GosNIIgenetika,
RUSSIA

Analysis of differentiation, metabolism, genetic properties in microorganisms after spaceflights on satellites Foton-M2 and Foton-M3.

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OBJECTIVES

To detect genetic changes and modification of *Streptomycetes* caused by spaceflight factors, e.g. microgravity, radiation

- Plates prepared in Moscow
- Shipped to Kazakhstan at 5-15ºC/1 day
- Kept at 2-10ºC/1 day
- Stowed onboard at 22-24ºC/3 days (mycelium)
- On orbit at 15-20ºC/16 days (sporulation?)
- Shipped to Moscow, returned to lab at 2-10ºC/27 hours).
- Radiation ca. 200-300 mRads on orbit

In 2005 and 2007, experiments with streptomycetes were conducted during 16- and 12-day flights of the Russian Foton-M2 and Foton-M3 spacecrafts.

Why streptomycetes are very suitable for studying spaceflight effects on microbes?

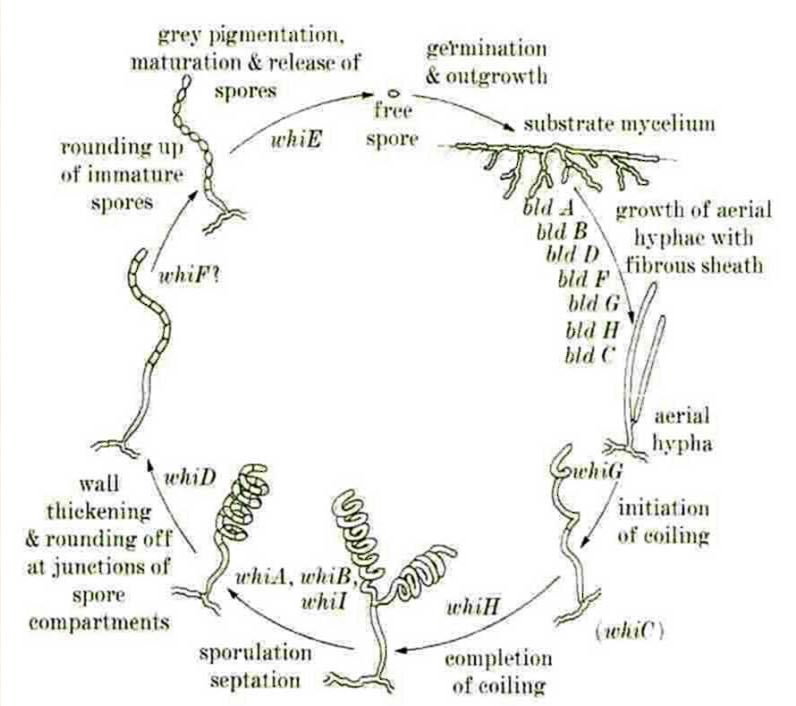
- ***They have a complex developmental cycle.***
- ***They produce a large number of biologically active compounds.***
- ***They are characterized by genome instability.***
- ***These microorganisms are well-studied genetically and analysis of crossing and recombinant progeny is possible.***

