J Microbiol Immunol Infect
2005;38:96-104

Antitumor features of *Bacillus oligonitrophilus* KU-1 strain

Sergey V. Malkov[1], Vladimir V. Markelov[2], Gleb Y. Polozov[1], Larisa I. Sobchuk[1], Natalia G. Zakharova[1], Boris I. Barabanschikov[1], Alexander Y. Kozhevnikov[3], Rauf A. Vaphin[1], Maxim V. Trushin[1,3]

[1]Department of Genetics, Kazan State University, Kazan; [2]Kazan Municipal Rehabilitation Medical Health Center "Sanatorium Krutushka", Kazan; and [3]Kazan Institute of Biochemistry and Biophysics, Kazan, Russia

Received: August 20, 2004 Revised: November 10, 2004 Accepted: November 26, 2004

Chemotherapy and radiotherapy remain mostly palliative methods for metastatic cancer treatment. Limitations in efficacy and safety of established treatments continue to underline the need for improved treatments for malignancy. Results with some probiotics with antitumor activity have been promising. Here, we report that oral reception of *Bacillus oligonitrophilus* KU-1 resulted in the prolongation of lives in cancer patients with terminal prognosis and stabilization of cancer growth. The theoretical basis for the phenomena observed is discussed.

Key words: *Bacillus subtilis*, genome, neoplasm metastases, probiotics

In the last decade, the appearance of new fields in chemotherapy such as neoadjuvant or induction chemotherapy [1], concurrent chemoradiation [2] and intra-arterial chemotherapy [3] was reported. In radiotherapy, a few new technologies were also introduced in clinical practice (e.g., stereotactic fractionated radiotherapy [4]; radio-surgery [5]; and intensity-modulated radiotherapy [6]). However, all of these remain principally palliative, especially in patients with distant metastases. In addition, the new anticancer drugs and treatment procedures are expensive and beyond the reach of many populations, especially in developing countries, and up to now, surgical operation remains one of the basic methods of cancer treatment. The above-mentioned reasons are the driving force for discovery of fundamentally new methods of cancer treatment. In this regard, biotherapy seems to be most promising due to its safety and cheapness.

We report the anticancer activity of *Bacillus oligonitrophilus* KU-1 strain in patients with distant cancerous metastases of various origin. In addition, we attempt to explain the possible mechanisms of the observed phenomena.

Corresponding author: Maxim V. Trushin, Department of Genetics, Kazan State University, Kremlyovskaya Street 18, Kazan 420008, Russia.
E-mail: mtrushin@mail.ru

Materials and Methods

Description of bacterial strain

Identification of *Bacillus oligonitrophilus* KU-1 strain was made as has been described by Krasilnikov [7]. This strain is a representative of the *Bacillus g*enus. The cells of the species are motile, $3.7 \times 0.8\text{-}1.2$ μm in size, and occur singly or in short chains. This species is able to produce oval spores (0.7-0.8 μm). There is significant growth on media with mineral nitrogen sources (in this case colonies are mucilaginous); on protein media or starch, the growth is poor (plain, whitish colonies). Sources of carbon and energy in aerobic conditions include glucose, lactose, sucrose, mannitol, inositol, sorbite, arabinose, and maltose. It does not form indol, hydrogen sulphide, and acetylmethyl carbinol. Growth is possible at pH 5.0 to 9.0. This strain is totally resistant to rifampicin, monomycin, oleandomycin phosphate, ristomycin, cephalexin and strongly inhibited by chloramphenicol, kanamycin, and neomycin.

Culture preparation

Bacillus oligonitrophilus KU-1 strain was isolated from soil of Kazan city, Russia. 300 mg of soil was re-suspended in 2 mL of modified liquid medium of Alexandrov [8] without potassium (Na_2HPO_4 10 g/L, $(NH_4)_2SO_4$ 2 g/L, $MgSO_4$ 0.5 g/L, SiO_2 0.15 g/L, $CaCO_3$ 0.05 g/L, pH 8.0) and inoculated on agar plates with the

Table 1. Results of the Ames test for the effect of *Bacillus oligonitrophilus* KU-1 culture broth on *Salmonella typhimurium* BA 13.

