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EXPERIMENTAL ARTICLES

Induction of Programmed Lysis in *Streptomyces lividans* Culture by the Inhibitors of Eukaryotic Type Serine/Threonine Protein Kinases

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Abstract—Programmed death (PD) of the mycelium of *Streptomyces lividans*, namely, its delayed lysis in response to treatment with indolylmaleimide derivatives, which inhibit actinobacterial serine/threonine protein kinases (STPK), is described. Delayed lysis of mycelial cell was accompanied by DNA damage similar to PD in differentiating *S. lividans* mycelium. Two-dimensional electrophoresis and mass spectrometry were used to identify proteins up-regulated by a PD-inducing STPK inhibitor. Most of these proteins are known to be implicated in responses to various stress stimuli. Thus, our model of delayed cell lysis of actinobacteria upon STPK inhibition may serve for unveiling the molecular mechanisms of bacterial PD and for antimicrobial drug design.

Keywords: actinobacteria, delayed lysis, programmed death, serine/threonine protein kinases, inhibitors.
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Programmed death (PD), or apoptosis, has been described in human and other eukaryotic cells [1, 2]. In recent years, mechanisms of bacterial cell death somewhat similar to apoptosis in eukaryotes have been described [3–5]. In prokaryotes, the homologues of the eukaryotic genes involved in initiation and realization of PD were revealed [6]. In bacteria, as in many multicellular organisms, the examples of programmed cell death may be found. These include autolysis of maternal cells upon sporulation, with the involvement of autolysins [5], or death of vegetative cells upon formation of a fruiting body in myxobacteria [3].

In bacteria, special toxin–antitoxin modules exist [6]. Under balanced growth conditions the toxin is constantly neutralized by the antitoxin; however, certain changes in metabolism (e.g., amino acid starvation) disturb the balance so that the toxin poisons the cell. As a result, some of the cells in a population stop proliferating, allowing for survival of the remaining bacteria [7].

A phenomenon similar to a programmed suicide may be observed in the course of differentiation of actinobacteria, particularly streptomycetes [3, 4, 8, 9]. In *Streptomyces antibioticus*, most hyphal segments are lysed 8–10 h after spore plating onto agar; the remaining intact segments give rise to aerial mycelium growing vertically above the agar surface. After 25 h, a second round of hyphal death occurs, resulting in a thinner aerial mycelium, and after 48–96 h, sporulation starts. Mechanisms similar to those mediating apoptosis in eukaryotes contribute to these processes of mycelium interchange, in particular, the involvement of serine/threonine Ca^{2+}-dependent protein kinases (STPK) [10]. Investigation of the mechanisms and inductors of bacterial cell programmed lysis is of both scientific and practical interest.

STPK are known to be involved in induction of apoptosis in human cells [11]. Inhibitors of STPKs that selectively induce apoptosis have been revealed [12]. Therefore, search for the agents inducing prokaryotic cell death by intervention with signal transduction seems reasonable.

The eukaryotic type STPKs were identified in all actinobacteria including pathogenic mycobacteria species [13]. These enzymes modulate such essential phenotypic features as virulence, pathogenicity, drug resistance, and persistence [14] and are considered among potential biotargets in development of new-generation drugs.

The aim of the present work was to study the possible role of STPKs in induction of programmed cell lysis in the model strain *Streptomyces lividans*.

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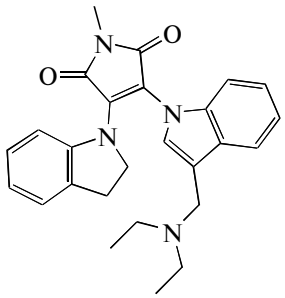
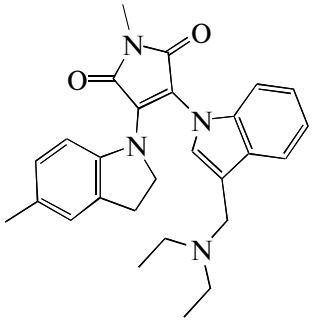
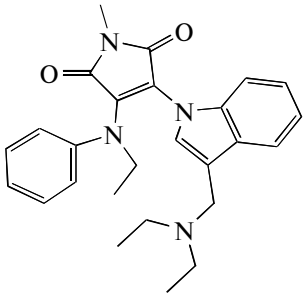
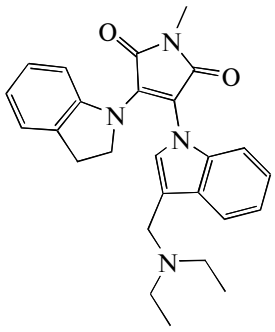
Table 1. Induction of secondary lysis of *S. lividans* cells by STPK inhibitors

Compound	Formula	Experimental concentration, nmol/disc	Growth inhibition zone after 20 h, mm	Secondary lysis zone, mm
L-I		1000	12	34
L-II		1000	13	33
L-III		1000	13	40
L-IV		2000	14	14

MATERIALS AND METHODS

Streptomyces lividans strain TK24 (66) [http://www.ncbi.nlm.nih.gov/sites/entrez?Db=genome&Cmd=ShowDetailView&TermToSearch=6276] was the object of the study.