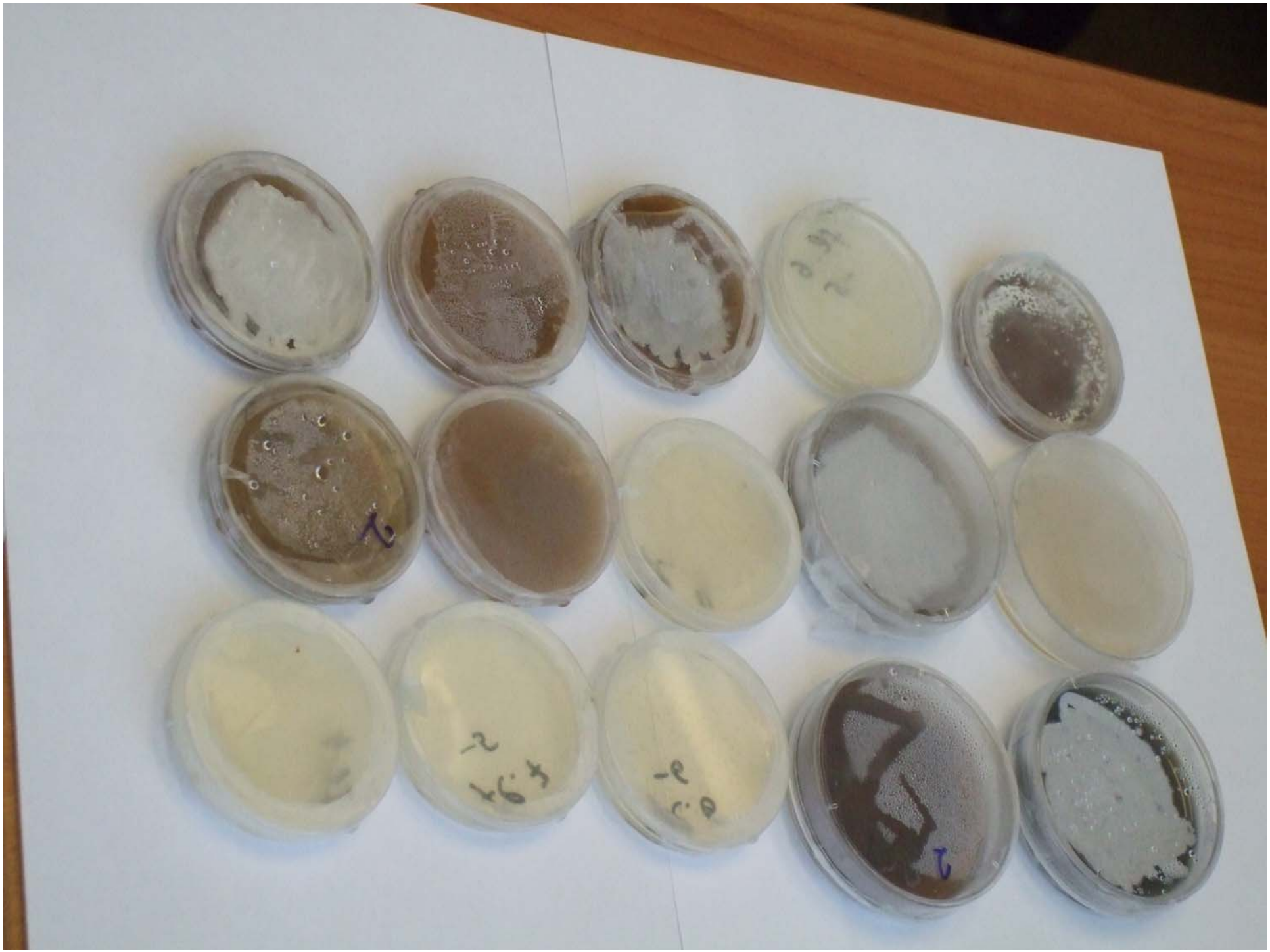
Streptomyces Life Cycle

- **Optimal temperature range 25-30ºC**
- **Spore germination & outgrowth produces substrate mycelium within 5-7 days**
- **Growth and coiling of aerial hyphae by day 14**
- **Sporulation, septation, maturation & spore release, days 15-21**

Typical Development Cycle of *Streptomyces* on Solid Substrates

(according to: Hopwood at al., 1986)






PARAFILM "M"
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Плазмида
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Регенерация
/ тритоны – контроль /
32
#004
REGENERATION
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Улитка
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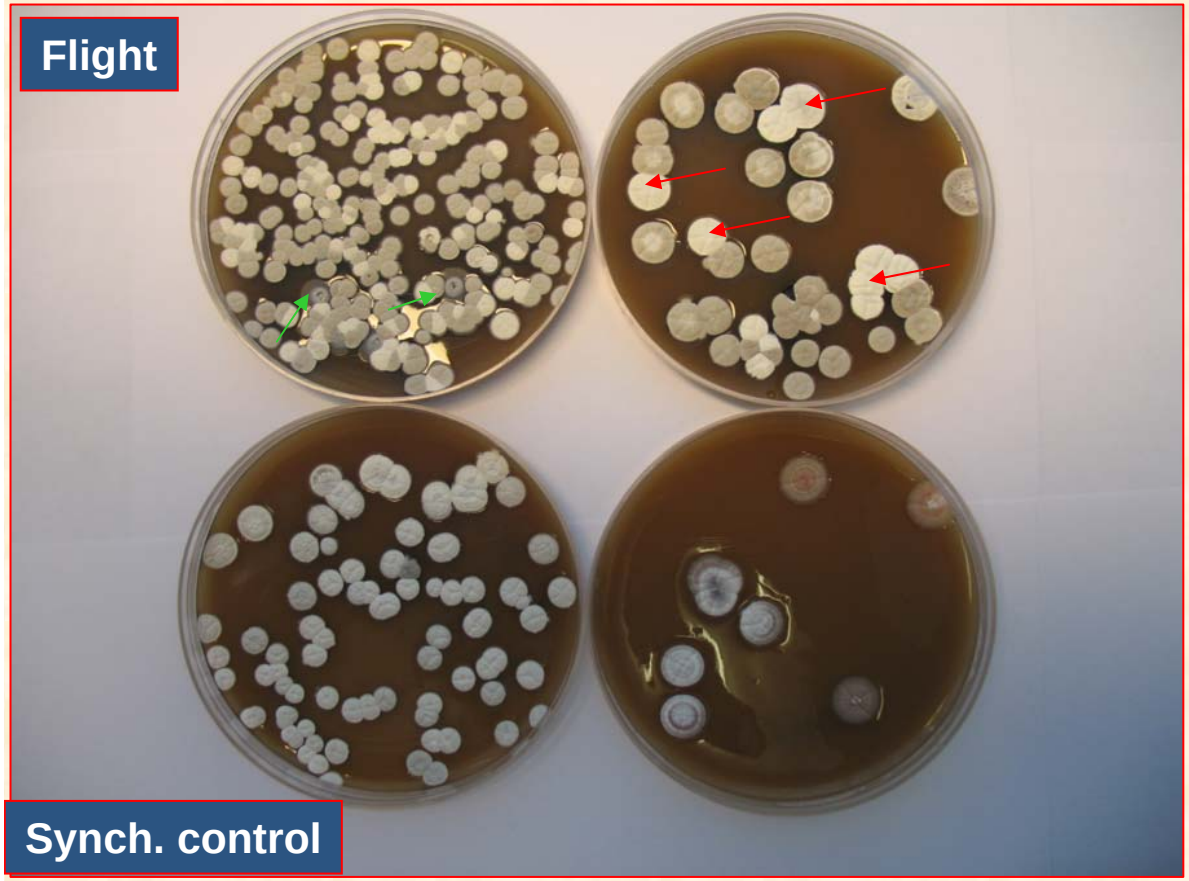
Differentiation and synthesis of biologically active compounds in Streptomyces lividans 66

