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**The Effect of Metabolic Products of *Bacillus galleriae* No 7 J. Jarosz  
on Four Saprophytic Acid-fast Tubercle Bacilli**

The question of whether an active substance or substances from the larvae of *Galleria mellonella* L., acting upon *Mycobacterium tuberculosis*, are produced by the animal organisms or the bacterial flora of these larvae, is still unsettled (1, 2, 3, 4, 5, 7). This was the reason of the investigation of the intestinal microflora of the larvae of the wax moth. The experiments were performed on: 1. *Bacillus galleriae* No 7 J. Jarosz (6) isolated by Hübner (5) from the alimentary tract of larvae of *Galleria mellonella* L. 2. Saprophytic acid-fast bacilli: *Mycobacterium smegmatis*, *Mycobacterium phlei*, *Mycobacterium pellegrino* and *Mycobacterium 279*.

*Bacillus galleriae* No 7 was grown on Sauton's medium in Roux bottles, at a temperature of 37°C. The strain was incubated for 24, 48, 72, 96 and 120 hrs. After 24 hrs. incubation a white gray film of *Bacillus galleriae* No 7, thickening with time, spread over the whole surface of the medium. The colour of the medium did not change.

The colour of the medium changed from yellow to brown-red. The filtrate from the culture of *Bacillus galleriae* No 7 was prepared by separating the film formed by the bacteria by centrifuging (8.000.G for 20 minutes) and filtering through Schott's bacterial filter 17 G-5.

The sterile filtrate of a 1, 2, 3, 4 and 5-day culture of *Bacillus galleriae* No 7 was added to Sauton's medium in the following combinations:

1. 2.5 ml Sauton's medium + 2.5 ml filtrate (50% × 50%)
2. 3.0 ml „ „ + 2.0 ml „ (60% × 40%)
3. 3.5 ml „ „ + 1.5 ml „ (70% × 30%)
4. 4.0 ml „ „ + 1.0 ml „ (80% × 20%)
5. 4.75 ml „ „ + 0.25 ml „ (95% × 5%)

The suspensions of *Mycobacterium smegmatis*, *Mycobacterium phlei*, *Mycobacterium pellegrino* and *Mycobacterium 279* were ground in bacterial mortars

in order to obtain single cells. Then they were sown first on Sauton's medium and second on the medium with the metabolites in question. The initial concentration of the tubercle bacilli suspension was 1 mg/ml. Tubercle bacilli were sown on the medium with metabolites and without metabolites in quantities of 0.1 ml, i.e. 0.1 mg of fresh weight of bacilli. The bacilli on the medium without metabolites acted as control. The results were checked after 5 days. The experiment was repeated 3 times.

## RESULTS

The effect of the products of metabolism of *Bacillus galleriae* No 7 isolated from the alimentary tract of larvae of *Galleria mellonella* L. on *Mycobacterium smegmatis*, *Mycobacterium phlei*, *Mycobacterium pellegrino* and *Mycobacterium 279*.

Products of metabolism obtained after 48 hrs. of incubation of *Bacillus galleriae* No 7 affected the growth of *Mycobacterium smegmatis* and *Mycobacterium phlei* in such a way that they caused the change in the form of growth from the surface film into the sediment. This was found to occur at the following concentrations of metabolites: 50%, 40%, and 30%. *Mycobacterium pellegrino* formed suspensions at the concentrations of 50%, 40%, 30% and 20% of metabolites in the medium, whereas with *Mycobacterium 279* such a growth was observed at concentrations of 50%, 40% and 30%. The addition of 20% of the metabolites in question gave a growth of *Mycobacterium 279* in the form of both film and sediment. After 72 hours of incubation of the intestinal bacteria, *Bacillus galleriae* No 7 gave the following results:

The growth of *Mycobacterium smegmatis* in the form of sediment occurred at concentrations of 50%, 40%, 30% and 20% of metabolites in the medium, while for *Mycobacterium phlei* a sediment appeared at concentrations of 50%, 40% and 30% of metabolites. The strains of *Mycobacterium pellegrino* and *Mycobacterium 279* formed a sediment at concentrations of 50%, 40%, 30% and 20% of metabolites.

After 96 hours of incubation of strain *Bacillus galleriae* No 7, the effect of metabolites can be described as follows: *Mycobacterium smegmatis* in the form of sediment at concentrations of 50%, 40%, 30% and 20%; at a concentration of 10% of metabolites the formation of both the film and sediment was observed. *Mycobacterium phlei* gave sediment at concentrations of 50%, 40% and 30% of metabolites, while 20% gave both film and sediment. *Mycobacterium pellegrino* and *Mycobacterium 279* at concentrations of 50%, 40%, 30%, 20% and 10% grew in the medium in the form of sediment.

