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EXPERIMENTAL ARTICLES

# Effect of Distant Interactions on Growth and Development of Streptomycetes

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**Abstract**—Regulation of streptomycete growth and development by distant interactions of physical nature was shown using a vial-in-vial experimental setup, providing chemical isolation of the inducer and detector cultures. Some effects of distant interaction were observed with *Streptomyces netropsis* proliferating submerged culture as an inducer and a surface culture of the same strain as a detector. The growth rate of the detector strain doubled. Spore germination was stimulated, as was indicated by a 30% increase in the number of colony-forming units (CRU) in the detector culture plated as a spore suspension. The phase variation spectrum also changed, with an increased share of the colonies of the morphotype predominant under the standard conditions.

*Keywords*: streptomycetes, distant interactions, viability, phase variation spectrum

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Investigation of the patterns and mechanisms responsible for the regulation of growth and development in living organisms is important for understanding of the mechanisms and forms of their adaptation and survival, as well as for biotechnologies based on the living systems of different levels of organization. Three main channels of signal transmission are known: mechanical (contact), the least studied one (Konev and Mazhul', 1977); chemical, the best studied (Winans and Bassler, 2008; Voloshin and Kaprel'yants, 2004); and the one mediated by the physical fields of living objects. Communication mediated by physical fields is also known as "distant interaction" (DI) (Kaznacheev and Mikhailova, 1981), "mitogenetic radiation" (Gurvich, A.G. and Gurvich, A.D., 1945), "secondary biogenic radiation" (Kuzin, 1997), or "physical signal" (Matsuhashi et al., 1995). This terminological variety may be explained by the scattered nature of the groups of researchers working in the field, as well as by the difficulty of signal registration. DI was determined as "interaction of organisms remote by a considerable distance," so that isolation prevents chemical and mechanical contacts (Kaznacheev and Mikhailova, 1981).

Biotesting is the main method used in DI investigation. The source of radiation and the organism perceiving this radiation as a signal are termed *emitter* (inducer) and *detector*, respectively. Reaction of the detector to the emitter is used to assess the existence and biological role of the signal. The biodetector method is presently the only direct way to confirm information transmission between organisms by a carrier of a physical nature.

The possibility of information transfer between living organisms mediated by physical fields was originally demonstrated by Gurvich in the early 20th century using onion roots as both emitter and detector (Gurwitsch, A. and Gurwich, L., 1925). Growing onion roots were shown to emit the rays stimulating cell division (mitoses) in the roots of another plant isolated from the first one. Due to its key property, i.e., the ability to stimulate mitoses, this radiation was termed mitogenetic radiation (MGR). Physically, this is very weak ultraviolet light (tens to hundreds quanta per second) with wavelengths within the 200–300-nm range (Gurvich, A.G. and Gurvich, A.D., 1945). MGR emission was shown for germinating seeds of a number of plants, eggs of insects and sea urchins, the cells of hydra, frog and mammalian blood cells, dissected muscles, bacteria, and yeasts. A total of over 30 biological objects belonging to all kingdoms of living organisms (Rahn, 1936; Wolff and Ras, 1931) were represented. Thus, it was shown that, rather than being species-specific, this radiation is a universal medium for communication between living objects of different taxonomic groups. Yeasts were subsequently accepted as the most convenient MGR detector (Gurvich, A.G. and Gurvich, A.D., 1945; Rahn, 1936). Since the early works, MGR was found to affect, apart from stimulation of cell division, also modifications in morphology and biochemistry of the organisms (Rahn, 1936). Later the physical fields were shown to induce various pathological changes in tissue cultures of higher animals, while, apart from UV, visible light,

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infrared radiation, and radio waves were shown to act as physical carriers of the signal (Kaznacheev and Mikhailova, 1981).

After γ-irradiation, plant seeds and other biological objects were shown to emit another, *secondary biogenic* radiation, which stimulates the germination and development of seeds and other biodetectors (Kuzin, 1997). The authors of the present work have shown the effect of DI on behavior of *Pseudomonas fluorescens* cells, which were used as both emitters and detectors (Nikolaev, 2000). The DI effect was assessed as the number of the cells attached to glass in a submerged detector culture. In the variants when the emitter and detector cultures could exchange information by both physical and chemical signals, the number of free-moving (unadsorbed) cells was 2 to 30 times (9 times on average) higher than in the control. The chemical signal alone (a gaseous antiadhesive compound) caused only a 10% increase in the number of unadsorbed cells. Action of the emitter culture alone resulted in a 50% increase in the number of free-floating cells. Thus, synergism of DI and chemical regulators occurred.

