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Antibiotic production in Streptomyces is organized by a division of labor through terminal genomic differentiation

Journal club

Supervisor: Dr. Yadollah Bahrami
Presented by: Hosna Hatami
Department of Medical Biotechnology

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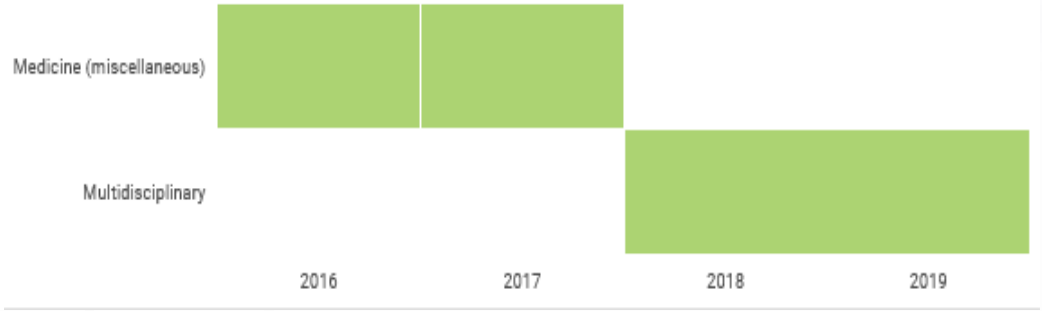


INTRODUCTION

- Social insects provide some of the most compelling examples of divisions of labor, with extremes in morphological differentiation associated with highly specialized functions and reproductive sterility in all colony members, except the queen.
- However, conditions that select for division of labor are not limited to animals, and it has become increasingly clear that microbes offer unique opportunities to identify and study the mechanistic underpinnings of divisions of labor.

INTRODUCTION

- First, microbes are typically clonal, which helps ensure that a division of labor is favored by kin selection.
- Second, microbial populations are highly social, often cooperating to carry out coordinated behaviors such as migration or biofilm formation that require the secretion of metabolically expensive public goods that can be shared among clonemates.
- If these conditions are met, and investment in public good secretion trades off with fitness, divisions of labor are predicted to evolve.

INTRODUCTION

- Here, we describe the cause and evolutionary benefits of a unique division of labor that has evolved in colonies of the filamentous actinomycete Streptomyces coelicolor.
- After germinating from unichromosomal spores, these bacteria establish multicellular networks of vegetative hyphae, reminiscent of fungal colonies.
- Vegetative hyphae secrete a broad variety of public goods, such as chitinases and cellulases that are used to acquire resources, as well as a chemically diverse suite of antibiotics that are used to kill or inhibit competing organisms.

MATERIALS AND METHODS

- All strains were derived from a single isolate of S. coelicolor A3(2) M145 (designed as WT).
- Briefly, samples from a frozen spore stock were diluted and plated onto SFM agar to obtain single colonies.
- After 5 days of growth, single colonies with WT morphology were diluted and plated onto another SFM plate.
- From each plate, single colonies with conspicuously mutant phenotypes were picked into sterile water and plated at appropriate dilutions onto SFM agar (n = 3 per colony), from which we estimated the frequency of different mutant phenotype classes.
- Each derived type was plated to confluence on SFM agar, and after 7 days of growth, spores were harvested to generate spore stocks, which were stored at −80°C in 20% glycerol.

MATERIALS AND METHODS

- To quantify mutation frequency, single colonies were grown for 5 days on three different media, and then we picked the colonies with WT morphology, diluted, and plated them onto the corresponding media.
- Mutation frequency was scored on the basis of the phenotypes after 3 to 5 days.

Table S3. Mutant strains used in this study.

This table lists mutant strains used in this study that were derived from the same WT ancester.

Strain	Experiment							
	CFU production	Antibiotic production (against *B. subtillis*)	^{1}H NMR profiling	PFGE	Pacbio sequencing	Proteomics	Antibiotic production (against soil isolates)	Fitness estimates (competition assay)
2H1A	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
8H1B	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
9H1B	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
9H1A	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
2H1I	Yes	Yes	Yes (including one extra strain derived from 2H1I)	Yes	Yes	Yes		
9H1C	Yes	Yes	Yes (including one extra strain derived from 9H1C)	Yes	Yes			
2H1G	Yes	Yes	Yes	Yes	Yes			
21H1B	Yes	Yes	Yes	Yes	Yes			
2H1F	Yes	Yes	Yes	Yes				
14H1B	Yes	Yes	Yes	Yes				
14H1E	Yes	Yes	Yes	Yes				
17H1A	Yes	Yes	Yes	Yes				
17H1C2	Yes	Yes	Yes	Yes				
17H1D	Yes	Yes	Yes	Yes				
2H1B	Yes	Yes	Yes	Yes				
2H1D	Yes	Yes	Yes	Yes				
5H1C	Yes	Yes	Yes	Yes				
5H1F	Yes	Yes	Yes	Yes				
8H1F	Yes	Yes	Yes	Yes				
8H1H	Yes	Yes	Yes	Yes				
21H1D2	Yes	Yes	Yes	Yes				
2H1C	Yes	Yes	Yes	Yes				
2H1E1	Yes	Yes	Yes	Yes				
2H1E2	Yes	Yes	Yes	Yes				
5H1E2	Yes	Yes	Yes	Yes				
21H1C	Yes	Yes	Yes	Yes				
2H1H1	Yes	Yes	Yes	Yes				
2H1H3	Yes	Yes	Yes	Yes				
9H1E2	Yes	Yes	Yes	Yes				
14H1G	Yes	Yes	Yes	Yes				

DISCUSSION

- Streptomycetes are prolific producers of antibiotics, with genomes typically containing more than 20 secondary metabolite gene clusters that comprise more than 5% of their entire genome. They invest heavily in these products, and their biosynthesis and secretion are costly.
- Our results suggest that, by limiting antibiotic production to a fraction of the colony through division of labor, S. coelicolor can eliminate the overall costs of biosynthesis while maximizing both the magnitude and diversity of their secreted antibiotics.
- Although this comes at a large individual cost, it increases group fitness by improving the ability for S. coelicolor to inhibit their competitors.
- Moreover, our results reveal that the range of conditions that select for a division of labor are quite broad, because colony-wide fitness is unaffected, even if mutant strains are as frequent as ~50%.