	Revertant (mean ± SD)
Control	59 ± 2.23
1.25 μg of 2-nitrofluorene (control with mutagen)	330 ± 5.45
10 μL *B. oligonitrophilus* KU-1 culture broth	54 ± 1.54
30 μL *B. oligonitrophilus* KU-1 culture broth	54 ± 1.39
60 μL *B. oligonitrophilus* KU-1 culture broth	42 ± 1.27

Abbreviation: SD = standard deviation

same content supplemented with orthoclase (0.5 g/L). The full-grown mucilaginous colonies were checked for growth ability on liquid Alexandrov's medium without potassium and supplemented with orthoclase. After identification, *B. oligonitrophilus* KU-1 cells were grown at 20°C without shaking during 2 days. Stationary phase *B. oligonitrophilus* KU-1 culture ($0.5\text{-}1.0 \times 10^9$ cells per mL) was used for cancer treatment.

Testing of toxicity and genotoxicity

Toxicity and genotoxicity of *B. oligonitrophilus* KU-1 was controlled in the standard test of Ames [9], and in cytogenetic testing with onion *Allium cepa* [10]. In rats, blood indexes were analyzed [11]. The results for Ames test are presented in Table 1. In this test, stationary phase culture (approximately 0.75×10^9 cells per mL) was filtered (pore size 0.2 μm). Culture broth (without cells) was mixed with growth medium [9]. It is clear from Table 1 that there was no mutagenic effect of *B. oligonitrophilus* KU-1 culture broth. In this case, the difference between control and experimental samples was non-significant.

In cytogenetic testing with *Allium cepa*, evaluation of chromosomal aberrations at anaphase was performed as recommended by Fiskesjö [10]. Stationary phase cultures of *B. oligonitrophilus* KU-1 with the same cell titer were diluted with distilled water to concentrations of 10%, 1%, and 0.1% (v/v). The results of this experiment, presented in Table 2, suggest that *B. oligonitrophilus* KU-1 cultures did not increase the level of genetic instability.

In experiments with rats (race "August"), bacterial culture (5 mL per day, approximately 0.75×10^9 cells per mL) was given with usual food (control food samples contained nutrient medium without cells). Blood samples taken from the tail artery revealed no significant alterations in blood indexes after 1 and 3 months' *B. oligonitrophilus* KU-1 oral administration (Table 3). It was found that experimental animals were playful, and all of them had keen appetite. However, there was increased bleeding in females during child-birth (probably due to increased blood pressure).

Scheme of therapy

This scheme was originally developed at the Department of Genetics Kazan State University (Kazan, Russia). Stationary phase *B. oligonitrophilus* KU-1 culture with the above-mentioned concentrations was used for oral administration according to the following plan. Day 1, 2.5 mL; day 2, 5 mL; day 3, 10 mL; day 4, 20 mL; day 5, 40 mL; day 6, 60 mL; day 7, 80 mL; day 8, 100 mL; day 9, 120 mL; day 10, 140 mL; day 11, 160 mL; day 12, 180 mL; day 13, 200 mL. A dose of 200 mL per day was used in the case of highly differentiated and mildly differentiated tumors. In the case of undifferentiated tumor or another aggressive forms of tumors, daily administration of *B. oligonitrophilus* KU-1 culture could be increased up to 500 mL. The indicated doses could be diluted with warm milk, juice or soda solution ($NaHCO_3$ 20 g/L). In the case of intolerance, the daily dose was decreased to 50 mL. In the absence of intolerance, treatment duration was continual for at least 3 months. Women aged over 60 years with high-grade differentiated or mildly differentiated mammary gland adenocarcinomas with bone metastases or metastases in lymph nodes should receive annual prophylactic administration of *B. oligonitrophilus* KU-1 culture (50-100 mL per day) on alternate weeks for at least a 3-month period.