Culture media YSP, YEME [15], and MG [16] were used for the cultivation of *S. lividans*. All reagents were obtained from Sigma, unless stated otherwise.

STPK inhibitors of the indolylmaleimide type used in the present work, including L-I, L-II, L-III, and L-IV (Table 1), were synthesized in the Labora-

tory of chemical transformation of antibiotics (Gause Institute of New Antibiotics, Russian Academy of Medical Sciences) and kindly provided by M.N. Preobrazhenskaya.

Evaluation of STPK inhibitors. Selection of potential PD inducers among STPK inhibitors was performed as follows. Suspensions of *S. lividans* spores in semisolid agar were poured into petri dishes with agarized MG medium. After solidification of the medium, paper discs soaked in the solution of a compound under study were put on the agar surface. The plates were incubated for 20 h at 28°C. Growth inhibition zones (halo) were registered around the paper discs and the dishes were incubated for another 24 h. Then, the zones of culture lysis, surrounding the zones of primary growth inhibition, were registered. All tested compounds were applied in the amount of 1000 and 2000 nmol/disc (Table 1).

Effect of STPK inhibitors on mycelium growth on agarized medium. *S. lividans* strain was grown in petri dishes with agarized YSP medium at 28°C during 48 h on a Hybond nitrocellulose (Amersham) support. Nitrocellulose discs with the mycelium were transferred to petri dishes containing agarized MG medium with and without STPK inhibitors. The plates were incubated for 24 h until partial lysis of the substrate mycelium was observed in the presence of STPK inhibitors [9]. Then, mycelia of the control (without STPK inhibitors) and experimental samples were collected with a spatula, transferred to centrifuge tubes, and washed with 1 M Tris–HCl buffer three times using centrifugation at 10000 *g*. The obtained samples were used for DNA isolation.

Effect of STPK inhibitors on actinobacterial mycelium in a liquid medium. *S. lividans* strain was grown at 28°C in 750-mL Erlenmeyer flasks containing 50 mL YEME medium on a shaker (250 rpm) during 20 h. The early-exponential culture was supplemented with an STPK inhibitor and incubated for 15 h. The mycelium was collected, centrifuged at 5000 *g* for 20 min, and used for electrophoretic protein separation. The mycelium grown in the absence of STPK inhibitors was used as a control.

Isolation of total DNA of *S. lividans* was performed according to the technique described in [15]. DNA was isolated from mycelia not subjected to autolysis. Integrity of the DNA molecules was confirmed by electrophoresis in 0.9% agarose gel [9].

Protein PAGE. For proteome analysis, mycelium of *S. lividans* was washed three times with 1.5 mL 0.1 M Tris–HCl buffer, pH 7.5. The cells were lysed with 1.5 mL lysis buffer (50 mM Tris–HCl, pH 7.5, 150 mM NaCl, 1 mM $MgCl_2$, 5 mM 2-mercaptoethanol, 10% glycerol, 1 mM phenylmethylsulfonyl fluoride) supplemented with lysozyme at a final concentration of 1 mg/mL. Mycelial suspension was incubated for 1 h at 37°C under constant stirring. The lysates were sonicated at 22 kHz (Branson Digital 250) three times for 15 s with intervals of 30 s. Cell debris were precipitated by centrifugation at 14000 *g*, 4°C for 20 min. Proteins in the supernatant were precipitated with five volumes of acetone, redissolved in the buffer (10% glycerol, 5% 2-mercaptoethanol, 2.3% SDS, 0.0625 M Tris–HCl, pH 6.8), and separated by PAGE in 12% gel [17]. The gels were fixed with 10% acetic acid solution in 50% ethanol and stained with CBB-G 250. Protein concentration was determined according to Bradford [18].

2-D electrophoresis. Proteins of the lysed mycelium were separated by two-dimensional isoelectric focusing according to O'Farrel with slight modifications [19]. Trypsin hydrolysis of proteins in polyacrylamide gel and sample preparation for mass spectrometry were performed according to the manufacturer's recommendations (In Gel Digest Protocol, Promega). Mass spectra were obtained by the method recommended by the manufacturer on an UltrafleXtreme Maldi Tof/Tof MS (Bruker Daltonics) analytical mass spectrometer in the Department of proteomic studies (Orekhovich Research Institute of Biomedical Chemistry, Russian Academy of Medical Sciences). Protein identification was performed with Mascot software (www.matrixscience.com). Protein search was performed in the NCBI database (*Streptomyces coelicolor* and *S. lividans* sub-bases).