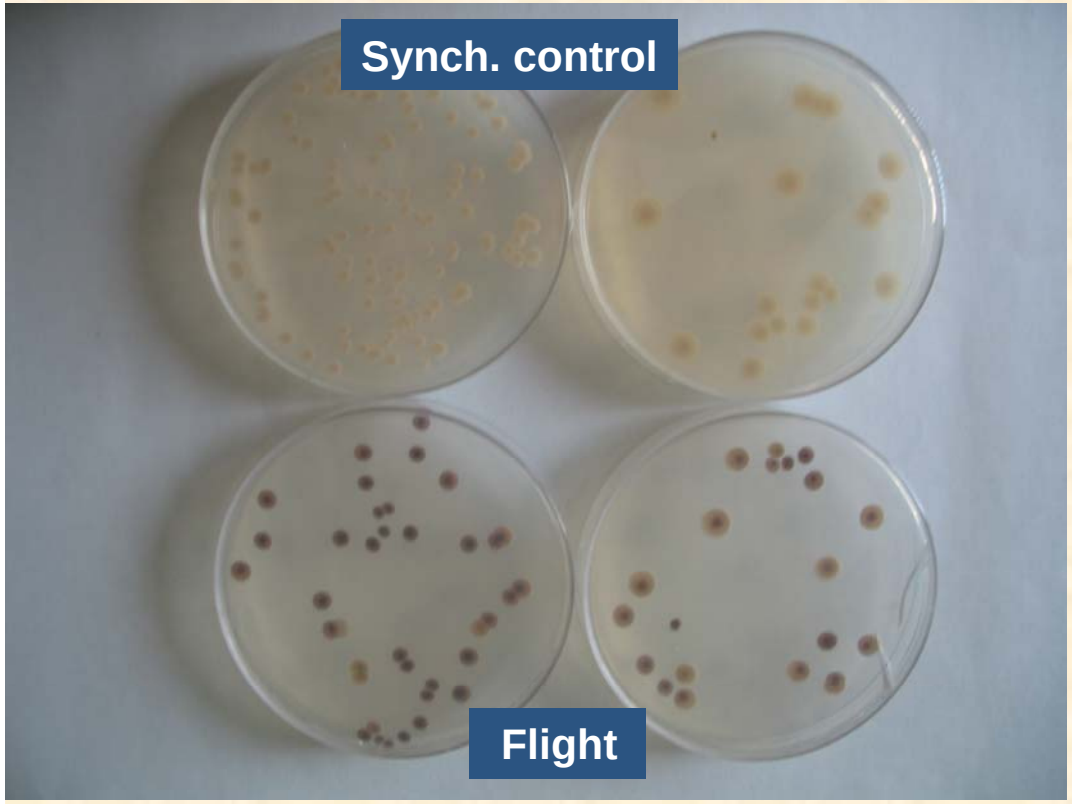
1. Segregation of atypical colonies.



The frequency of occurrence of white, poor-sporulating colonies was 35-40% in Flight versus 1-5% in the Synchronous control samples.