DISCUSSION

- Division of labor is predicted to be favored in this system for several reasons.
- First, Streptomyces colonies emerge from a single spore and are clonal.

DISCUSSION

- Second, the costs of antibiotic production via large and dedicated multistep biosynthetic pathways, e.g., nonribosomal peptide or polyketide synthases, are likely to be highest at the initiation of antibiotic production but diminish thereafter, meaning that producing cells become more efficient at making antibiotics through time. furthermore, we show that antibiotic production trades off with reproduction.

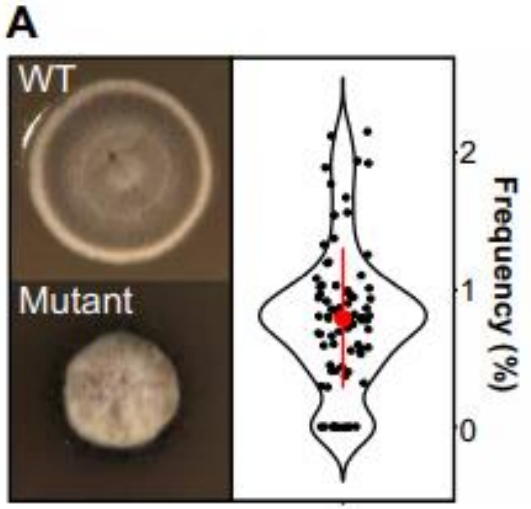
DISCUSSION

- Last, many antibiotics are secreted, so the entire colony, but not susceptible competitors, can benefit from the protection they provide.

RESULTS AND DISCUSSION

- **Genomic instability and phenotypic heterogeneity are coupled**

  - we quantified the phenotypic heterogeneity arising within 81 random single colonies of S. coelicolor M145 by harvesting the spores of each of these colonies and then replating the collected spores onto a new agar surface. Although most progeny are morphologically homogeneous and similar to the wild type (WT), notably aberrant colonies.