Statistics

All data were analyzed by Origin® version 6.1 software (Origin Lab Corporation, MA, USA). To compare

Table 2. Results of cytogenetic testing with onion roots (*Allium cepa*): effect of *Bacillus oligonitrophilus* KU-1 on the level of chromosomal aberrations

	Control (distilled water)	*B. oligonitrophilus* KU-1 (10 %)	*B. oligonitrophilus* KU-1 (1%)	*B. oligonitrophilus* KU-1 (0.1%)
Total chromosomal aberrations (%) [mean ± SD]	2.23 ± 0.84	1.36 ± 0.56	2.45 ± 0.7	2.65 ± 0.47

Abbreviation: SD = standard deviation

Table 3. Blood indexes in rats receiving *Bacillus oligonitrophilus* KU-1 as a food supplement

Duration of administration	Hb (%)	ESR (mm/h)	Er (/μL) [× 10^6]	Leu (/μL) [× 10^3]	Leukocytic formula (%)				
					E	SN	SL	L	M
Control	9.98 ± 0.84	2.2 ± 0.61	6.4 ± 0.21	17.7 ± 1.66	0.8 ± 0.25	1.5 ± 0.66	15 ± 1.9	79.7 ± 1.94	3.1 ± 1.7
1 month	11.25 ± 0.5	3.1 ± 0.41	7 ± 0.38	21 ± 2.4	2.25 ± 0.75	1.2 ± 0.49	20.55 ± 1.94	74 ± 2.64	1.75 ± 0.48
3 months	11.15 ± 0.48	2.75 ± 0.75	6.88 ± 0.34	20.2 ± 2.6	2.75 ± 1.1	0.2 ± 0.2	24.5 ± 5.2	66.25 ± 9.6	1.25 ± 0.25

Abbreviations: Hb = hemoglobin; ESR = erythrocyte sedimentation rate; Er = erythrocytes; Leu = leukocytes; E = eosinophils; SN = stab neutrophils; SL = segmentonuclear leukocytes; L = lymphocytes; M = monocytes.

differences in overall survival, we used paired Student's *t* test. A *p* value of <0.05 was considered significant.

Ethics

The current work was performed according to standards of ethical research published online at http://icmr.nic.in/ethical.pdf.

Results

This retrospective study involved 13 patients who had earlier undergone treatment at cancer hospitals in Kazan, Samara and Moscow, Russia. These patients were then given *B. oligonitrophilus* KU-1 in the outpatient setting.

Patient 1

Female, born in 1937. In 1998, rectal adenocarcinoma with non-operative metastases into greater omentum was diagnosed ($T_3N_1M_1$). Primary tumor ablation and formation of sigmoid-anal anastomosis was performed at Tatarstan Republican Hospital, Kazan. The patient rejected chemotherapy (5-fluorouracil). Two months after the operation, the oral administration of *B. oligonitrophilus* KU-1 culture (200-250 mL per day) was started. Four months after the beginning of bacteria treatment in June 1999, cancer antigen (CA) 19-9 oncomarker reached normal value (9.02 U/mL). Since that time, prophylactic *B. oligonitrophilus* KU-1 culture was taken during February to June every year, and during this time, CA 19-9 level was under control (October 1999, 788 U/mL; February 2000, 10.74 U/mL; September 2000, 4.38 U/mL; March 2001, 7.06 U/mL; March 2002, 5.56 U/mL; November 2003, 29.3 U/mL). All this time, blood indexes were at the normal level. In February of 2004, a polyp (0.6 cm) was detected in the stomach with the use of gastroendoscopy. In June 2004, CA 19-9 level was 31.1 U/mL and the patient remains alive.

Patient 2

Female, born in 1929. In 1996, sigmoid colon tumor was detected. Primary tumor ablation was performed at Tatarstan Republican Hospital. Two years later (in December 1998), tumor relapse with metastases into left ureter and loops of thin bowel was determined. A tumor (15 × 9 cm) was observed at bifurcation of the aorta. The patient rejected chemotherapy. Since that time, minimal doses of *B. oligonitrophilus* KU-1 culture were received. In 1999, the bacterial dose was increased to 50 mL per day, and was subsequently decreased to 20 mL 1 year later, resulting in stabilization of hemoglobin level. The patient died from stroke in November 2000.