2. Synthesis of actinorhodin.

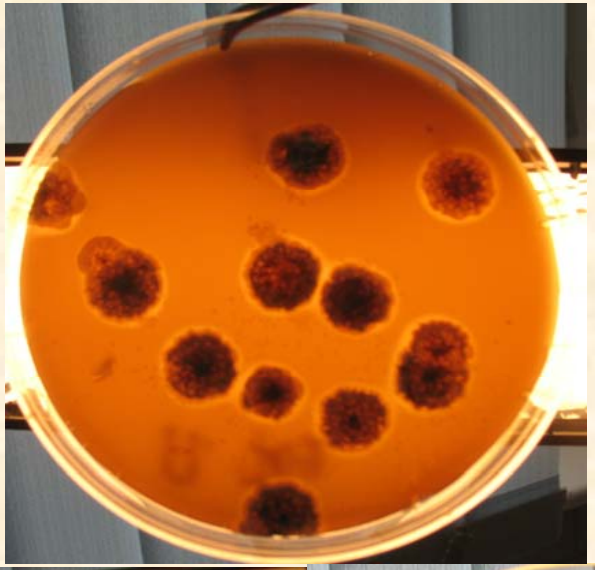
S. lividans has the capability to synthesize actinorhodin, an antibiotic that can render pink to violet color to the substrate mycelium and agar media.



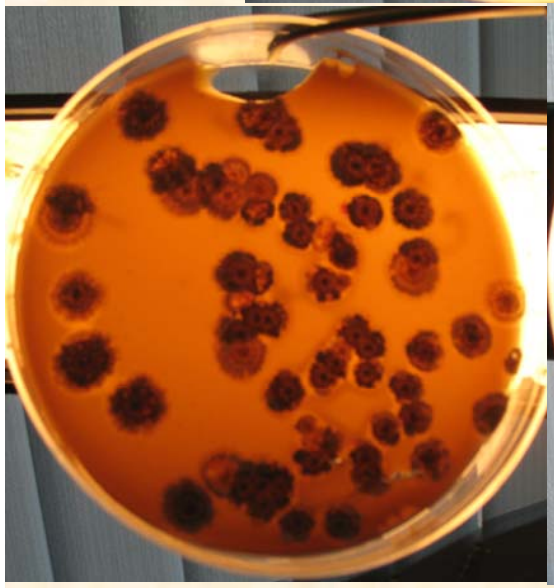
The color of actinorhodin intensity of the Flight colonies was greater than that of the Synch. control colonies.

It can therefore be inferred that spaceflight effects can impact actinorhodin gene expression.

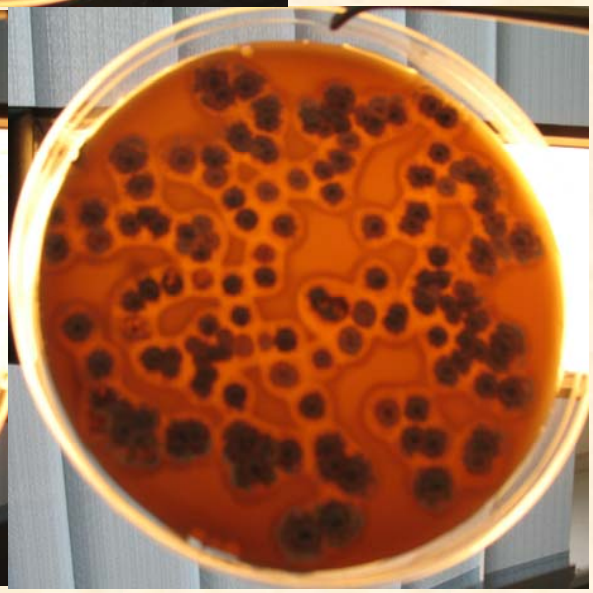
3. Synthesis of amylo-proteolytic enzymes.