It should be noted that a number of authors doubt the existence of mitogenetic radiation (Wolpert, 1992; Hollander and Claus, 1937; Quickenden and Tilbury, 1985), due to data scattering, the capricious nature of DI phenomenon, and insufficient experimental evidence.

Search for the experimental systems providing for reproducible and easily recordable effects of DI is therefore of importance. It was previously concluded that DI effects were more pronounced under stress conditions or during the change of the stages of microbial growth (Matsuhashi et al., 1995; Nikolaev, 2000a, 2000b). For example, the DI effect was convincingly shown for a model of bacillar spore germination (Matsuhashi et al., 1995, 1996a, 1996b, 1997a, 1997b). Under unfavorable conditions (1% KCl and elevated temperature of 44°C), spore germination and growth of *Bacillus carbonifillus* were found to increase several fold by the physical signal emitted by actively growing cultures of the same or other bacterial species. Efficiency of spore germination (percentage of spores germinating under optimal conditions) was up to 100%, while almost no germination was observed in the absence of the physical signal. The authors determined the sonic nature of the signal stimulating spore germination (Matsuhashi et al., 1998).

Streptomycetes are a convenient model for investigation of development of microbial cultures. While chemical regulation of sporulation and antibiotic synthesis by members of this microbial group are well studied (Khokhlov, 1988), no information exists on DI-mediated control. Moreover, investigation of the role of DI in the regulation of development of these biotechnologically important mycelial actinobacteria is of considerable applied interest.

The goal of the present work was to investigate the effects of distant interactions of growth and development of the cultures of *Streptomyces netropsis* ISP-5259, which are poorly preserved in collections.

## MATERIALS AND METHODS

**The subject of research** was *Streptomyces netropsis* strain ISP-5259 from actinomycete culture collection of the Winogradsky Institute of Microbiology. The strain belongs to the group of poorly stored cultures (Kuznetsov and Rodionova, 1971). The culture was maintained on agarized oat medium (Kuznetsov, 1972). Submerged cultures were grown in 250-mL conical Erlenmeyer flasks with 50 mL of no. 2 maize medium (Kuznetsov, 1972), on a rotor shaker (120 rpm) at 28°C.

Submerged culture of *S. netropsis* grown for one day with agitation and subsequently incubated under static conditions for 24 h at 28°C was used as an emitter (DI source). By the onset of the experiment, the culture was in the stage of growth deceleration and transition to the stationary phase (according to biomass accumulation data). The cultures prepared in other ways had no effect on growth of the detector culture.

The culture of the same streptomycete strain obtained on no. 2 agar medium inoculated with a spore suspension stored previously for four months at 10°C was used as a detector. The spore suspension was prepared by scraping off the surface of a 7-day culture grown on a solid medium, resuspending the spores in tap water, and filtration through an SF-4 glass filter (Kuznetsov, 1972).

**The experimental setup** was constructed using the vial-in-vial principle (Fig. 1). The emitter culture (5 mL) prepared as described above was transferred into a quartz vial (17 mm diameter, 37 mm height, 1 mm wall thickness) and sealed with a rubber stopper with 2–3 mL of air in the headspace. In the control variants, the quartz vial was filled with 5 mL of sterile no. 2 medium or water. The vial with the inducer culture was placed in the center of a Koch dish (88 mm diameter, 50 mm height) upon a fresh lawn of the detector culture. The lawn was prepared by pouring into the dish the melted oat medium (40 mL) inoculated with 0.1 mL *S. netropsis* cell suspension stored for four months at 10°C.

**Number of viable streptomycete spores** was determined by plating spore suspensions on solid oat medium and enumerating the colony-forming units (CFU). The plates were incubated for 7 days at 28°C.

