

- At equilibrium $Q = 0$:

$$\frac{\partial \dot{A}}{\partial A} = -k_{out}E_h$$

With respect to P

$$\frac{\partial \dot{A}}{\partial P} = 0$$

With respect to M

$$\frac{\partial \dot{A}}{\partial M} = 0$$

With respect to Q

$$\frac{\partial \dot{A}}{\partial Q} = -k_{out}\beta A$$

- At equilibrium:

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

Derivatives of $\dot{P}$

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

With respect to A

$$\frac{\partial \dot{P}}{\partial A} = r_P G'(A)P$$

- At equilibrium $P = 0$:

$$\frac{\partial \dot{P}}{\partial A} = 0$$

With respect to P

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

- At equilibrium $P = 0$:

$$\frac{\partial \dot{P}}{\partial P} = r_p G(A) - c_P$$

With respect to M

$$\frac{\partial \dot{P}}{\partial M} = 0$$

With respect to Q

$$\frac{\partial \dot{P}}{\partial Q} = 0$$

Derivatives of $\dot{M}$

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

With respect to A

$$\frac{\partial \dot{M}}{\partial A} = 0$$

With respect to P

$$\frac{\partial \dot{M}}{\partial P} = k_m$$

With respect to M

$$\frac{\partial \dot{M}}{\partial M} = -\delta_m$$

With respect to Q

$$\frac{\partial \dot{M}}{\partial Q} = 0$$

Derivatives of $\dot{Q}$

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

With respect to A

$$\frac{\partial \dot{Q}}{\partial A} = 0$$

With respect to P

$$\frac{\partial \dot{Q}}{\partial P} = 0$$

With respect to M

$$\frac{\partial \dot{Q}}{\partial M} = k_q$$

With respect to Q

$$\frac{\partial \dot{Q}}{\partial Q} = -\delta_q$$

B4. Jacobian Matrix Eigenvalues

As a result, the Jacobian evaluated at the plasmid-free equilibrium has a block upper-triangular structure:

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

The eigenvalues are given by the diagonal blocks. In particular, the eigenvalue governing plasmid invasion is:

$$\lambda_P = r_P G(A^*) - c_P$$

- 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.
- This condition depends entirely on the equilibrium growth rate $G(A^*)$, which is controlled by intrinsic host efflux activity and extracellular antimicrobial concentration.

B5. Plasmid Invasion Critical Thresholds

Plasmid invasion occurs when:

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

The invasion threshold is therefore defined implicitly by:

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

The growth inhibition function for equilibrium intracellular antimicrobial concentration is:

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

Thus, substituting $G(A^*)$ into the invasion condition yields:

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

Rearranging:

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

Solving for E_h, gives the critical intrinsic efflux activity:

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

- If $E_h < E_h^{crit}$, intracellular drug accumulation suppresses growth too strongly for plasmids to increase.
- If $E_h > E_h^{crit}$, intrinsic efflux reduces drug levels sufficiently to allow positive plasmid growth.

Alternatively, for fixed intrinsic efflux activity, the maximum drug concentration permitting plasmid invasion can be derived.

Starting again from:

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

and solving for A_{ext}, we obtain:

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

- If $A_{ext} < A_{ext}^{crit}$, intrinsic efflux enables sufficient growth for plasmid amplification.
- If $A_{ext} > A_{ext}^{crit}$, plasmids cannot invade even if present.