RESULTS

We have previously developed a cell test system *S. lividans aphVIII*+ for screening of the STPK inhibitors of eukaryotic type [20]. The key element of the system was aminoglycoside phosphotransferase type VIII (AphVIII), an enzyme inactivating aminoglycoside antibiotics. The activity of AphVIII is regulated by STPKs. Thus, phosphorylation of the enzyme increases the resistance of *S. lividans* to aminoglycoside antibiotics, while STPK inhibitors increase the sensitivity of bacteria to these antibiotics. Quantitative evaluation of *S. lividans* sensitivity to kanamycin upon treatment with STPK inhibitors makes it possible to carry out the primary selection of the inhibitors. Using the test system, 21 low molecular weight derivatives of indolylmaleimide were screened; inhibition of STPK was also studied in a panel of human protein kinases [20, 21]. All compounds exerted activity in vitro and in the *S. lividans aphVIII*+ test system at various degrees. In particular, L-I, L-II, L-III, and L-IV were active against protein kinase C (PKCalpha). The half maximal inhibitory concentrations for PKCalpha (IC_{50}) were 87 ± 11, 133 ± 15, 250 ± 29, and 130 ± 19 nM, respectively [21]. At the present stage of investigation it is not clear which one of the 33 known *S. lividans* STPKs are inhibited by the compounds under study. It is reasonable to consider them inhibiting protein kinase Pk25, which phosphorylates AphVIII [16] and, due to low selectivity, other STPKs of *S. lividans*. We

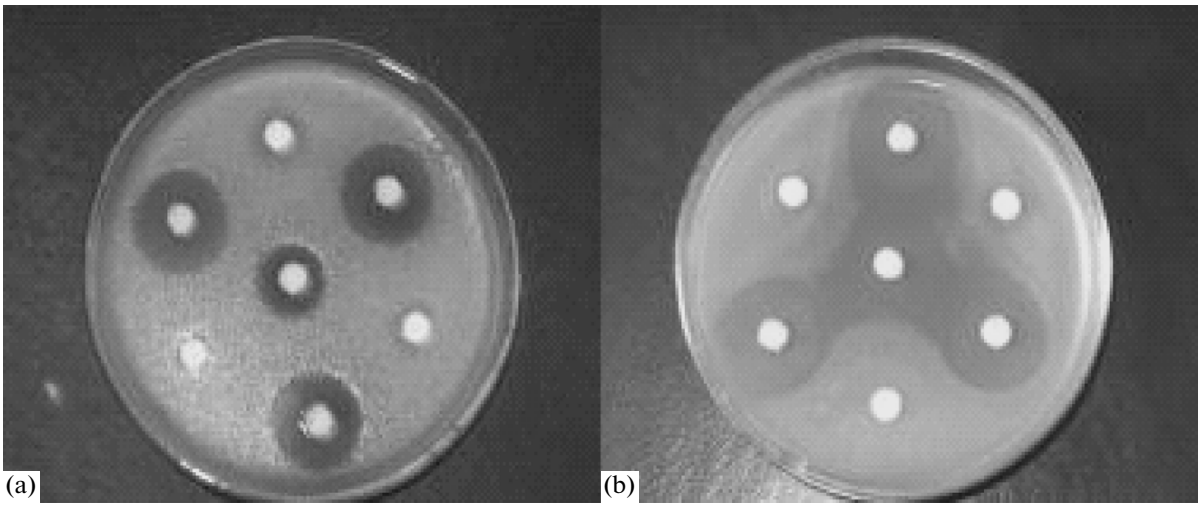


Fig. 1. Effect of an STPK inhibitor on *S. lividans* culture. Growth inhibition zone after a 20 h cultivation in the presence of L-III, an inhibitor of STPK, on paper discs (a) and secondary lysis zone after 44 h of cultivation (b).

assumed that inhibition of one or several STPKs may trigger PD in bacterial cells.

Study of delayed cell lysis induction by inhibitors of bacterial STPKs of the indolylmaleimide type. A series of 21 indolylmaleimide derivatives selected as inhibitors of bacterial STPKs [20, 21] were studied for their ability to induce a delayed cell lysis. It was demonstrated that only some inhibitors of STPK possessed the activity. As follows from Figure 1a, a zone of growth inhibition was formed around the disc soaked with an inhibitor after 20 h of incubation. The same petri dish 44 h after introduction of the STPK inhibitor is shown in Fig. 1b. An increase of the zone diameter due to delayed lysis of *S. lividans* mycelium can be seen. Table 1 summarizes the data on the activity of 4 out of 21 compounds studied. Three of them, namely, L-I, L-II, and L-III, caused the delayed lysis of *S. lividans* cells. Treatment with other 18 inhibitors, including L-IV, did not result in an increase of the growth inhibition zone.