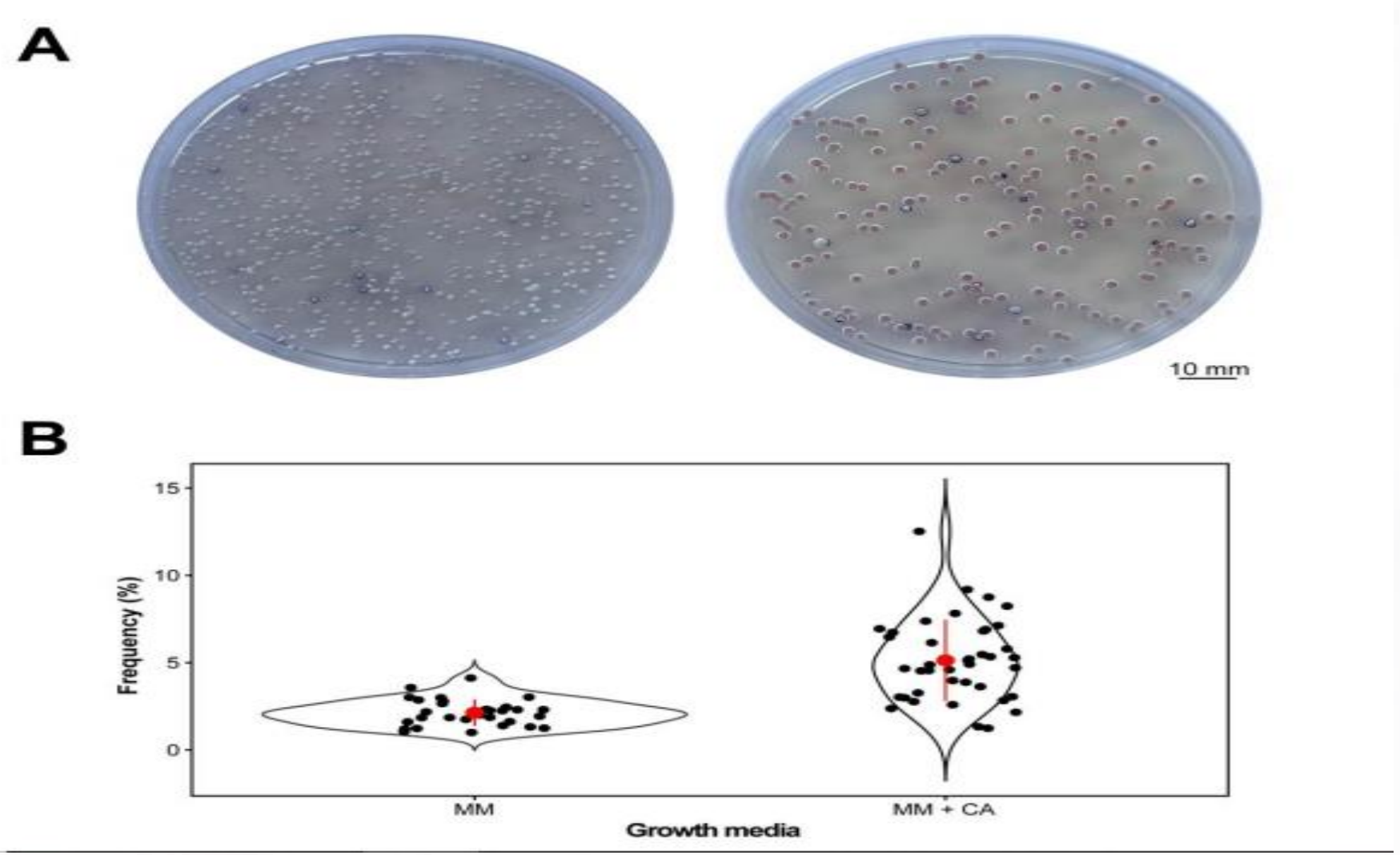


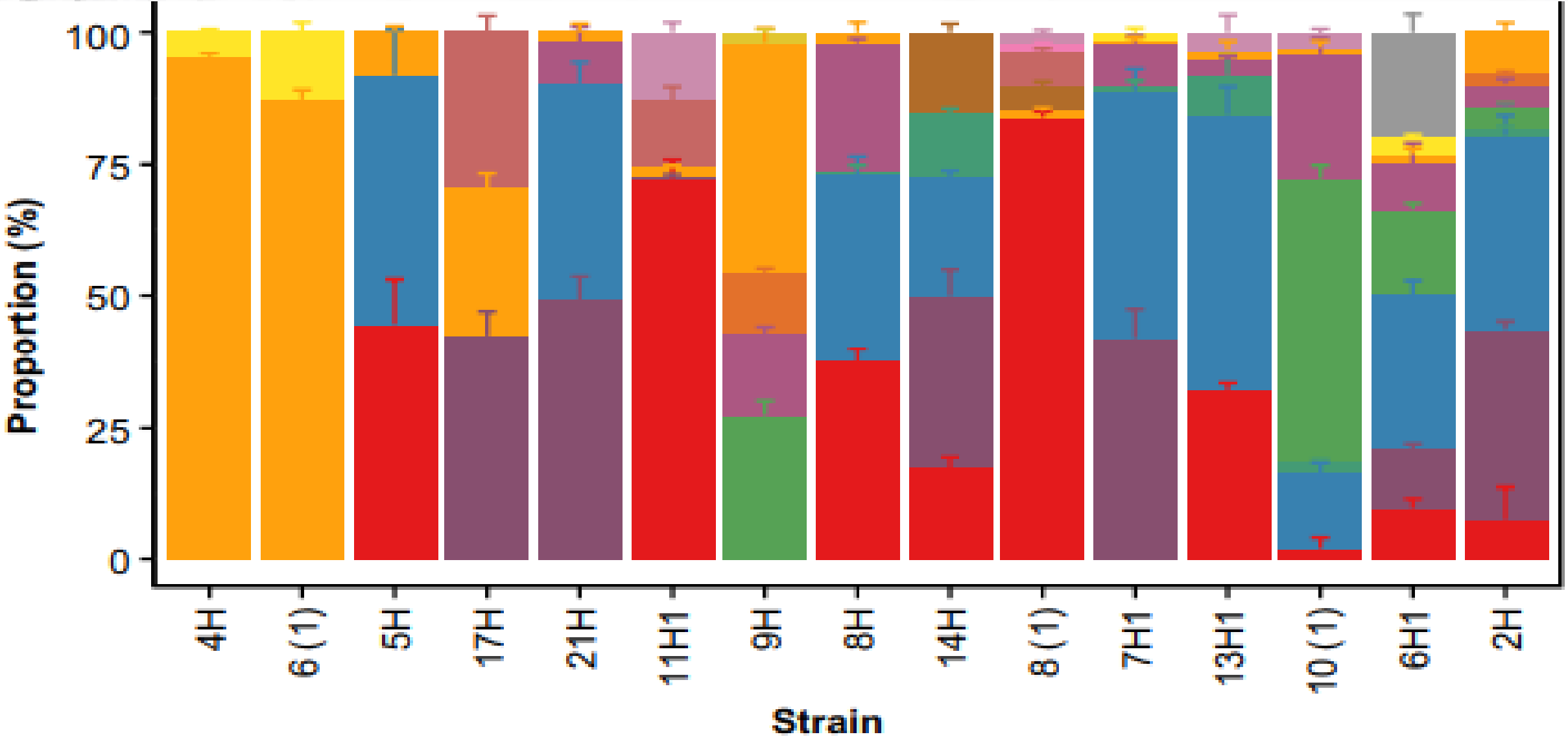
Fig. S1. Mutant frequencies in different media. Phenotypes (A) and mutation frequencies (B) of colonies grown on minimal media (MM) or minimal media supplemented with casamino acids (MM + CA). (A) Examples of mutant and wildtype colonies growing on minimal media (MM) (Left) or minimal media supplemented with casamino acids (MM + CA) (Right). (B) The frequency of mutants emerging from WT colonies on both media types.

RESULTS AND DISCUSSION

- thereby ruling out the possibility that these mutations are an artifact of rapid growth on rich resources.
- The differences we observe on these two media types also suggest that the mutant frequencies we estimate based on spore counts may underestimate their values within growing colonies, given that mutants may be compromised in growth or sporulation.
- This is supported by the nearly twofold difference in mutant frequencies on MM + casamino acids compared to unsupplemented MM, where auxotrophs arising by mutation would be unable to persist.