**Assessment of the grown streptomycete colonies.** Since the substrate mycelium of streptomycetes grown in surface culture penetrates into the medium, assessment of the biomass of these microorganisms is difficult. Therefore an indirect method of digital photodensitometry was developed for the purpose. Koch dishes with the colonies were photographed using a Canon A95 digital camera and the percentage of the

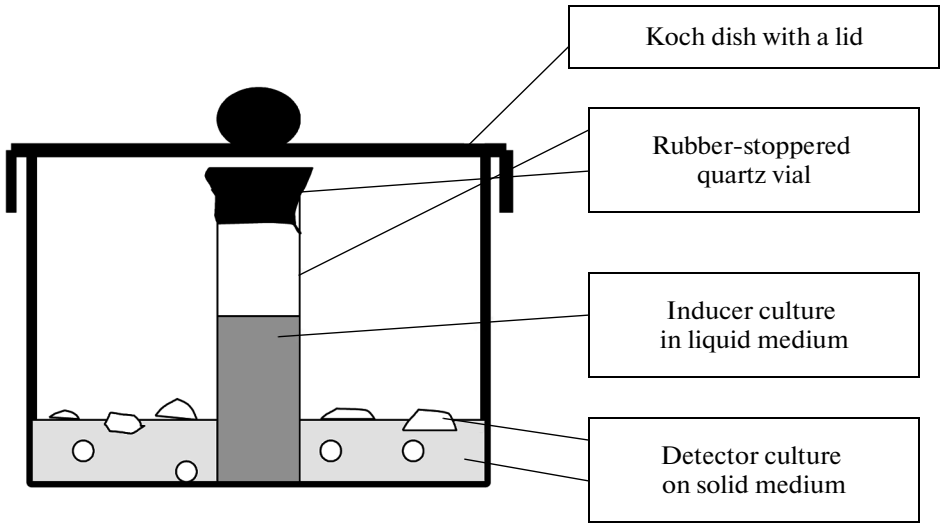


**Fig. 1.** Experimental setup for investigation of distant interaction in streptomycetes (see description in the text).

area occupied by colonies was determined using the Adobe Photoshop 7.0 software package. The ratio of the areas occupied by the streptomycete colonies grown under the experimental and control conditions was calculated and used as an indicator of the effect of the emitter culture signal on the growth of the detector culture.

**The results were processed** using the Microsoft Excel and Statistica 6.0 (StatSoft). Since the scattering of the experimental data was considerable, both parametric and nonparametric statistical techniques (*t*-test for paired values, sign tests, and Wilcoxon test) were used (Hollander and Wolfe, 1973; Kobzar', 2006). In the series of experiments, the difference between the experimental and control variants was always statistically significant at $p < 0.05$. The experiments were carried out in three repeats with two repeats in each series.

## RESULTS AND DISCUSSION

**Effect of distant interactions on the germination of *S. netropsis* spores.** The spores of *S. netropsis* respond poorly to storage, so spore survival decreased significantly after 4-month incubation at 10°C (the number of CFU/mL was as low as 1% of the value obtained prior to incubation. The CFU decrease could, however, result from the state of deeper dormancy of the spores, rather than from a loss of viability. In this case, an external signal is required for spore germination. The DI effect was observed only with the spore suspension with the titer of $10^5$ CFU/mL. The following parameters were recorded: colony number, the area of the medium occupied by streptomycete colonies, and the morphological characteristics of the colonies (size, presence of spores and aerial mycelium, and pigmentation).

In the presence of the emitter culture, the number of colonies increased by 25–50% (by 30% on average) compared to the control (Table 1). A typical picture of growth of the control and experimental variants is presented on Fig. 2. Calculation of the colony area according to the procedure described in Materials and Methods revealed a 20–270% greater area (70% on average) occupied by the colonies developing in the presence of an emitter culture compared to the control. Since the number of colonies increased by 30%, while their relative area (which is proportional to the biomass) increased by 70%, it may be concluded that the growth rate in the variants with the inducer culture was approximately twice that of the control (70 : 30 = 2.33). Colony size varied significantly in the control and experimental variants. In the control, the share of small colonies ($d \sim 0.5$ mm) was higher, 15–20%. In the experimental variant, the share of small colonies

**Table 1.** Colony number and colony area of *Streptomyces netropsis* cultures in the absence (control) and presence of DI (experiment), % of the control

| Parameter | In the presence of DI |
|---|---|
| Colony number on the surface of the medium | 125–150 (**130**)* |
| Area occupied by the colonies on the surface of the medium determined by digital photodensitometry | 120–370 (**170**) |

* The numerals show the spread of values, with the average value in parentheses.