After 120 hours' incubation of *Bacillus galleriae* No 7, metabolites at concentrations of 50%, 40%, 30% and 20% changed the growth of strains

Table 1. Metabolites of *Bacillus galleriae* No 7 obtained after 24 hours of incubation, at 37°C on Sauton's medium, did not affect the growth of the tubercle bacilli

Strain	Incubation time of strain no 7	Sauton's medium/ metabolites									Control
		50%/50%	60%/40%	70%/30%	80%/20%	90%/10%	95%/5%				
<i>Mycobacterium smegmatis</i>	24 h										
	48 h	■	■	■	■	■	■	■	■	■	
	72 h	■	■	■	■	■	■	■	■	■	
	96 h	■	■	■	■	■	■	■	■	■	
	120 h	■	■	■	■	■	■	■	■	■	
<i>Mycobacterium phlei</i>	24 h										
	48 h	■	■	■	■	■	■	■	■	■	
	72 h	■	■	■	■	■	■	■	■	■	
	96 h	■	■	■	■	■	■	■	■	■	
	120 h	■	■	■	■	■	■	■	■	■	
<i>Mycobacterium pellegrino</i>	24 h										
	48 h	■	■	■	■	■	■	■	■	■	
	72 h	■	■	■	■	■	■	■	■	■	
	96 h	■	■	■	■	■	■	■	■	■	
	120 h	■	■	■	■	■	■	■	■	■	
<i>Mycobacterium 279</i>	24 h										
	48 h	■	■	■	■	■	■	■	■	■	
	72 h	■	■	■	■	■	■	■	■	■	
	96 h	■	■	■	■	■	■	■	■	■	
	120 h	■	■	■	■	■	■	■	■	■	

film     
  precipitate     
  film and precipitate

of *Mycobacterium smegmatis* from film to sediment. 10% and 5% concentrations of metabolites allowed the formation of both film and sediment. *Mycobacterium phlei* in the presence of the liquid obtained from the culture medium of *Bacillus galleriae* No 7 at 50% and 40% concentrations formed sediment, while concentrations of 30%, 20%, 10% and 5% of metabolites allowed the bacterium to produce film and sediment. *Mycobacterium pellegrino* and *Mycobacterium 279* formed sediment at concentrations of 50%, 40% and 30%. The concentration of 20% brought about the appearance of film and sediment.

## DISCUSSION

The results of 5 successive experiments indicated that the metabolites of *Bacillus galleriae* No 7 isolated from the alimentary tract of larvae of *Galleria mellonella* L. added to Sauton's medium, affected the growth of *Mycobacterium smegmatis*, *Mycobacterium phlei*, *Mycobacterium pellegrino* and *Mycobacterium* 279. The change in the growth pattern of tubercle bacilli from a film to the form of sediment occurred after the addition of the metabolites obtained from a 2-day-old culture of bacteria of *Bacillus galleriae* No 7.

It was noted that the longer the time of incubation the smaller quantity of the metabolites added to Sauton's medium affected the growth of tubercle bacilli. Products of metabolism obtained after 120 hrs. incubation of *Bacillus galleriae* No 7 changed or weakened the growth of the tubercle bacilli in each quantity added to the medium. This is probably connected with increase at the concentrations of metabolites as the incubation time of *Bacillus galleriae* No 7 is prolonged. A change in the form of the tubercle bacilli colonies on a medium with metabolites allows me to assume the existence of an active substance secreted into the medium by bacteria of *Bacillus galleriae* No 7. This substance probably disturbs the cell metabolism, as a result of which the tubercle bacilli form a sediment.

## Conclusions

1. *Bacillus galleriae* No 7 of intestinal bacteria of the larvae of *Galleria mellonella* L. secretes a substance or substances which weaken and change the growth of *Mycobacterium smegmatis*, *Mycobacterium phlei*, *Mycobacterium pellegrino* and *Mycobacterium* 279.

2. The activity of the products of the metabolism of *Bacillus galleriae* No 7 depends upon the time of incubation of this strain.

3. Metabolites of *Bacillus galleriae* No 7 incubated for 24 hours do not change the growth of the tubercle bacilli used in the experiment.

4. The optimal time of incubation of this strain varies between 72 and 96.

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## Wpływ produktów metabolizmu *Bacillus galleriae* nr 7 J. Jarosz na cztery saprofityczne prątki kwasooporne

### Streszczenie

Celem pracy było zbadanie wpływu produktów metabolizmu *Bacillus galleriae* No 7 wyizolowanego z przewodu pokarmowego larw *Galleria mellonella* L. na *Mycobacterium smegmatis*, *Mycobacterium phlei*, *Mycobacterium pellegrino* i *Mycobacterium* 279. Wykazano, że metabolity *Bacillus galleriae* No 7 osłabiają bądź zmieniają wzrost z kozucha na osad badanych, saprofitycznych prątków kwasoopornych. Ilość i działanie metabolitów uzależnione są od czasu inkubacji *Bacillus galleriae* No 7.

## Влияние продуктов метаболизма *Bacillus Galleriae* № 7 J. Jarosz на четыре сапрофитные кислотоустойчивые палочки

### Резюме

Целью настоящей работы было изучение влияния продуктов метаболизма *Bacillus Galleriae* № 7, выделенного из пищеварительного тракта личинок восковой моли, на *Mycobacterium smegmatis*, *Мyc. phlei*, *Мyc. pellegrino*, *Мyc. 279*. Установлено, что метаболиты *Bacillus Galleriae* № 7 ослабляют или изменяют рост кислотоустойчивых сапрофитных палочек. Вместо мехообразного поверхностно растущего слоя бактерий наблюдается образование бактериального осадка в питательной среде. Количество метаболитов и их активность зависят от срока инкубации *Bacillus Galleriae*.