RESULTS AND DISCUSSION

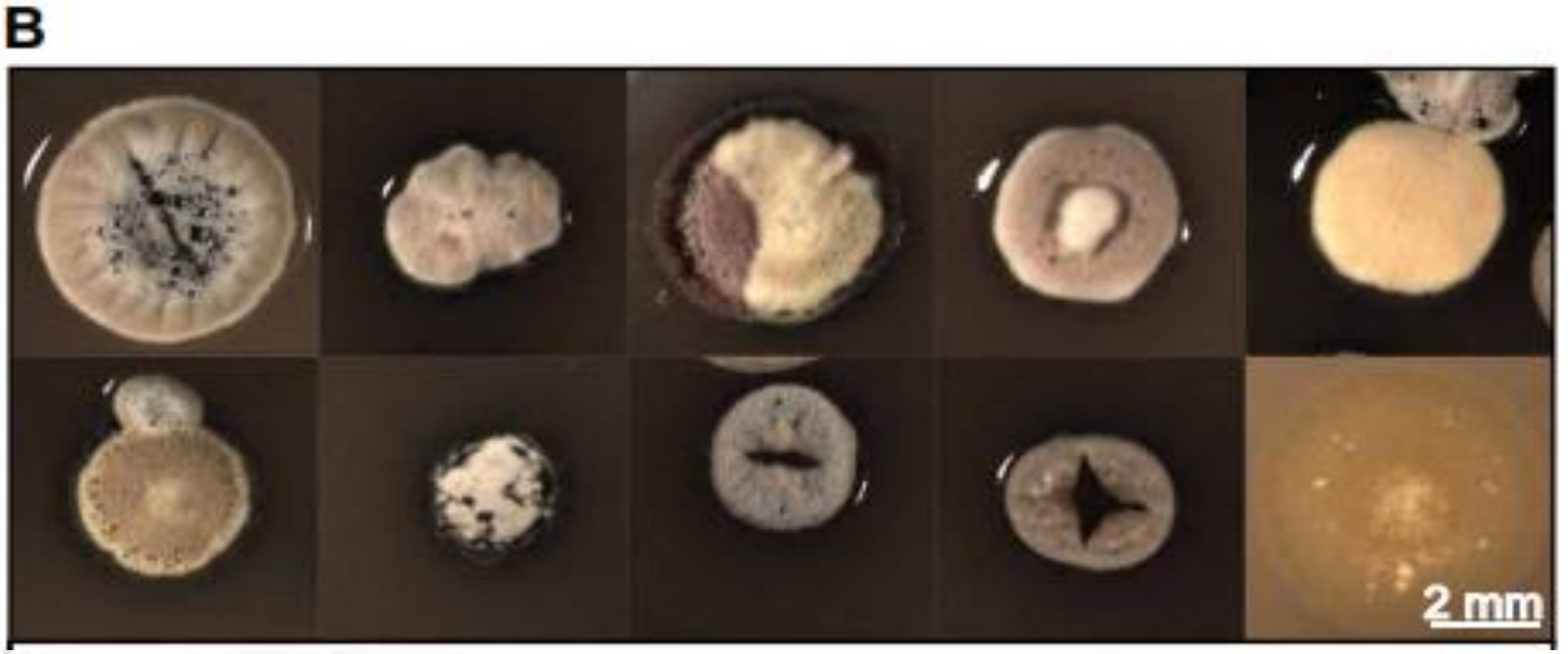
To determine the heritability of these aberrant phenotypes, we restreaked 15 random colonies from different plates onto a new agar plate, which revealed remarkable variability in colony morphology.



(B) Phenotypically diverse progeny (top) emerges after restreaking mutant colonies that vary in size, shape, and pigmentation. Representative colonies are shown. The bottom graph depicts the range of distinct morphologies that emerge after restreaking 15 random colonies. Each color represents a distinct colony phenotype.