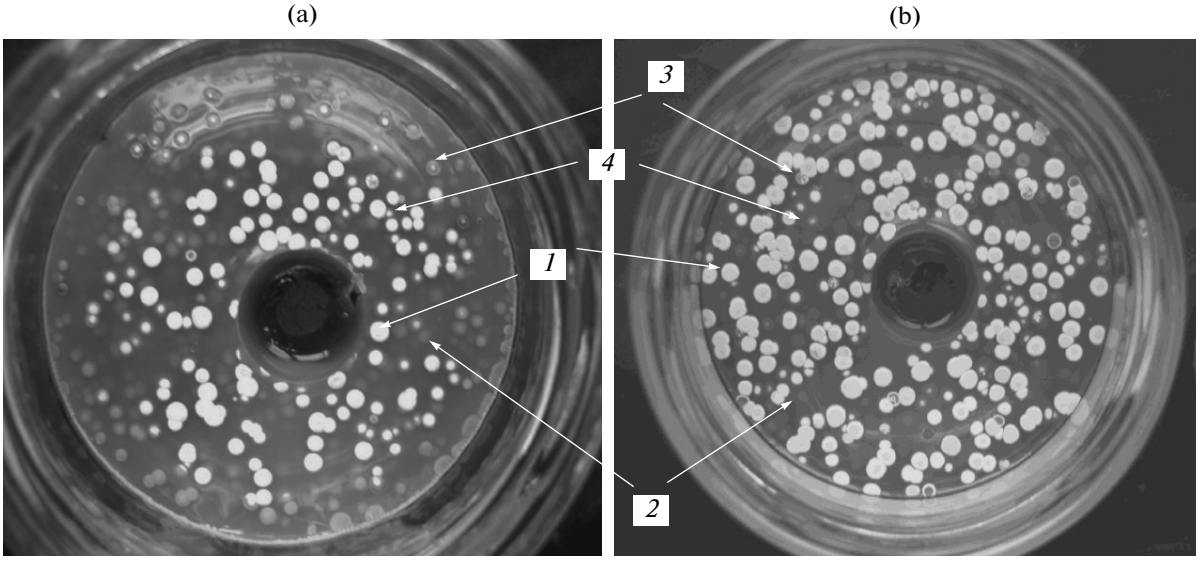


**Fig. 2.** Effect of the inducer culture on CFU and morphotype diversity of the colonies of *Streptomyces netropsis* ISP-5259 populations: control (a) and experiment (b). Colony morphotypes: basic (*1*), asporogenic (*2*), oligosporic (*3*), and small colony-forming (*4*).

was 10–15%, i.e., 25% less than in the control (Table 2).

**DI effect on the composition of *S. netropsis* populations.** Streptomycetes are characterized by high population heterogeneity in terms of colony morphology. Plating of a fresh spore suspension (prior to storage) revealed four main types within the *S. netropsis* ISP-5259 population: the dominant (basic), oligosporic, asporogenic, and dwarf (forming small colonies) ones (Kuznetsov, 1987), which accounted for 60, 20, 10, and 10% of the total colony number, respectively (Table 2). Importantly, the dwarf variant colonies varied in their ability to form the pigment and the aerial mycelium. According to the classification scheme (Kuznetsov, 1987), the dwarf variant contains several subtypes according to the degree of aerial mycelium formation and pigment production. However, since it was difficult to differentiate between these subtypes under conditions of the present experiment, we determined only the *small* morphotype with the colony diameter not exceeding 0.5 mm. Colonies of the *basic* type had an even edge, a well-developed aerial mycelium on which spores were formed, and a brown substrate mycelium. The *oligosporic* variant formed similar colonies, but the aerial mycelium was formed only in the center. The colonies of the *asporogenic* variant were characterized by the absence of aerial mycelium and weak pigmentation.

