## Writing exercise S03: Formulating a hypothesis

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You will want to end your introduction with the aims of your project and the hypothesis that you will be testing. (You should have developed a logical argument through the course of the introduction which introduces the reader to the topic and helps them to see why your aims/hypothesis are a logical next step to research in this field.)

### A good scientific hypothesis is:

* Testable
* Clear and easy to understand
* Logical (grounded in the previous scientific literature)

You may find it helpful to use the framework “PICOT” to help shape/formulate your hypothesis.

* P: Population
* I: Intervention
* C: Comparison
* O: Outcome
* T: Time

Not all hypotheses will include every element in this framework, but you may find in many cases that using “PICOT” will help you refine/improve your hypothesis so that it is a clear, specific, and testable.

**Example**:

For overly stressed students, switching from coffee to herbal tea will reduce the numbers of nervous breakdowns compared to continuing with high doses of coffee over exam week.

**P**: overly stressed students
**I**: switching from coffee to herbal tea
**C**: compared to continuing with high doses of coffee
**O**: reduce the numbers of nervous breakdowns
**T**: over exam week

Is this hypothesis specific enough? (which students? What type of tea? etc.) Is it testable?

Sometimes the aims/hypothesis in published scientific papers will be left rather implicit (i.e. “The effect of switching from coffee to herbal tea over exam week in overly stressed students is not known.”), but for your thesis you really should try to make the aims and hypothesis as explicitly clear as possible.

### Exercise

Read each sample of scientific writing and ask yourself:

* What is the hypothesis? Is it clear, testable, and logical (based on the provided text/your knowledge of the scientific literature?)
* How might the hypothesis be improved (e.g. written more clearly/explicitly, made more specific, etc.)?

**Sample 1** 1

To reduce costs and increase crop yields, multiple studies have focused on understanding the helper effect or the promotion of mycorrhization in leguminous plants that hold economic importance [[17](https://link.springer.com/article/10.1007/s00284-024-03928-x#ref-CR17), [21](https://link.springer.com/article/10.1007/s00284-024-03928-x#ref-CR21)]. For example, the use of bacterial strains (e.g., *Pseudomonas, Bacillus, Rhizobium*) to improve fruit quality and/or productivity in boysenberry (blackberries) crops [[22](https://link.springer.com/article/10.1007/s00284-024-03928-x#ref-CR22)]. In northwestern Patagonia, Argentina, raspberry (*Rubus idaeus*) crops are very abundant and economically important, as well as, other “berries” crops [[23](https://link.springer.com/article/10.1007/s00284-024-03928-x#ref-CR23)]. However, the utilization of actinobacteria and/or mycorrhizae in these regional crops has not yet been explored. For this reason, this study aims to investigate the interaction between actinobacteria and mycorrhizal colonization in raspberry young plants that could in the future contribute to eco-friendly raspberry crops. In order to do this, we conducted an experiment postulating the hypothesis that the co-inoculation of actinobacteria and arbuscular mycorrhizal fungi (AM) on young raspberry plants improves the mycorrhization parameters probably as a consequence of bioactive metabolites produced by the actinobacteria that help AM colonization and enhance plant growth. Additionally, these inoculated microorganisms will interact positively with the microbiota present in the soil. To test this hypothesis, we used a strain of a known bacterium with plant growth-promoting traits (PGP), *Streptomyces* sp. (SH9) and a mycorrhizal fungus, *Rhizophagus intraradices*, carrying out an experiment of single and double inoculations in sterile and natural soil (non-sterile), expecting a lower plant growth and lower mycorrhization parameters in raspberry plants growing in sterile than in non-sterile soil. Additionally, young plants of Raspberry without inoculation will show lower mycorrhization and growth parameters than inoculated plants, being the co-inoculation the treatment with the highest effect on these variables. Lastly, control plants growing in sterile soil will show the lowest growth and no mycorrhization compared to the other treatments and substrates, and the highest effect is expected with the co-inoculation in natural soil.

**Sample 2** 2

We previously established that *E. coli* and *Salmonella* PhoB/PhoR systems can be activated in the presence of high Pi, when these organisms undergo translational arrest resulting from either cytoplasmic Mg2+ starvation, or treatment with antibiotics that inhibit protein synthesis ([3](https://journals.asm.org/doi/10.1128/mbio.01642-24#core-B3), [35](https://journals.asm.org/doi/10.1128/mbio.01642-24#core-B35), [36](https://journals.asm.org/doi/10.1128/mbio.01642-24#core-B36)). These results implied that this two-component system responds to an intracellular signal that is normally generated during growth in low Pi but that can be brought about by these other physiological disturbances. In the current study, we conduct experiments to specifically test this hypothesis. We show that if *Salmonella* is provided with an alternative P source that is metabolized in a PhoB-independent manner, PhoB/PhoR remains inactive during growth in Pi-free media, thereby demonstrating that PhoB/PhoR activity is repressed by an intracellular P sufficiency signal ([Fig. 1B](https://journals.asm.org/doi/10.1128/mbio.01642-24#F1)).