Lab. control



Synch. control



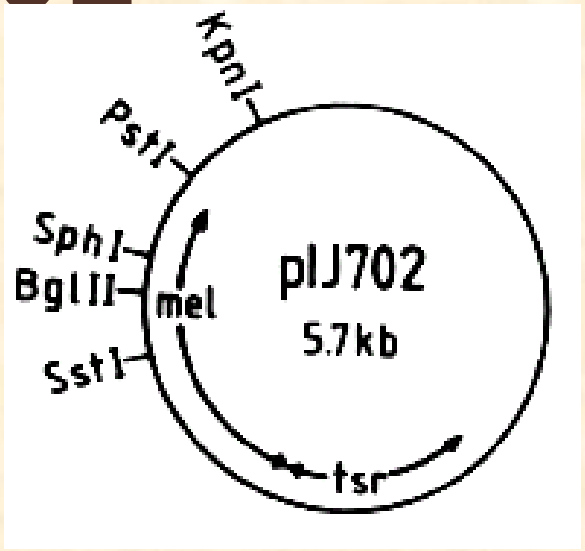
Flight

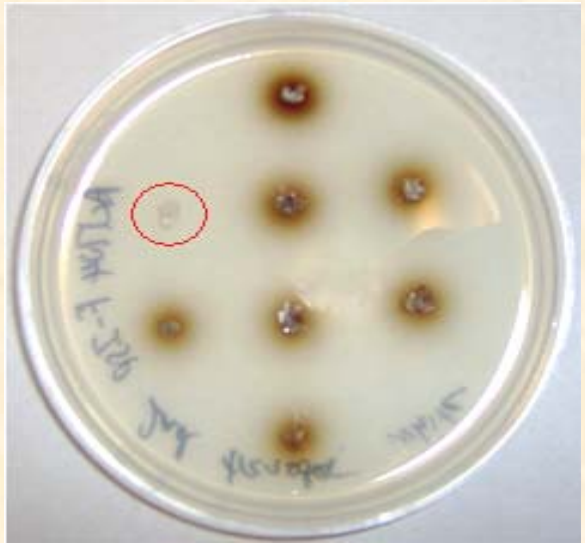
Amylo-proteolytic enzymes produced by cells can be detected on agar media by light areas around colonies.

Light areas of the media were distinctly visible around colonies formed from Flight spores and hardly seen around colonies from the Synch. and Lab. control colonies.

Streptomyces lividans with plasmid pIJ702

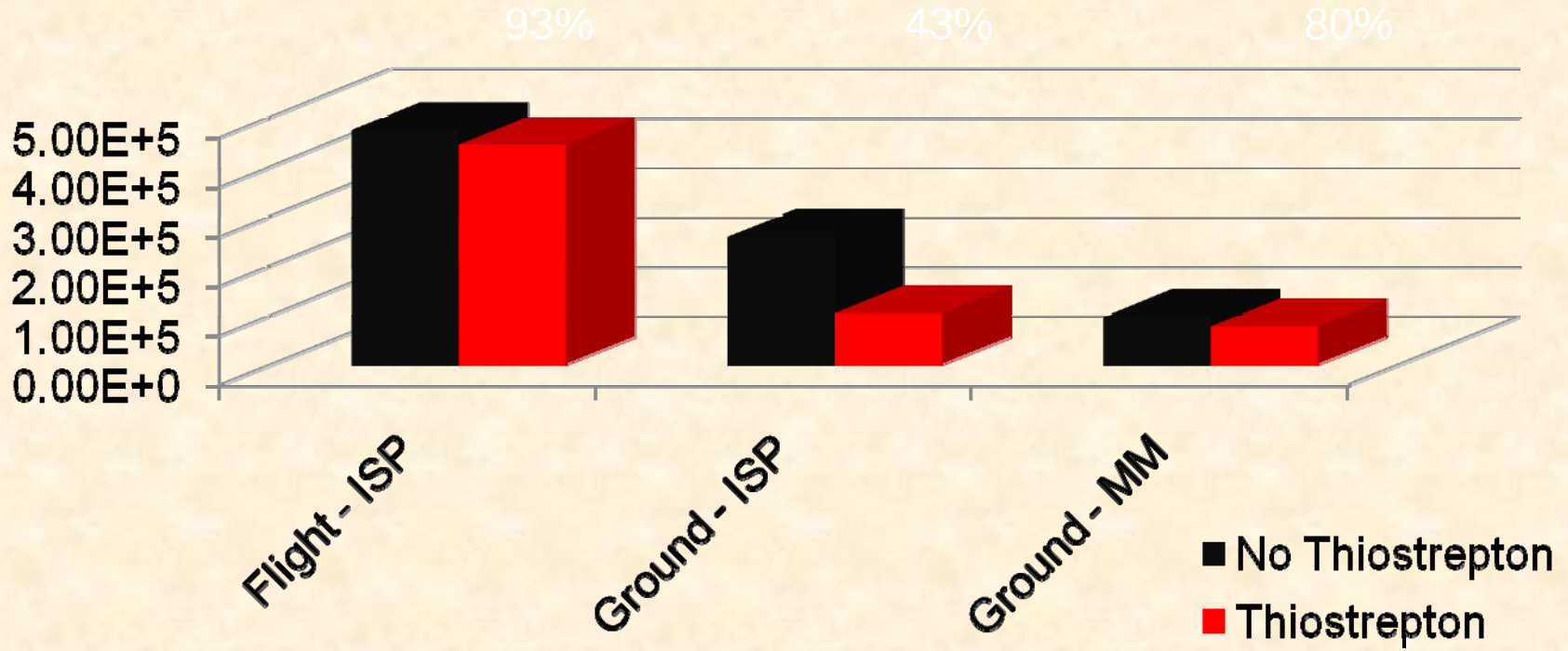
- Plasmid includes marker for thiostrepton (tsr) resistance
- Melanin (mel) gene codes for brown pigment production