RESULTS AND DISCUSSION

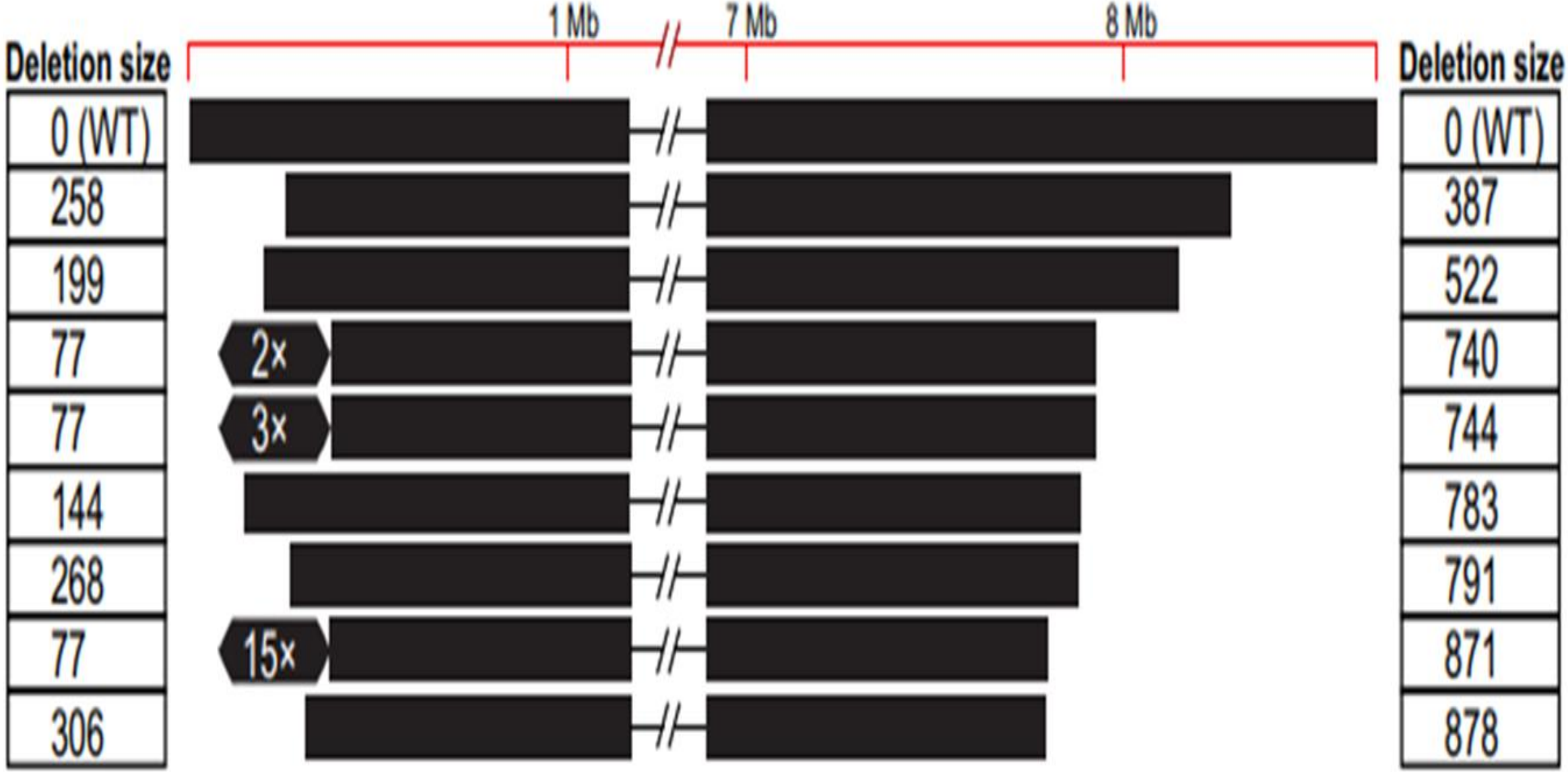
- Rather than reverting to the WT morphology, as would be anticipated if the initial heterogeneity was due to phenotypic plasticity or another form of bistability, the colonies derived from mutant colonies are themselves hypervariable, giving rise to up to nine diverse phenotypes from any single colony.
- Thus, in the course of two cycles of colony outgrowth, an array of colony types that differ in size, shape, and color emerged.