Since not all of the STPK inhibitors induced the delayed mycelium lysis after 2 days of incubation, only one or several STPKs out of the 33 annotated in the *S. lividans* genome may be assumed to be the cellular targets of the inhibitors. Inhibition of these STPKs probably induces PD of the *S. lividans* growing mycelium, which is manifested in the formation of the zones of delayed lysis on the agar medium.

Electrophoresis of DNA isolated from *S. lividans* treated with STPK inhibitors. Programmed death of the substrate mycelium is known to be accompanied by the activation of intracellular nucleases and DNA degradation [9]. A method used in [9] was applied to analyze DNA integrity in *S. lividans* mycelium treated with an STPK inhibitor. In particular, the effect of L-III on DNA degradation was studied since it was the most efficient inducer of delayed cell lysis in *S. lividans* culture after 44 h of growth (Fig. 2). Mycelium samples grown: (a) in the presence of DMSO without the STPK inhibitor or (b) in the presence of DMSO and the inhibitor L-IV not inducing PD of *S. lividans* mycelium were used as controls.

No DNA degradation was revealed upon treatment with 100 μM L-III, but at higher concentrations (250–1000 μM), a dose-dependent decrease in the mean length of the DNA fragments was observed. On

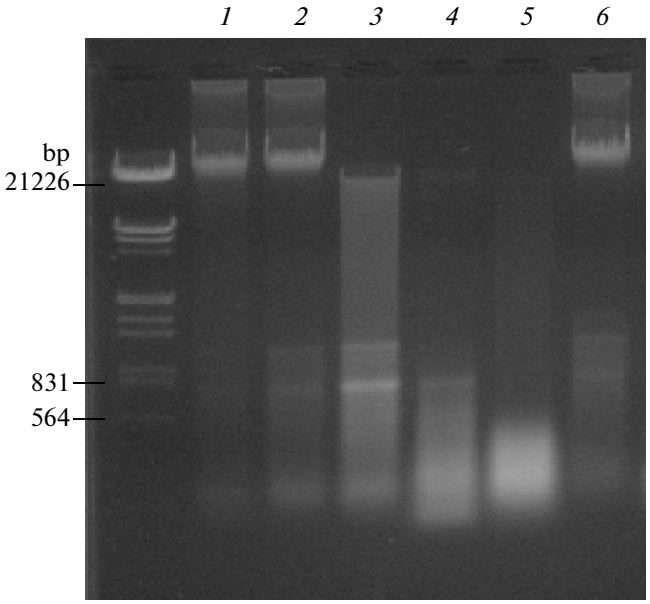


Fig. 2. Analysis of DNA integrity upon bacterial cell death. DNA isolated from the mycelium grown in the absence of STPK inhibitors (*1*), in the presence of L-III at concentrations of 100, 250, 500, and 1000 μM, respectively (*2*–*5*), and in the presence of 250 μM L-IV (an STPK inhibitor which does not induce PD) (*6*). Marker, phage λ DNA digested with restriction endonucleases *Hin*dIII + *Eco*RI.