PLASMID RETENTION

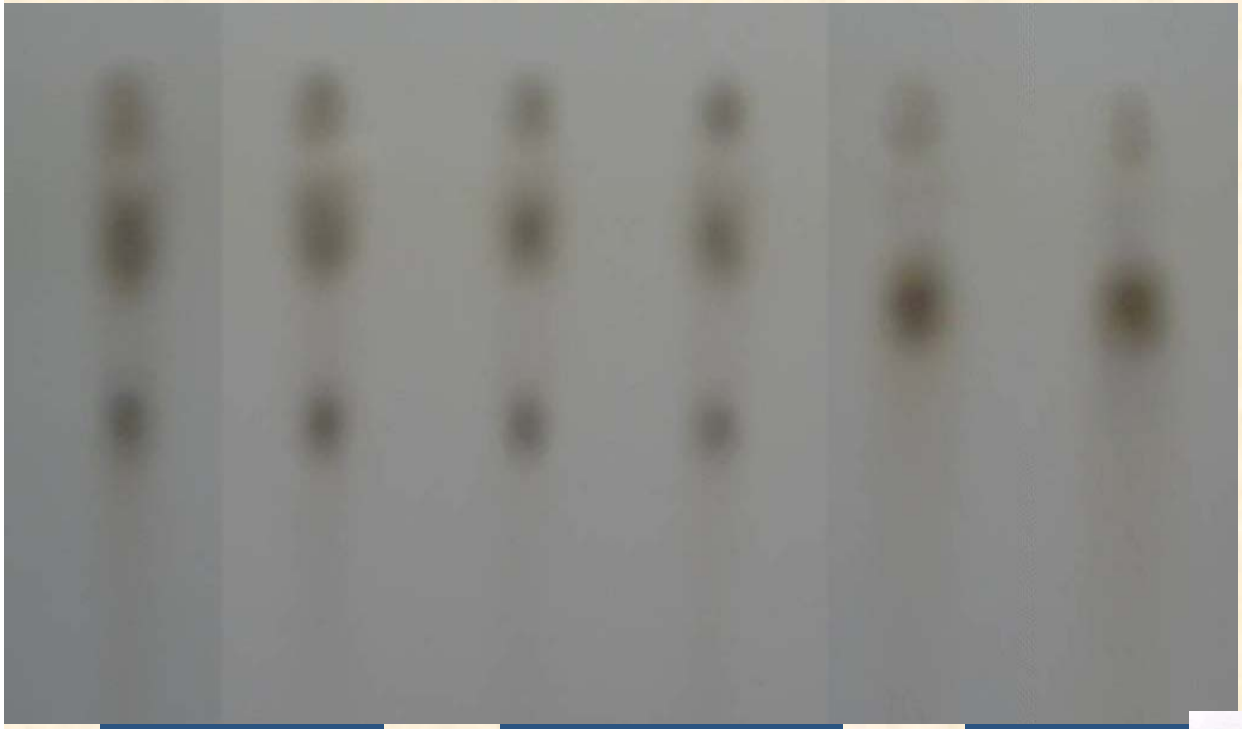
***Streptomyces lividans* Plasmid Retention**