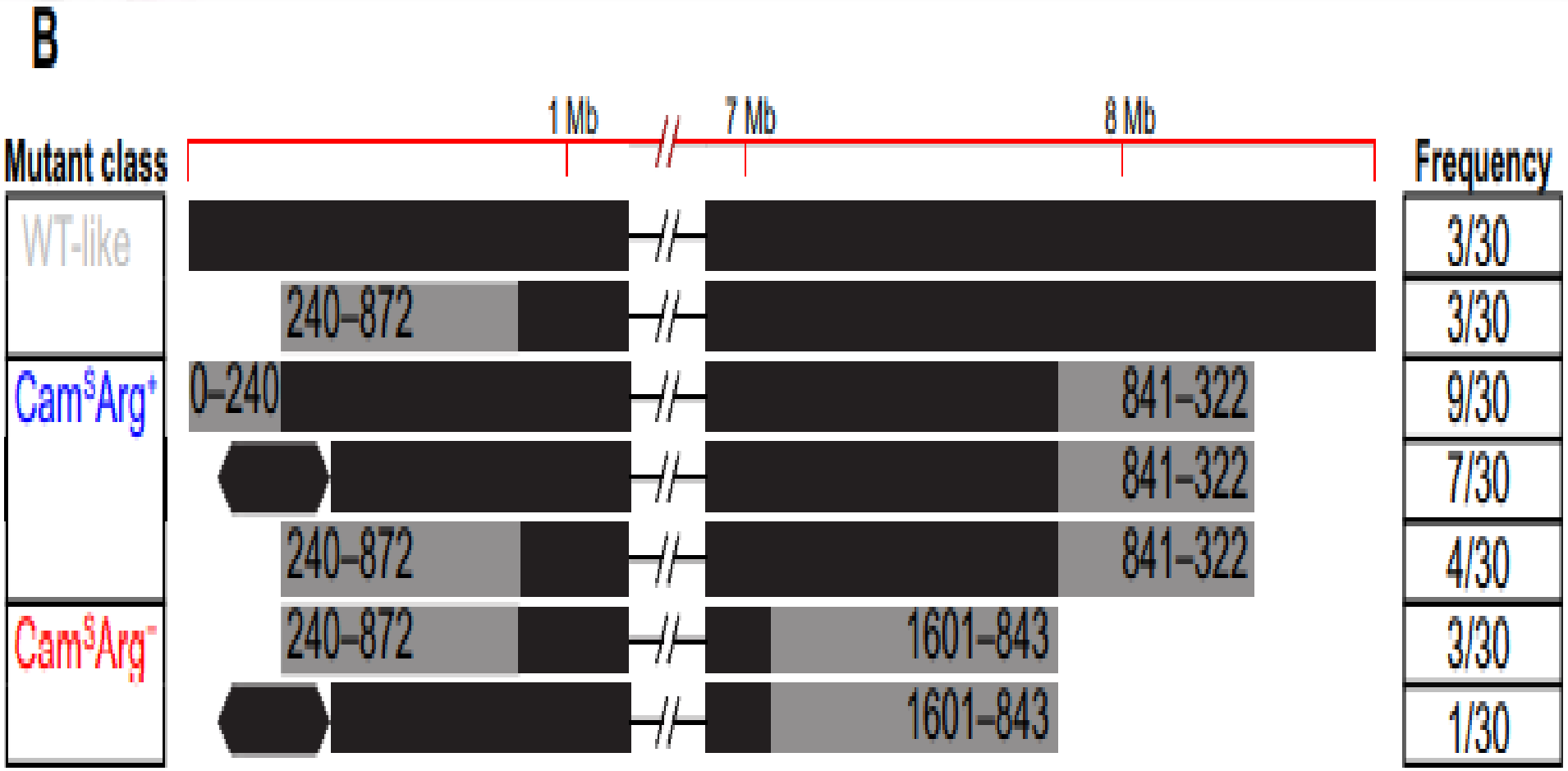
RESULTS AND DISCUSSION

- Using whole-genome sequencing of eight random mutants, we confirmed that these isolates contained profound chromosomal changes.
- large genome deletions were observed at the chromosome ends in all eight strains.
- In three cases, we found an ~297-kb amplification on the left chromosomal arm flanked by the Insertion Sequence IS1649, encoded by SCO0091 and SCO0368. Average sequence coverage of the amplified region suggests that it contains between 2 and 15 copies of this amplification

A


1 Mb
7 Mb
8 Mb
Deletion size
0 (WT)
258
199
77
77
144
268
77
306
2×
3×
15×
Deletion size
0 (WT)
387
522
740
744
783
791
871
878

- Sequencing results were expanded using pulsed-field gel electrophoresis (PFGE) analysis of 30 mutant isolates



RESULTS AND DISCUSSION

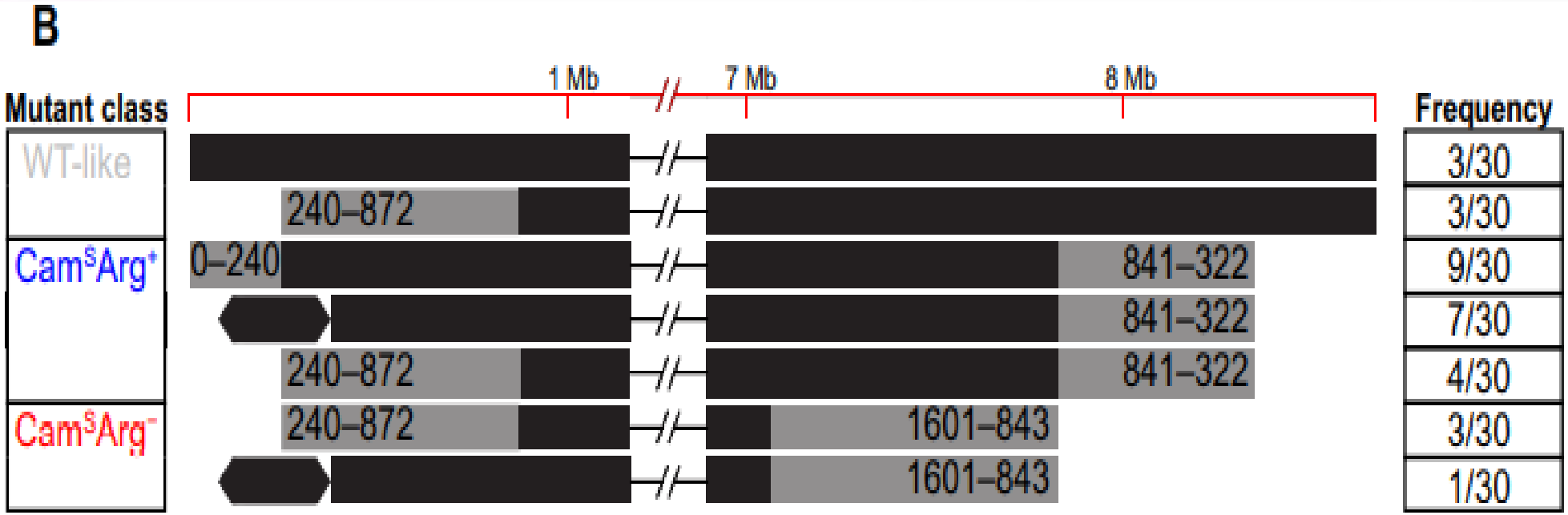
- Consistent with our sequencing results, this analysis revealed that mutants contained variably sized deletions of up to ~240 or ~872 kb on the left chromosome arm and up to 1.6 Mb on the right chromosome arm, deleting more than 1000 genes.
- In addition, 8 of 30 strains contained the same large amplification between copies of IS1649.
- These strains are conspicuously yellow, which might be caused by the overproduction of carotenoids due to the amplification of the crt gene cluster.

RESULTS AND DISCUSSION

- In addition to this and other phenotypic effects associated with these changes, deletions to the right chromosome arm cause the loss of two loci, argG (SCO7036) and cmlR1 (SCO7526)/ cmlR2 (SCO7662), that result in two easily scorable phenotypes:
- arginine auxotrophy and chloramphenicol susceptibility, respectively.