**Sample 3** 3

Under RIP-permissive conditions *in vitro*, TcpP is sensitive to proteolysis by tail-specific protease (Tsp; site-1 protease) and subsequently by YaeL protease (site-2 protease) ([25](https://journals.asm.org/doi/10.1128/mbio.00721-24#core-B25)[–](https://journals.asm.org/doi/10.1128/mbio.00721-24#core-B26)[27](https://journals.asm.org/doi/10.1128/mbio.00721-24#core-B27)). RIP of TcpP is inhibited by its associated protein, TcpH, under specific *in vitro* conditions ([25](https://journals.asm.org/doi/10.1128/mbio.00721-24#core-B25)[–](https://journals.asm.org/doi/10.1128/mbio.00721-24#core-B26)[27](https://journals.asm.org/doi/10.1128/mbio.00721-24#core-B27)). In cells lacking TcpH, TcpP is constitutively degraded via RIP ([25](https://journals.asm.org/doi/10.1128/mbio.00721-24#core-B25)[–](https://journals.asm.org/doi/10.1128/mbio.00721-24#core-B26)[27](https://journals.asm.org/doi/10.1128/mbio.00721-24#core-B27)). However, the mechanism by which TcpH inhibits RIP and how TcpH-dependent RIP inhibition is modulated by extracellular stimuli remains unknown.

**Sample 4** 4

We previously identified and characterized a small membrane protein called MspA ([26](https://journals.asm.org/doi/10.1128/mbio.01512-24#core-B26), [27](https://journals.asm.org/doi/10.1128/mbio.01512-24#core-B27)). Inactivation of *mspA* in both the MRSA strain JE2 and the MSSA strain SH1000 was shown to cause a significant decrease in phenol soluble modulins toxin production and cytotoxicity due to downregulation of the accessory gene regulatory system. The *mspA* mutant was shown to have a reduced content of the membrane carotenoid pigment staphyloxanthin, to be more susceptible to components of the innate immune response such as fatty acids and AMPs, and to have decreased membrane stability when challenged with the detergent SDS. Interestingly, both systems for uptake (IsdC, IsdB, and FhuC) and export (HrtAB) of heme, the major iron source for *S. aureus* during infection, were more abundant in the *mspA* mutant compared to the wild type. The mutant also had increased levels of intracellular iron, suggesting dysregulation of iron homeostasis. These pleiotropic effects observed *in vitro* resulted in attenuation of the mutant in superficial and systemic infection mouse models ([27](https://journals.asm.org/doi/10.1128/mbio.01512-24#core-B27)).

Given the predicted location of MspA and the pleiotropic effects its inactivation has on virulence and pathogenicity, we hypothesized that MspA could have a structural role, contributing to the synthesis of the membrane or cell wall or supporting the stability of proteins involved in such processes. Therefore, virulence defects showed by the mutant could be a downstream consequence of perturbations in cellular structural integrity.

**Sample 5** 5

As in other fungi, light sensing of *B. cinerea* is primarily mediated by the BcWCL1 and BcWCL2 protein complex. The interaction between BcWCL1 and BcWCL2 proteins occurs through their PAS domains, independent of light conditions ([44](https://journals.asm.org/doi/10.1128/mbio.00133-24#core-B44)), and BcWCL1 and BcWCL2 could both be located in the nuclei ([45](https://journals.asm.org/doi/10.1128/mbio.00133-24#core-B45)). The loss of BcWCL1 was found to be associated with increased sporulation and reduced secretion of oxalic acid, resulting in a decreased ability to acidify culture mediums during light-dark (LD) conditions. This, in turn, affected virulence under light conditions and the regulation of light-responsive genes, such as *Bcltf1*, *Bcltf2*, and *Bcltf3* ([46](https://journals.asm.org/doi/10.1128/mbio.00133-24#core-B46)). Among these genes, BcLTF1 transcription factor plays a crucial role in maintaining ROS homeostasis, regulating secondary metabolism, and influencing virulence ([47](https://journals.asm.org/doi/10.1128/mbio.00133-24#core-B47)). However, only some of the light-inducing genes in *B. cinerea* were found to rely on the presence of BcWCL1 ([48](https://journals.asm.org/doi/10.1128/mbio.00133-24#core-B48)), leaving open the question whether and how BcWCL2 targets unique downstream genes that are associated with virulence characteristics of this necrotrophic pathogen.