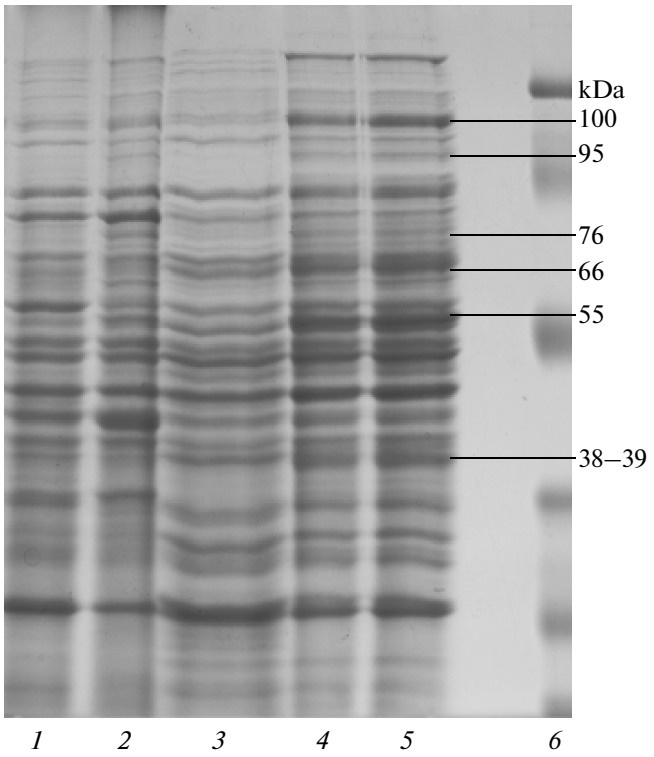


Fig. 3. Electrophoresis of total cell lysates of *S. lividans*. Proteins of the cells grown in the presence of 100 μM L-IV (*1*), 50 μM L-III (*2*), 100 μM L-III (*4*), 250 μM L-III (*5*), without PD inductors (*3*), and molecular weight markers: β-galactosidase (118 kDa), bovine serum albumin (90 kDa), ovalbumin (50 kDa), carbonic anhydrase (34 kDa), β-lactoglobulin (26 kDa), and lysozyme (19 kDa) (*6*).

the contrary, L-IV at concentrations of 250 and 500 μM did not cause DNA degradation. These results suggest that cell lysis induced by STPK inhibitor L-III possesses the features of PD similar to allolysis of a *S. coelicolor* strain in the process of differentiation [6].

Protein composition of the extracts of lysed *S. lividans* mycelium treated with L-III. The protein composition of the extracts of *S. lividans* mycelium grown in liquid medium in the presence of L-III was determined. Figure 3 demonstrates that in the extracts of the cells grown in the presence of L-III (50, 100, and 250 μM, lanes *2*, *4*, and *5*, respectively), additional major protein bands with molecular masses of 100, 66, and 55 kDa were revealed. Moreover, proteins of 95, 76, and 38–39 kDa, absent in the extracts of the control mycelia, appeared.

Using two-dimensional gel electrophoresis of the total protein extracts from *S. lividans* mycelium treated with 250 μM L-III and grown in the absence of the inhibitor (Figs. 4a and 4b), five proteins of 25, 34, 37, 55, and 66 kDa were demonstrated to be up-regulated upon treatment with L-III.

Finally, the mass spectrometric identification of the additional protein fractions of the extracts from the mycelium of *S. lividans* treated with L-III was carried out. According to the analysis of N-terminal sequences and their comparison with the databases, the following proteins were identified: PhoU (25.4 kDa), a translation elongation factor Tu (37.6 kDa), molecular chaperone DnaK (66.1 kDa), 30S ribosomal protein S1 RpsA (54.9 kDa), malate dehydrogenase Mdh (34.6 kDa), and glyceraldehyde-3-phosphate dehydrogenase Gap1 (36.3 kDa) (Table 2).

DISCUSSION

Actinobacteria with complex cell differentiation in their life cycle are known to undergo programmed lysis, or allolysis, which is accompanied by DNA deg-

Table 2. Up-regulated proteins identified in *S. lividans* cells treated with L-III

Identified protein		Molecular mass, Da	Calculated isoelectric point, pI	Cell function	Reference
DnaK	Molecular chaperone	66140	4.7	Heat shock protein 70	[33]
PhoU	Regulator of the phosphate transport system	25441	4.93	Phosphate transport system regulatory protein	[29]
Tu	Elongation factor in translation	37602	5.08	Translation elongation factor	[32]
RpsA	30S ribosomal protein S1	55034	4.35	RPS1, a component of the small ribosomal subunit	[33]
Mdh	Malate dehydrogenase	34622	5.00	Catalyzes malate oxidation to oxaloacetate in cell respiration	[36]
Gapl	Glyceraldehyde-3-phosphate dehydrogenase	36267	5.09	Glucose metabolism in cell respiration	[35]