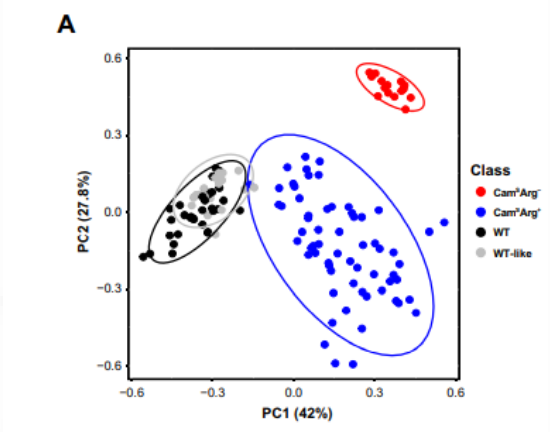
RESULTS AND DISCUSSION

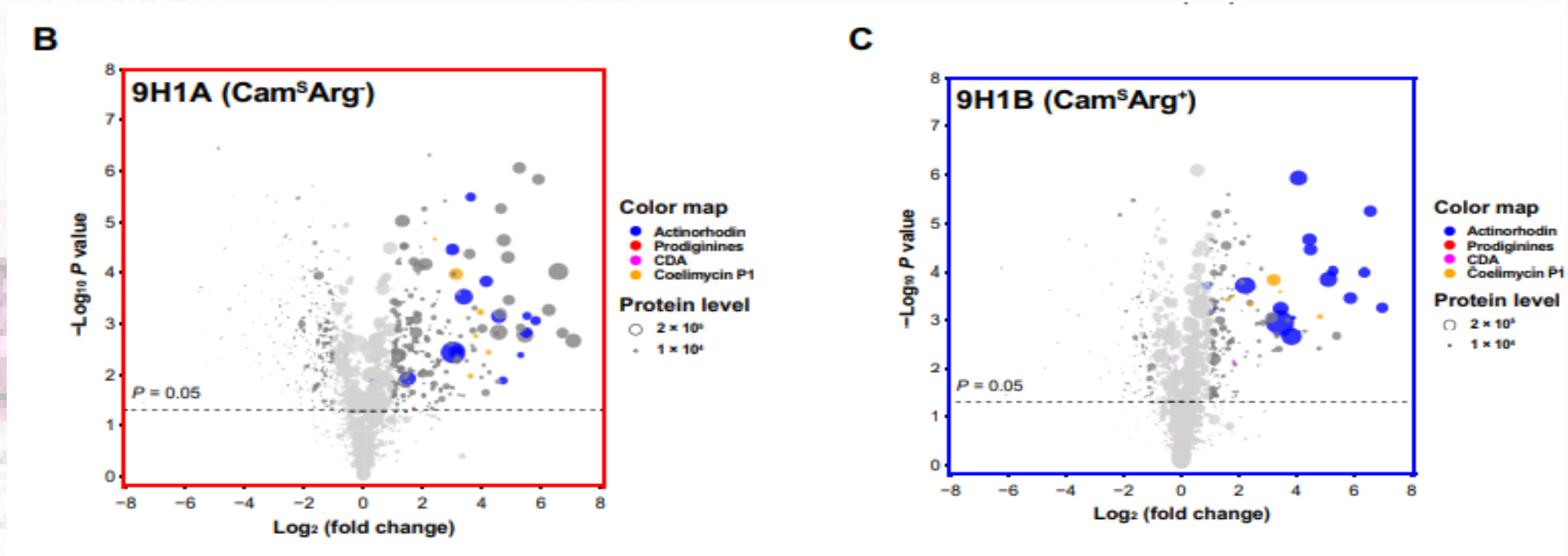
- Scoring these phenotypes allows rapid determination of the minimal size of the deletion on the right chromosome arm in the absence of molecular characterization.
- Chloramphenicol susceptibility indicates a deletion of at least 322 kb, while the addition of arginine auxotrophy indicates a deletion of at least 843 kb.



❑ Mutants increase the production and diversity of antibiotics

- Mutant strains were conspicuously pigmented when compared to their parental WT strains. Because several antibiotics produced by S. coelicolor are pigmented, namely, actinorhodin, prodigines, and coelimycin P1, which are blue, red, and yellow, respectively, we tested whether mutant strains had altered secondary metabolite and inhibitory profiles.
- Secreted metabolites from mutant and WT strains grown on agar surfaces were analyzed using quantitative H nuclear magnetic resonance (NMR) profiling. Principal components analysis.

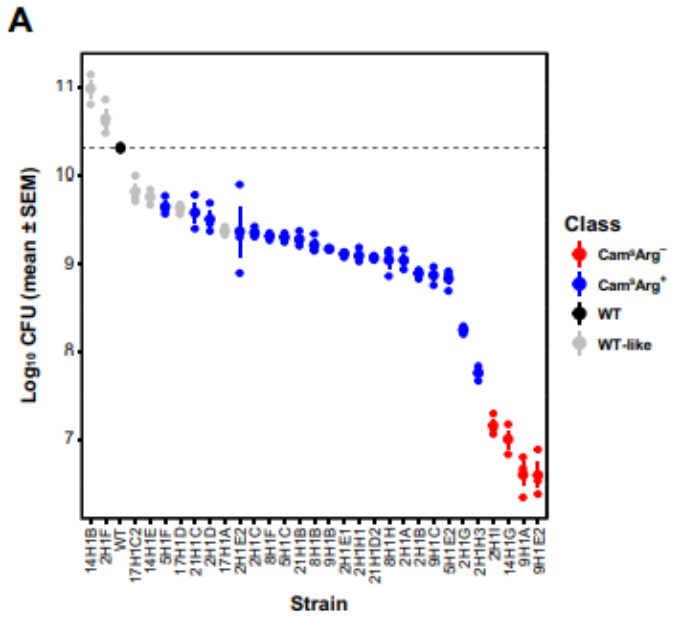


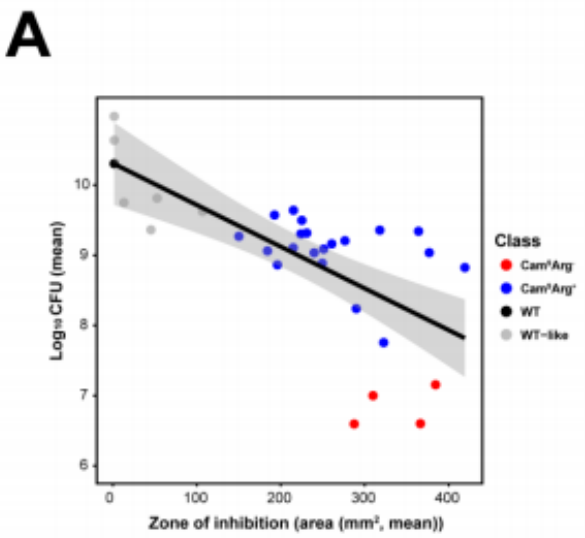


(B and C) Volcano plots of MS-based quantitative proteomics of two representative strains 9H1A (CamS Arg−) (B) and 9H1B (CamS Arg+) (C). Protein level is indicated by the size of the dot, and genes with ≤2-fold change and/or P ≥ 0.05 are grayed out.