Patient 3

Female, born in 1930. In early 1997, mammary gland adenocarcinoma with vertebral column metastases, metastases in ribs and upper extremities was determined ($T_3N_1M_1$). The associated illnesses were diabetes, allergy, and renal calcinosis. Primary tumor ablation was performed at the All-Russian Oncological Center, Moscow. In March 1997, the patient was started on *B. oligonitrophilus* KU-1 culture (400 mL per day). Three-and-a-half months later, the stabilization of metastases was observed, and bacteria administration was stopped. However, tumor relapse occurred subsequently, and the patient died in November 1998.

Patient 4

Female, born in 1957. In late 1999, ovarian tumor with metastases into liver and abdominal cavity as well as undifferentiated rectal tumor was diagnosed. The level of CA 19-9 oncomarker was more than 400 U/mL. In February 2000, ablative surgery was performed at the Government State Service "Oncology", Kazan; sigmostoma and rectum were not ablated. Predicted disease-free survival was no more than 6 months. In May 2000, treatment with *B. oligonitrophilus* KU-1 culture (200 mL per day) was started. The dose was increased to 400 and 600 mL in August and October, respectively. Topotecan was administered simultaneously. In December 2000, oncomarkers CA 125 and CA 72-4 were at normal levels. Because of lack of availability, topotecan was stopped in January 2001. The level

of CA 19-9 was increased more than 400 U/mL. During this time, the patient received 500-700 mL of *B. oligonitrophilus* KU-1 culture daily. The patient died in July 2001 due to liver collapse.

Patient 5

Male, born in 1955. In 2000, rectal adenocarcinoma with metastases into liver was revealed ($T_3N_1M_1$). Primary tumor ablation was performed at Samara Oncology Center, Samara. The patient rejected chemotherapy. Predicted time of death was summer 2002. There was irregular administration of *B. oligonitrophilus* KU-1 culture (200-300 mL per day). In autumn 2001, there was a significant increase in CA 19-9 level (up to 500 U/mL) and the patient began to take bacteria routinely (300 mL per day). In February 2002, lung metastases were revealed. The patient deteriorated from May 2003, when the daily intake of bacteria was 200-250 mL. The patient died in September 2003 due to pulmonary edema and stroke.

Patient 6

Female, born in 1939. In January 1999, mildly differentiated tumor of maxillary sinus was revealed ($T_3N_0M_0$). Despite chemotherapy and radiotherapy, the patient deteriorated. In September 1999, the patient rejected chemotherapy and radiotherapy and began treatment with *B. oligonitrophilus* KU-1 culture (300-400 mL per day). Her condition subsequently stabilized. In spring 2001 and 2002, prophylactic *B. oligonitrophilus* KU-1 culture was administered. In August 2002, nephritis developed and was treated with antibiotics. In December 2002, there was relapse of maxillary sinus tumor and undifferentiated form of that tumor was diagnosed. Several surgeries were performed at Samara Oncology Center. All this time, treatment with *B. oligonitrophilus* KU-1 culture was continued. Blood indexes were at normal levels. In November 2003, there was a drastic worsening of health state, and the patient died in January 2004.

Patient 7

Male, born in 1925. In March 2001, metastatic backbone tumor was revealed (tumor nidus 1.5 × 5 cm in D9 vertebral body). There was an edge destruction of C10 vertebral body. Primary tumor ablation was performed at Kazan State Oncological Center. The patient rejected chemotherapy and radiotherapy. The initial dose of *B. oligonitrophilus* KU-1 culture was 100 mL per day. All that time, blood indexes were at normal levels, but an increase in hemoglobin was observed (to 146-157 g/L). In February 2004, the patient stopped taking *B. oligonitrophilus* KU-1 culture as he considered himself healthy. The patient remains alive.