ISP – Rich medium

MM – Minimal medium

5. Study of melanin synthesis and analysis of pigment fractions.



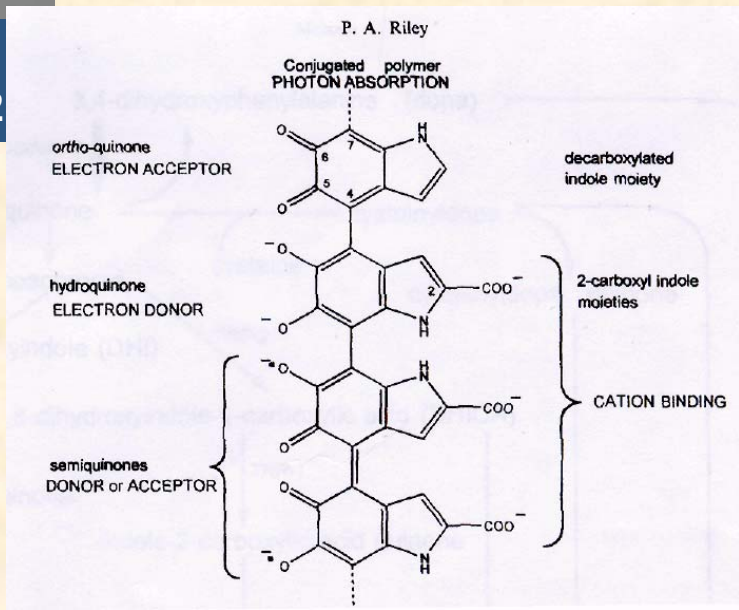
1- extract from agar

2 - extract from fermentation medium

Lab. control		Synch. Control		Flight	
1	2	1	2	1	2

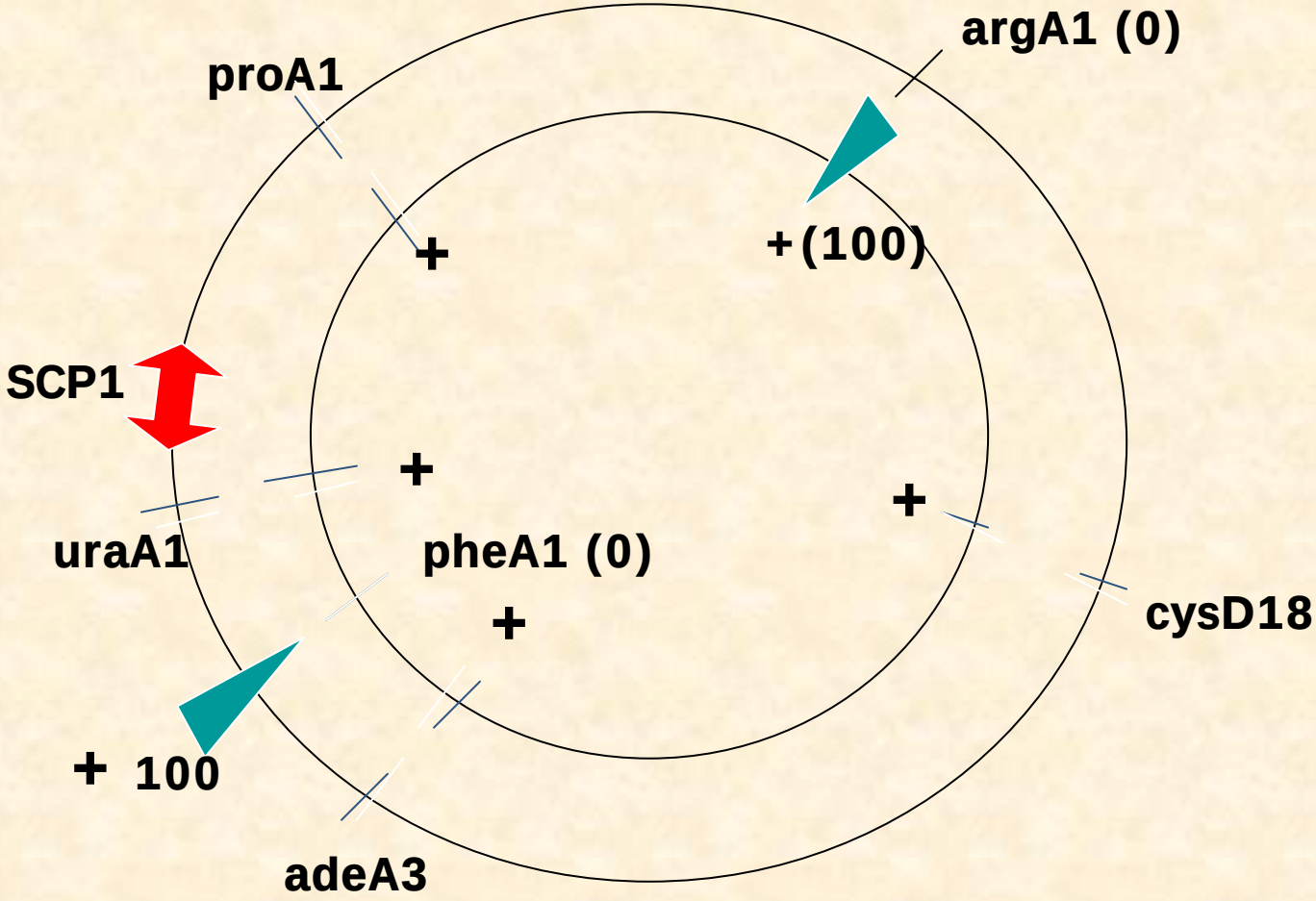
Control melanin samples detected 3 fractions with Rf 0.85, 0.80, and 0.64. Percent content of each fraction: 16.7, 50.6, and 28.8%.