In the control variants grown from spore suspensions stored for 4 months, the basic type colonies constituted 30–40% of the total colony number, i.e., half of their share prior to storage. The population was represented mainly by the colonies of nondominant morphological types (asporogenic and small-colony variants). The oligosporic variant was responsible for up to 10% of the total colony number. DI resulted, apart

**Table 2.** Composition of *Streptomyces netropsis* populations on agar medium in the control (without DI) and experimental (in the presence of DI) variants, % of the total colony number

| Morphological type | Before storage | After storage | |
|---|---|---|---|
| | | control without DI | in the presence of DI |
| Dominant | 60 | 30–40 (**35**)* | 70–90 (**80**) |
| Asporogenic | 10 | 30–40 (**35**) | 5–15 (**10**) |
| Oligosporic | 20 | 2–8 (**5**) | 2–8 (**5**) |
| Small colony-forming | 10 | 20–25 (**25**) | 2–8 (**5**) |

* The numerals show the spread of values (minimum to maximum), with the average value in parentheses. For the culture before storage, only the average values are presented.

from accelerated growth, in the stabilized composition of *S. netropsis* grown from stored suspension. In the experimental variant, most of the population (up to 80%) was represented by the basic type, with not more than 10% of the colonies belonging to the asporogenic variant (Table 2, Fig. 3).

Thus, a signal of a physical nature emitted by *S. netropsis* proliferating culture affected the population of the same strain, resulting in a CFU/mL increase by 30% on average, doubling of colony growth, and stabilization of the phenotypic composition of the population.

It should be noted that DI effect was observed only when specific conditions of the cultivation of the inducer and detector cultures were strictly maintained and that the state of the inducer culture affected the magnitude of DI effect.

Conditions favorable for DI effect—i.e., stress and changes in the growth stage of microbial cultures—have been previously described in the literature (Matsuhashi, 1995; Nikolaev, 2000b). Our results are in agreement with these observations. In the case of *S. netropsis*, DI effect was weak if the spore suspension was stored for less than 4 months and had a relatively high titer of viable spores. This dependence of DI effect on the quality of detector cells probably results from the fact that the culture achieved a dormant state so deep that it may be characterized as unculturable. Germination of such spores requires resuscitating treatment. This suggestion is based on the previously reported effect of a considerable increase of the number of germinating spores (CFU/mL spore suspension) in the presence of a chemical regulator, factor A (Gruzina et al., 2003). This butyrolactone was originally described as a factor controlling sporulation and antibiotic synthesis in actinomycetes (Khokhlov, 1988).

Our results broaden the range of objects for which the biological effects of DI have been demonstrated. They are important for streptomycete biology, since this microbial group is characterized by high phenotypic heterogeneity, which may be observed as a diversity of morphological types in their populations and in association with biosynthetic activity of these morphotypes (Kuznetsov, 1972; Kämpfer, 2004). Increasing the population stability of streptomycetes, most of which are producers of practically used biologically active compounds, is an important task (Egorov, 2004).

The subject of our study, *S. netropsis*, is among the species that quickly die off and react poorly to storage conditions. Storage of such species results in a drastic decrease in the number of viable spores, accompanied by a decrease in the share of the dominant basic variant (which is usually the most productive one), up to its complete loss (Kuznetsov et al., 1992). For such strains, enhancing their viability and stabilization of the composition of their populations is of high importance. Our results indicate that DI may be used to recover the germinating ability of the spores and to stabilize the population structure.

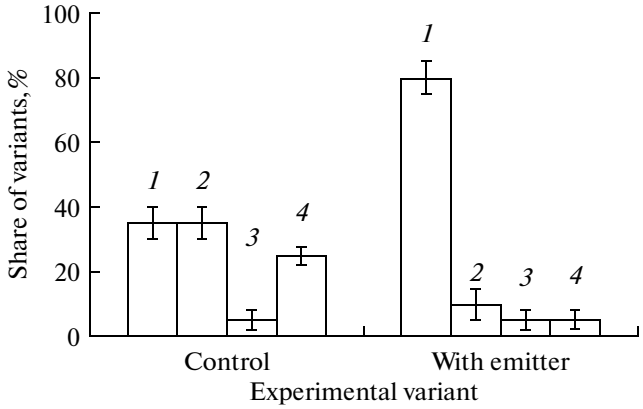


**Fig. 3.** Composition of the streptomycete population affected by the inducer culture. Colony morphotypes: basic (*1*), asporogenic (*2*), oligosporic (*3*), and small colony-forming (*4*).

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