Patient 8

Female, born in 1947. In 2003, so-called cancer in situ was determined. In early spring 2003, CA 19-9 level was 149 U/mL. *B. oligonitrophilus* KU-1 culture was given from June 2003 (150 mL per day), but was stopped subsequently because of increased blood pressure. CA19-9 level was 28 U/mL in December 2003. The patient is alive up to now.

Patient 9

Female, born in 1935. In autumn 2003, mildly differentiated stomach adenocarcinoma was revealed ($T_4N_xM_0$). After that, sub-total resection of the stomach was performed at Government State Service "Oncology". In May 2004, metastases were determined in the abdominal cavity. Blood and urine indexes had plural fluctuations (haemoglobin 70g/L, total protein 52g/L, leukocytes 3.2×10^9/L). The patient did not receive chemotherapy. *B. oligonitrophilus* KU-1 (200 mL per day) was begun in June 2004. By autumn 2004, the hemoglobin level had increased to 102 g/L. In September 2004, CA 19-9 level was 19 U/mL, hemoglobin 116 g/L, and blood and urine indexes were at normal values. Increase in blood pressure of 20-30 mm Hg was observed. Cancer stabilization was achieved and the patient remains alive.

Patient 10

Female, born in 1962. In August 2002, double-sided ovary cystadenocarcinoma with a wide distribution into peritoneum was determined ($T_4N_xM_1$). In addition, multiple metastases into the greater omentum were observed. Hysterectomy and ablation of greater omentum were performed at Samara Oncology Center. Cystatin was administered. From February 2003, the patient received *B. oligonitrophilus* KU-1 300 mL per day. Increased intracranial pressure was observed, while blood pressure was normal. During that period, CA 125 level was normal (less than 30 U/mL). After CA 125 normalization, a prophylactic regimen of *B. oligonitrophilus* KU-1 was given (50 mL per day, on alternate weeks). In autumn 2003, CA 125 level was normal (13 U/L), but increased to 225 U/mL in May 2004. Chemotherapy (cystatin 110 mg) was administered. From June 2004, the patient received

B. oligonitrophilus KU-1 (300 mL per day). In July 2004, CA 125 decreased to 50 U/mL. Ultrasound data indicated that the size of metaplastic cancer in cellular tissue of pelvis minor decreased to 30%. In September 2004, CA 125 level was normal (15 U/mL). The patient continues to receive *B. oligonitrophilus* KU-1 50 mL per day on alternate weeks and remains alive.

Patient 11

Female, born in 1932. In 1993, high-grade differentiated adenocarcinoma of mammary gland was determined ($T_4N_0M_1$) with metastases into lymph nodes. Cancer resection was performed at Kharzizsk Oncological Center, Ukraine. In 2003, tumour relapse was determined (metastases in lymph nodes and mediastinum). In December 2003, *B. oligonitrophilus* KU-1 (250 mL per day) was started, but was withdrawn in March 2004, due to a drastic increase in blood pressure associated with stopping an antihypertensive drug (clonidine). In March 2004, CA 15-3 was normal (18.5 U/mL). According to ultrasound data, lymph nodes were not present in the mediastinum; clavicular lymph nodes were also decreased. Since summer 2004, the patient has received *B. oligonitrophilus* KU-1 50 mL per day on alternate weeks, and remains alive.

Patient 12

Female, born in 1976. In 1997, high-grade differentiated adenocarcinoma of thyroid gland was revealed ($T_4N_1M_0$). Tumor resection was made at Tatarstan Republican Hospital. After operation, the patient received thyroxine replacement therapy. In 2002, tumor relapse occurred. Two operations were performed (ablation of stiff lymph nodes). In September 2002, the patient started *B. oligonitrophilus* KU-1 (300 mL per day). In March 2003, there was an increase in blood and intracranial pressure and *B. oligonitrophilus* KU-1 was stopped for 1 month. Since April 2003, *B. oligonitrophilus* KU-1 (75 mL per day on alternate weeks) was recommenced. In April 2003, the patient became pregnant, and in June 2003 *B. oligonitrophilus* KU-1 was stopped. In August 2003, ablation of lymph nodes from the neck was performed. In November 2003, she gave birth to 2 boys born prematurely without any pathology. Since autumn 2004, she has received thyroxine replacement therapy and remains free of relapse.