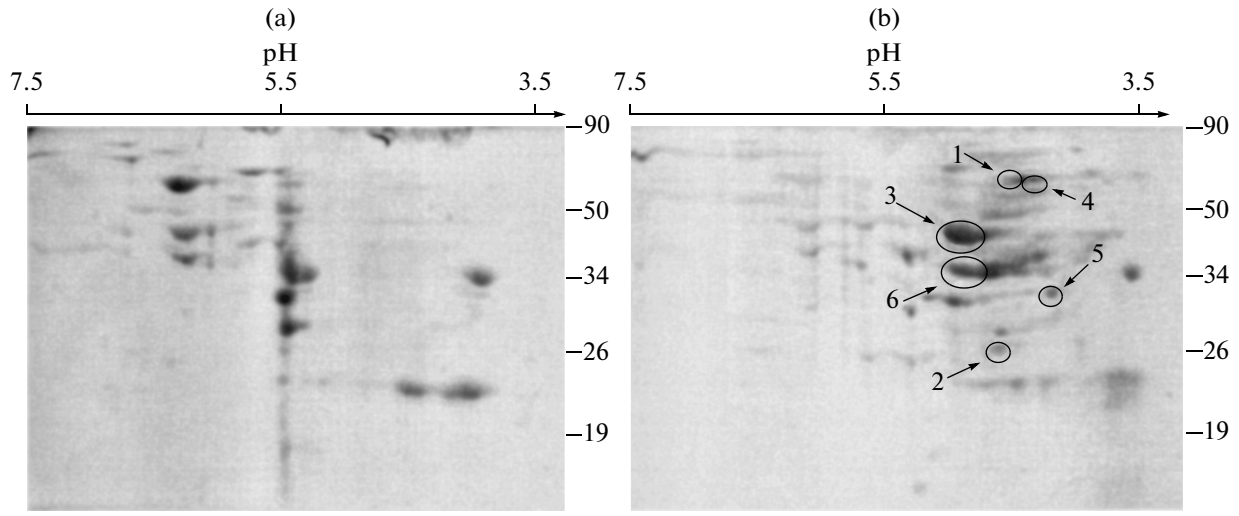


Fig. 4. 2D-PAGE of the *S. lividans* mycelial proteins. pH values are plotted against the horizontal axis, and the marker molecular weight values against the vertical axis. Lysates of the mycelium grown in the absence of indolyl maleimides (a) and in the presence of 250 μM L-III (b).

radation [4]. In *S. lividans* strain grown on solid medium and treated with an STPK inhibitor, DNA degradation was detected similar to that observed in the course of mycelium differentiation. It may be assumed that inhibition of certain protein kinases triggers a lytic mechanism similar to partial lysis of the aerial mycelium of *S. lividans* upon spore formation. The mass spectral analysis of proteins up-regulated upon treatment with the STPK inhibitor L-III revealed the proteins characteristic of bacterial stress response to starvation, heat, oxidative stresses, and energy inhibition. DnaK (SCO3671) involved in folding of the newly synthesized polypeptide chains and refolding of the incorrectly assembled proteins [22], as well as a protein of the phosphate transport system PhoU (SCO4228) [23], are both known as stress-induced proteins [24, 25]. L-III also activated the synthesis of proteins involved in translation. The protein S1 (RpsA), a component of the 30S ribosome subunit (SCO1998) participates in recognition and binding to mRNA during initiation of translation; the elongation factor Tu (SCO4662) regulates protein biosynthesis on the ribosomes. Synthesis of these proteins is activated in *S. coelicolor*, a species closely related to *S. lividans*, as well as in other microorganisms, upon their exposure to various stress stimuli [25–27]. The elongation factor Tu and molecular chaperones were revealed in proteomic analysis of the *S. coelicolor* mycelium undergoing PD [10]. We also detected the proteins Gap1 (SCO1947) and Mdh (SCO4827) involved in cell respiration. Synthesis of Gap1 is activated under stress [28]. Activation of malate dehydrogenase synthesis (Mdh) was observed in bacteria in response to a decrease of the ATP content in cells dying under aerobic conditions [29].

Therefore, the STPK inhibitor L-III is responsible for the delayed cell lysis in the mycelium of *S. lividans*. This process is accompanied by an apoptosis-like DNA degradation and accumulation of the proteins involved in stress response and PD. According to the experimental data, inhibition of one or several protein kinases probably induces a PD phenotype in *S. lividans*.

Phosphorylation of the AphVIII protein inactivating kanamycin forms the basis for the *S. lividans aphVIII*+ test system [20, 21]. Only 4 of 21 potential STPK inhibitors selected in the test system induced delayed mycelium lysis in *S. lividans*. This is in accordance with the data obtained in the *E. coli* test system, allowing for selection of the most potent inhibitors of the STPK pk25 (PknA) [30] and its homologues. Apparently, the latter STPK and its homologues are not involved in PD during *S. lividans* differentiation. Probably, L-III is a non-selective inhibitor capable of modulating several STPKs, including those which regulate PD. Potential targets of secondary lysis induction should be sought among the STPKs regulating cell differentiation, for example, pk17 [31]. Further comparative studies of the phosphokinome of *S. lividans* [32, 33] with various selective STPK inhibitors could contribute to better understanding of these issues.