Flight samples detected only two fractions with Rf 0.85 and 0.76 and peak intensity 9.0 and 84.0%



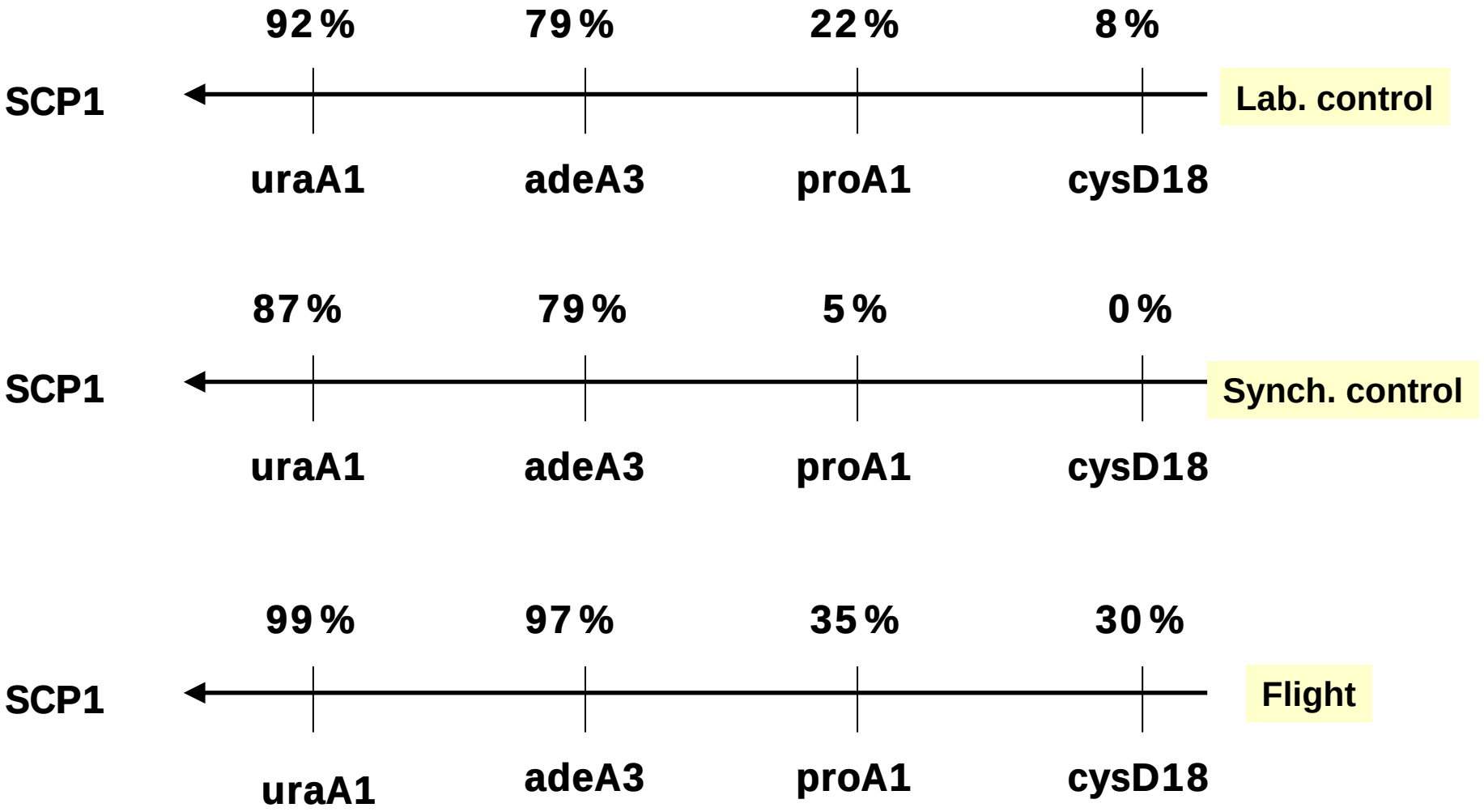
6. Genetic analysis of *S. coelicolor* A3(2) strain crossing.

The Scheme of Crossing



Two circles represent chromosomes of parental strains with genetic markers. Selective alleles are marked with green triangles.

Frequency of the donor-to-recipient transfer of auxotrophic markers



CONCLUSIONS

In space:

- *genetic instability of microbes increases. This is evidenced by changes in their growth, differentiation and sporulation rates.*
- *microbes become capable of synthesizing biologically active compounds at a higher rate as demonstrated by enhanced synthesis of amylo-proteolytic enzymes and actinorhodin.*
- *microbes develop changes in their synthesis and chemical structure of metabolites. It has been found that melanin components change and that specific yield decreases while biomass increases.*
- *gene transfer between different microbial strains occurs at a higher rate, which may produce microbes that have new gene combinations and different physiological and biochemical characteristics.*

ACKNOWLEDGEMENTS

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