Patient 13

Male, born in 1946. In April 2003, mildly differentiated adenocarcinoma of descending colon was noted ($T_4N_2M_0$), and this was operated on in June 2003 at Government State Service "Oncology". From August 2003, the patient received *B. oligonitrophilus* KU-1 (100 mL per day). In October 2003, CA 19-9 level was 208 U/mL. According to ultrasound data, there were metastases in the liver. In November 2003, the dose of *B. oligonitrophilus* KU-1 was increased to 600 mL per day. In December 2003, CA 19-9 level increased to 1020 U/mL. Ultrasound findings showed metastases with fluid substances, a characteristic feature of undifferentiated tumor. In December 2003, *B. oligonitrophilus* KU-1 volume was decreased slightly, to 500 mL per day. In February 2004, *B. oligonitrophilus* KU-1 was decreased to 400 mL per day. In that period, the patient received 5-fluorouracil with leucovorin. In April 2004, multiple metastases (5 cm in diameter) in the liver were determined by ultrasound imaging. In April 2004, *B. oligonitrophilus* KU-1 was increased to 500 mL per day. In August 2004, it was found that liver metastases had increased to 7 cm in diameter. Additionally, metastases were observed in the greater omentum and abdominal cavity. In September 2004, there was further worsening of health. In September 2004, supporting therapy with vicasol and prednisolone was initiated. At the end of September 2004, the patient deteriorated rapidly, with development of jaundice and cachexy, loss of appetite, insomnia, and oedema of the legs. The patient died in October 2004.

Table 4. Survival periods for patients under study and comparison with predicted values

Patient no.	Observed overall survival (months)	Survival predicted by physician in charge of the case (months)	Survival according to literature data [12] (months)
2	48	0	6
3	18	3	12
4	18	6	6
5	36	18	6
6	60	24	18
13	17	12	12

Table 5. Student's *t* test for survival period

Type of comparison	*t* value
Observed survival vs survival predicted by physician in charge of the case	3.36 ($p<0.05$)
Observed survival vs survival according to literature data	3.22 ($p<0.05$)

Observed versus predicted survival

The overall survival for patients that died (patients 2, 3, 4, 5, 6, 13) and predicted survival times are presented in Table 4. For better comparison, we also present literature data [12] on survival period for each type of cancer observed in our patients (Table 5). As can be seen from Table 5, there was significant life prolongation in patients who received *B. oligonitrophilus* KU-1. All of the patients remaining alive have exceeded expected survival times based on literature data [12] (Table 6).

Side effects

Some of the patients above (e.g., patients 2, 7, 9, 10, 11, 12) had side effects (mainly increase in blood pressure) after receiving *B. oligonitrophilus* KU-1 culture. Side effects that have been associated with *B. oligonitrophilus* KU-1 culture can be summarized as follows:

1. Intolerance (nausea, retching, a drastic increase of severe pain)
2. Piesis and increased intracranial pressure
3. Worsening of allergy and autoimmune diseases
4. Gastritis induction (with long-term treatment).

Discussion

Prognosis and treatment effectiveness

All patients demonstrated the phenomenon of oncomarker reduction in association with *B. oligonitrophilus* KU-1 administration. The use of *B. oligonitrophilus* KU-1 allows avoidance of many possible side effects of chemotherapy and radiotherapy, such as diarrhea, stomatitis, alopecia, neutropenia and hyperbilirubinemia.

In the case of intolerance (daily dose volume less than 200 mL), there is a poor prognosis, and prolongation of life is rare. Although patient 2 received about 20-50 mL per day of bacterial culture, there was significant life prolongation. This case might be considered exceptional. In the absence of intolerance (daily dose volume 200-500 mL), prolongation of life (approximately 12-18 months in addition to standard prognosis) is possible in patients with undifferentiated tumors (e.g., patients 3, 4, 13). Life prolongation (no more than 2 years in addition to standard prognosis) is possible in patients older than 40 to 60 years with mildly differentiated tumors. This prognosis might correspond to patient 5. However, despite the metastases in the liver, more significant life prolongation was observed, and was presumably exceptional in this regard. Life prolongation (more than 2-3 years in addition to standard prognosis) is possible in patients with high-grade differentiated tumors in the absence of metastases in the vitally important organs (i.e., liver or lungs). In patients older than 60 years, the complete elimination of revealed metastases is possible (especially in the case of mammary gland adenocarcinoma). This prognosis corresponds to patients 1 and 6-12.