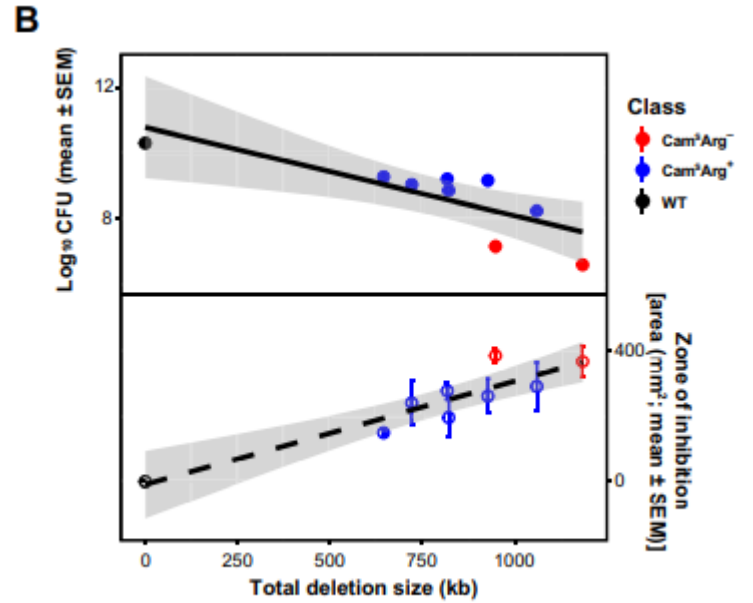
❑ Antibiotic production is coordinated by a division of labor

- ➢ we measured the fitness of each mutant strain by quantifying the number of spores they produce when grown in isolation.
- ➢ mutants produce significantly fewer spores than the WT strain.
- ➢ The reduction in spore production is significantly negatively correlated with antibiotic production.





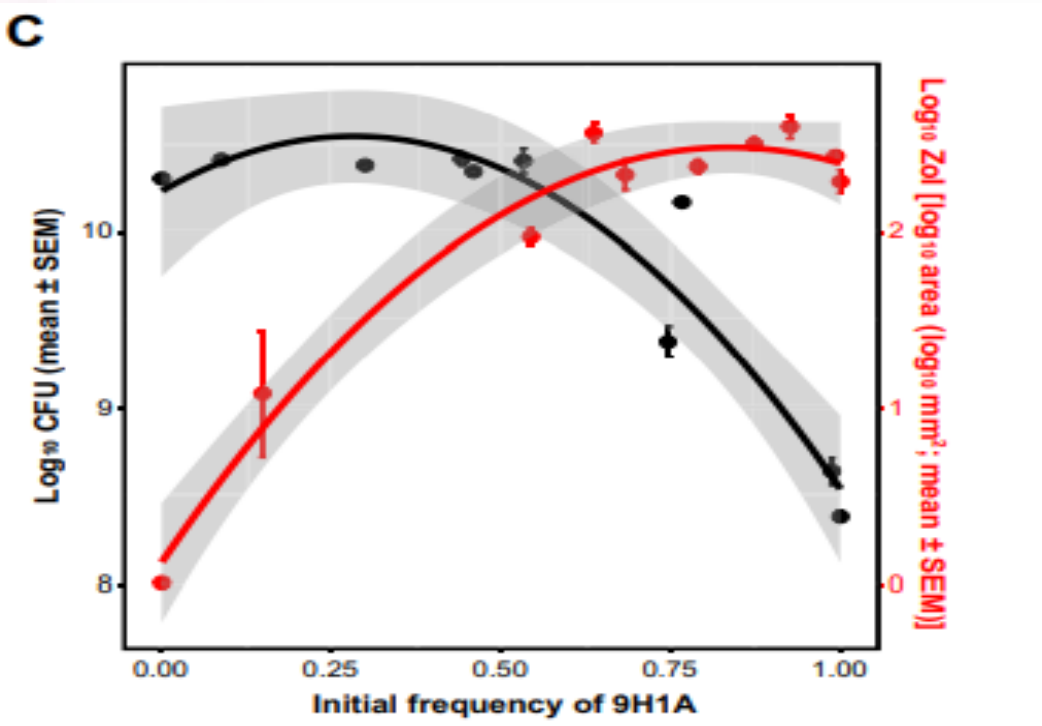
- we observed a significant negative correlation between the size of the genome deletion and colony-forming unit (CFU) and a positive correlation between deletion size and bioactivity against B. subtilis .



- To examine the effects of mutant strains on the colony as a whole, we mixed mutant strains with their WT parent at increasing frequencies and quantified colony-wide spore production and the ability of these mixtures to kill B. subtilis.

- Results of these experiments, support two important conclusions:

(i) Increasing fractions of mutants lead to increased antibiotic production
(ii) although mutant strains have individually reduced fitness



RESULTS

- Genomic instability and phenotypic heterogeneity are coupled
- Mutants increase the production and diversity of antibiotics
- Antibiotic production is coordinated by a division of labor

Thank you