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REFERENCES

1. Danial, N.N. and Korsmeyer, S.J., Cell Death: Critical Control Points, *Cell*, 2004, vol. 116, no. 2, pp. 205–219.
2. Ameisen, J.C., On the Origin, Evolution, and Nature of Programmed Cell Death: A Timeline of Four Billion Years, *Cell Death Differ.*, 2002, vol. 9, no. 4, pp. 367–393.
3. Lewis, K., Programmed Death in Bacteria, *Microbiol. Mol. Biol. Rev.*, 2000, vol. 64, no. 3, pp. 503–514.
4. Prozorov, A.A. and Danilenko, V.N., Allolysis in Bacteria, *Microbiology*, 2011, vol. 80, no. 1, pp. 1–9.
5. Gordeeva, A.V., Labas, Yu.A., and Zvyagil'skaya, R.A., Apoptosis in Unicellular Organisms: Mechanisms and Evolution, *Biokhimiya*, 2004, vol. 69, no. 10, pp. 1055–1066.
6. Koonin, E.V. and Aravin, L., Origin and Evolution of Eukaryotic Apoptosis: The Bacterial Connection, *Cell Death Differ.*, 2002, vol. 9, pp. 394 –404.
7. Prozorov, A.A. and Danilenko, V.N., Toxin–Antitoxin Systems in Bacteria: Apoptotic Tools or Metabolic Regulators?, *Mikrobiologiya*, 2010, vol. 79, no. 2, pp. 129–140.
8. Manteca, A., Mäder, U., Connolly, B.A., and Sanchez, J., A Proteomic Analysis of *Streptomyces coelicolor* Programmed Cell Death, *Proteomics*, 2006, vol. 6, no. 22, pp. 6008–6022.
9. Fernandez, M. and Sanchez, J., Nuclease Activities and Cell Death Processes Associated with the Development of Surface Cultures of *Streptomyces antibioticus* ETH 7451, *Microbiology* (UK), 2002, vol. 148, pp. 405–412.
10. Kennelly, P.J., Protein Kinases and Protein Phosphatases in Prokaryotes: A Genomic Perspective, *FEMS Microbiol. Lett.*, 2002, vol. 206, no. 1, pp. 1–8.
11. Lee, M.J., Chen, H.M., Tzang, B.S., Lin, C.W., Wang, C.J., Liu, J.Y., and Kao, S.H., *Ocimum gratissimum* Aqueous Extract Protects h9c2 Myocardiac Cells from h(2)O(2)-Induced Cell Apoptosis through Akt Signalling, *Evid. Based Complement. Alternat. Med.*, 2011. pii:578060.
12. Snowden, R.T., Sun, X.M., Dyer, M.J., and Cohen, G.M., Bisindolylmaleimide IX Is a Potent Inducer of Apoptosis in Chronic Lymphocytic Leukaemic Cells and Activates Cleavage of Mcl-1, *Leukemia*, 2003, vol. 17, no. 10, pp. 1981–1989.
13. Danilenko, V.N., Osolodkin, D.I., Lakatosh, S.A., Preobrazhenskaya, M.N., and Shtil, A.A., Bacterial Eukaryotic Type Serine-Threonine Protein Kinases: Tools for Targeted Anti-Infective Drug Design, *Curr. Topics Med. Chem.*, 2011, no. 11, pp. 1352–1369.
14. Cozzone A.J., Role of Protein Phosphorylation on Serine/Threonine and Tyrosine in the Virulence of Bacterial Pathogens, *J. Mol. Microbiol. Biotechnol.*, 2005, vol. 9, pp. 198–213.
15. Kieser, T., Bibb, M.J., Buttner, M.J., Chater, K.F., and Hopwood, D.A., *Practical Streptomyces Genetics*, Norwich: John Innes Foundation, 2000.
16. Bekker, O.B., Elizarov, S.M., Alekseeva, M.G., Liubimova, I.K., and Danilenko, V.N., Ca^{2+}-Dependent Modulation of Antibiotic Resistance in *Streptomyces lividans* 66 and *Streptomyces coelicolor* A3(2), *Microbiology*, 2008, vol. 77, no. 5, pp. 630–638.
17. Laemmli, U.K., Cleavage of Structural Proteins during the Assembly of the Head of Bacteriophage T4, *Nature*, 1970, vol. 227, no. 5259, pp. 680–685.
18. Bradford, M.M., A Rapid and Sensitive Method for the Quantitation of Microgram Quantities of Protein Utilizing the Principle of Protein-Dye Binding, *Anal. Biochem.*, 1976, vol. 72, pp. 248–254.
19. O'Farrell, P.H., High-Resolution Two-Dimensional Electrophoresis of Proteins, *J. Biol. Chem.*, 1975, vol. 250, no. 10, pp. 4007–4021.
20. Danilenko, V.N., Simonov, A.Y., Lakatosh, S.A., Kubbutat, M.H., Totzke, F., Schächtele, C., Elizarov, S.M., Bekker, O.B., Printsevskaya, S.S., Luzikov, Y.N., Reznikova, M.I., Shtil, A.A., and Preobrazhenskaya, M.N., Search for Inhibitors of Bacterial and Human Protein Kinases among Derivatives of Diazepines[1,4] Annelated with Maleimide and Indole Cycles, *J. Med. Chem.*, 2008, vol. 51, no. 24, pp. 7731–7736.
21. Danilenko, V.N., Lakatosh, S.A., Preobrazhenskaya, M.N., and Shtil', A.A., RF Patent no. 2388759 (2006).
22. Kilstrup, M., Jacobsen, S., Hammer, K., and Vogensen, F.K., Induction of Heat Shock Proteins DnaK, GroEL, and GroES by Salt Stress in *Lactococcus lactis, Appl. Environ. Microbiol.*, 1997, vol. 63, no. 5, pp. 1826–1837.
23. Ghorbel, S., Kormanec, J., Artus, A., and Virolle, M.-J., Transcriptional Studies and Regulatory Interactions between the *phoR-phoP* Operon and the *phoU, mtpA*, and *ppk* Genes of *Streptomyces lividans* TK24, *J. Bacteriol.*, 2006, vol. 188, no. 2, pp. 677–686.
24. Li, Y. and Zhang, Y., *PhoU* Is a Persistence Switch Involved in Persister Formation and Tolerance to Multiple Antibiotics and Stresses in *Escherichia coli, Antimicrob. Agents Chemother.*, 2007, vol. 51, no. 6, pp. 2092–2099.
25. Kiliç, N.K., Stensballe, A., Otzen, D.E., and DöNmez, G., Proteomic Changes in Response to Chromium(VI) Toxicity in *Pseudomonas aeruginosa, Bioresour. Technol.,* 2010, vol. 101, no. 7, pp. 2134–2140.
26. Teixeira-Gomes, A.P., Cloeckaert, A., and Zygmunt, M.S., Characterization of Heat, Oxidative, and Acid Stress Responses in *Brucella melitensis, Infect. Immun.*, 2000, vol. 68, no. 5, pp. 2954–2961.
27. Novotna, J., Vohradsky, J., Berndt, P., Gramajo, H., Langen, H., Li, H.M., Minas, W., Orsaria, M., Roeder, D., and Thompson, S.J., Protheomic Studies of Diauxic Lag in the Differentiating Prokaryote *Streptomyces coelicolor* Reveal a Regulatory Network of Stress-Induced Proteins and Central Metabolic Enzymes, *Mol. Microbiol.*, 2003, vol. 48, no. 5, pp. 1289–1303.
28. Ortiz-Ortiz, M.A., Morán, J.M., Ruiz-Mesa, L.M., Bravo-San Pedro, J.M., and Fuentes, J.M., Paraquat