As is shown in practice with terminally ill patients, undifferentiated tumor, black cancer, mesothelioma and medulloblastoma are badly suppressed by reception of silicate bacteria. Prognosis becomes worse in the presence of metastases into liver, lungs, and heart and in young age. Taking into account the cases of advanced disease considered here, it is possible to conclude that these silicate bacteria successfully block highly differentiated and mildly differentiated mammary gland adenocarcinoma with metastases into bones and lymph nodes.

Table 6. Overall survival for patients currently under observation

Patient no.	Observed overall survival (months)	Survival according to literature data [12] (months)
1	82	6
7	42	Unknown
8	20	Unknown
9	15	2
10	29	18
11	120	12
12	84	50

Mechanisms of action

It is well established that bacteria can be used to improve human health. The so-called probiotic bacteria provide specific health advantages when taken as a food component or supplement. According to Guarner and Schaafsma [13], probiotics do not necessarily colonize the human bowels. Probiotics taken orally have been shown to prevent antibiotic-associated diarrhoea [14]. In the case of diarrhoea caused by rotavirus infection, oral administration of probiotics can be also very effective [15]. Schiffrin et al found that medicinal effects

in rotavirus infection might be related to enhancement of immunoglobulin A production in infected children [16]. In healthy humans, probiotics were shown to enhance phagocytic activity of circulating leukocytes, supporting a role for enteric bacteria in increasing local and systemic immune response [17]. Among further health benefits, probiotics reduce colonization by *Helicobacter pylori* [18], relieve inflammatory bowel disease [19], and can be used for treatment and prevention of allergy [20].

Anticancer activity of various microorganisms has been discussed since the middle of the last century. It was shown that actinomycete is able to destroy the cells of ascitic tumor in vivo and in vitro [21]. Folmar and Knel found that various bacterial species (streptococcus, meningococcus, grass bacillus) possess cytotoxic activity against tumor cells [22]. Roberts et al found that L-asparaginase isolated from *Escherichia coli* is able to obliterate Gartner $6C_3$HED lymphosarcoma [23]. Carswell et al showed that *E. coli* endotoxins possess carcinolytic action [24]. Reddy et al showed that probiotics might interact directly with tumor cells in culture and inhibit their growth [25]. In mice and rats, anticancer activity of probiotics manifests via stimulation of immune function, resulting in increases in tumor necrosis factor-α, interferon-α and interleukin-10 [26]. In humans, probiotics can prevent recurrence of superficial bladder cancer [27] and may prolong survival in patients with cervical cancer [28].

The immunologic mechanisms of anticancer activity of probiotics are reviewed by Ouwehand and coauthors [29]. At the biochemical level, the cancer prevention effect may be connected with inhibition of certain enzymes (azoreductase, urease, nitroreductase, β-glucouronidase and glycocholic acid reductase) that are responsible for the transformation of procarcinogens into carcinogens [30]. Many bacterial genera (*Lactobacillus, Bifidobacterium, Propionobacterium, Bacillus, Escherichia, Enterococcus* and *Saccharomyces*) have probiotic action [31].