**Sample 6** 6

*Brucella* is a gram-negative facultative intracellular pathogen that causes brucellosis, a widespread zoonosis of animal and human health concern ([27](https://journals.asm.org/doi/10.1128/mbio.00726-24#core-B27), [28](https://journals.asm.org/doi/10.1128/mbio.00726-24#core-B28)). The hallmark of the infectious process of *Brucella* is its capacity to invade, survive, and replicate in professional and non-professional phagocytes ([29](https://journals.asm.org/doi/10.1128/mbio.00726-24#core-B29), [30](https://journals.asm.org/doi/10.1128/mbio.00726-24#core-B30)), activities that are many times dependent on effector proteins secreted or translocated by the Type IV (VirB) secretion system (T4SS) ([27](https://journals.asm.org/doi/10.1128/mbio.00726-24#core-B27), [29](https://journals.asm.org/doi/10.1128/mbio.00726-24#core-B29)). In the past decades, although many effector proteins have been identified in *Brucella* ([29](https://journals.asm.org/doi/10.1128/mbio.00726-24#core-B29)[–](https://journals.asm.org/doi/10.1128/mbio.00726-24#core-B30)[32](https://journals.asm.org/doi/10.1128/mbio.00726-24#core-B32)), the functions or molecular targets for most of them remain unknown. Particularly, to date, no virulence factors involved in actin modulation have been identified in *Brucella*.

**Sample 7** 7

Strains of *E. coli* that cause human disease are genetically diverse from non-pathogenic serotypes ([17](https://journals.asm.org/doi/10.1128/mbio.01048-24#core-B17), [18](https://journals.asm.org/doi/10.1128/mbio.01048-24#core-B18)). For example, Trg and Tap chemoreceptors, which direct bacteria toward ribose, galactose, and dipeptides ([19](https://journals.asm.org/doi/10.1128/mbio.01048-24#core-B19), [20](https://journals.asm.org/doi/10.1128/mbio.01048-24#core-B20)), are less prevalent and functional among UPEC isolates than fecal or commensal strains and even more so when compared to diarrheal strains ([21](https://journals.asm.org/doi/10.1128/mbio.01048-24#core-B21)). Because the genetic and phenotypic display of these pathogens are varied, it is likely that regulatory mechanisms of key processes that include bacterial motility are as well.

**Sample 8** 8

Recently, we determined that most isolates of *S. maltophilia* also encode a VirB/VirD4 type IV protein secretion system (T4SS) ([27](https://journals.asm.org/doi/10.1128/mbio.01198-24#core-B27)). Based on the characterization of a *virB10* mutant of the clinical isolate K279a, the *S. maltophilia* T4SS promotes apoptosis of macrophages while impeding the death of lung epithelial cells ([27](https://journals.asm.org/doi/10.1128/mbio.01198-24#core-B27)). Interestingly, the T4SS also has antibacterial activity, reducing, in a contact-dependent manner, the colony forming units (CFUs) of clinical and environmental isolates of *Pseudomonas aeruginosa*, *Pseudomonas mendocina*, and *Escherichia coli* (*Ec*) ([27](https://journals.asm.org/doi/10.1128/mbio.01198-24#core-B27), [28](https://journals.asm.org/doi/10.1128/mbio.01198-24#core-B28)). This antibacterial activity was confirmed by others, for example, in short-term co-incubations, *S. maltophilia* K279a lyses strains of *P. aeruginosa* and *E. coli* ([29](https://journals.asm.org/doi/10.1128/mbio.01198-24#core-B29)). Given that *S. maltophilia* and *P. aeruginosa* often co-exist in hospital water systems and various types of infections, especially those in the lungs ([30](https://journals.asm.org/doi/10.1128/mbio.01198-24#core-B30)[–](https://journals.asm.org/doi/10.1128/mbio.01198-24#core-B31)[42](https://journals.asm.org/doi/10.1128/mbio.01198-24#core-B42)), further knowledge of the antibacterial effects of the *S. maltophilia* T4SS was relevant for understanding human infection. We posited that the antimicrobial effect of the *S. maltophilia* T4SS is due to the delivery of toxic proteins (effectors) into target bacteria.

### References

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