Exposure Induces Nuclear Translocation of Glyceraldehyde-3-Phosphate Dehydrogenase (GAPDH) and the Activation of the Nitric Oxide-GAPDH-Siah Cell Death Cascade, *Toxicol. Sci., 2010*, vol. 116, no. 2, pp. 614–622.

29. Thorenoor, N., Lee, J.H., Lee, S.K., Cho, S.W., Kim, Y.H., Kim, K.S., and Lee, C., Localization of the Death Effector Domain of Fas-Associated Death Domain Protein into the Membrane of *Escherichia coli* Induces Reactive Oxygen Species-Involved Cell Death, *Biochemistry*, 2010, vol. 49, no. 7, pp. 1435–1447.
30. Bekker, O.B., Alekseeva, M.G., Osolodkin, D.I., Palyulin, V.A., Elizarov, S.M., Zefirov, N.S., and Danilenko, V.N., A New Test System for Screening of the Inhibitors of Serine–Threonine Proteine Kinases: the *E. coli* APHVIII/Pk25 Construct, *Acta Naturae*, 2010, vol. 2, no. 3(6), pp. 114–127.
31. Rajkarnikar, A., Kwon, H.J., Ryu, Y.W., and Suh, J.W., Catalytic Domain of AfsKav Modulates Both Secondary Metabolism and Morphologic Differentiation in *Streptomyces avermitilis* ATCC 31272, *Curr. Microbiol.*, 2006, vol. 53, pp. 204–208.
32. Maslov, D.A., Bekker, O.B., Alekseeva, M.G., and Danilenko, V.N., Development of the *E. coli* APHVIII/LPK17 Bacterial Test System for Screening of the Proteine Kinase AfsK Inhibitors, Inducers of Programmed Bacterial Death, *Proc. Congr. EvraziyaBio-2010*, 2010, Moscow, pp. 125–126.
33. Elizarov, S.M., Michurina, V.N., and Danilenko, V.N., Proteine Kinase Activity of the Seine–Threonine Type in *Streptomyces lividans* Cell-Free Extracts, *Antibiot. Khimioter.*, 1998, vol. 43, no. 2, pp. 3–8.