Our data strongly suggest that *B. oligonitrophilus* KU-1 is a more effective probiotic than previously found. The original hypothesis, was proposed over 30 years ago by a famous Russian embryologist, Tokin, who suggested that the main condition of cancer treatment might be enhancement of an organism's vitality, resulting in its integration (integration refers to cells forming organs and more complex systems). According to Tokin's theory, "everything that favors normal forming must counteract tumor growth and vice versa" [32]. In other words, the main reasons for malignant growth are the decrease in regeneration level within the whole organism or locally and aberration of normal forming (i.e., regeneration and malignant growth are antagonistic processes). Many facts support this idea. Namely, carcinogenic chemicals impede the process of regeneration and a decrease in regeneration level is observed during cancer formation. In cancer patients, aberration in wound repair and opening of joints after operation are the most frequent examples of regeneration reduction. At the same time, many chemotherapeutic agents (e.g., 5-fluorouracil, cyclophosphane, bleomycin, topotecan and many others) and radiation (in particular, X-ray) used frequently in tumor treatment possess carcinogenic and mutagenic activity, and thus pose a hazard if incorrect doses are used.

According to Tokin's suggestion, it is necessary to search for integrators that would increase the vitality of the organism. These might include probiotic bacteria. Gut microflora are able to prevent cancer formation, but these bacteria are probably not strong integrators. Some silicate-destroying bacteria (releasing biologically active silicon into environment) may be such integrators.

Genome rejuvenation hypothesis

B. oligonitrophilus serves as a donor of bioavailable silicon. The hypothesis of genome rejuvenation, developed at Kazan State University Department of Genetics, relies on the fact that silicon is an obligatory component of nucleic acids [33-38]. Using emission spectroscopy, Voronkov et al found that DNA contains approximately 0.31% and 0.26% of silicon (data for cow spleen and hunchback salmon milt, respectively [37]. Voronkov et al suggested that silicon in nucleic acids is isomorphous to phosphorus, and is present in DNA as shown in Fig. 1 [37]. With each replication cycle, chromosomal silicon is lost, resulting in its accumulation in blood. For example, the silicon concentration in blood increases 5- to 90-fold in older humans compared with younger individuals [35].

Genome rejuvenation is the reverse incorporation of silicon into DNA molecules. This process is possible in all living organisms. In plants, genome rejuvenation occurs during somatic embryogeny, the shoot-forming processes, syngenesis and cloning. In animals and humans, genome rejuvenation takes place during gametogenesis, formation of regeneration blastema, and in transplantable cellular cultures of animals and humans. In bacteria, the process of genome rejuvenation is probably connected with sporulation (e.g., in *Bacillus*

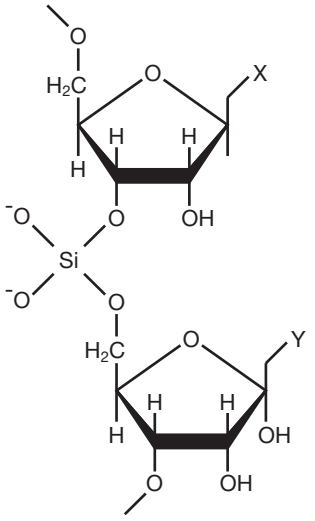

Fig. 1. Distribution of silicon (Si) atoms within the nucleic acids.

subtilis). In turn, aberration of rejuvenation resulted in competence development [39]. The development of competence might be considered as a process of senescence at the bacterial level; indeed, competent cells of *B. subtilis*, for example, have low vitality and can be easily damaged [40].

The most probable role of *B. oligonitrophilus* KU-1 within the organism is maintenance of integration processes, which results in introduction of silicon to DNA. Moreover, we have shown previously that this bacterial strain is able to decrease allergic reactions within the organism [41]. This is in agreement with data of other researchers [42]. Apart from the strengthening of integration processes of organisms (that is necessary, according to Tokin's theory, for successful antagonism of tumor cells), an anticancer effect can be connected with synthesis of specific antitumor agents by some enterobacterial species in cooperation with *B. oligonitrophilus* KU-1. Moreover, death of tumor cells might result from their "hypersilicosis" (only tumor cells in which the normal genome rejuvenation is broken will be hypersensitive to increased level of silicon).

In addition, Voronkov and collaborators reported in 1971 that some silicon compounds are able to inhibit malignant growth [43]. They found that silicon introduction in rats resulted in cancer necrosis and development of connective tissues due to increased collagen synthesis. It is very likely that bioavailable silicon released by *B. oligonitrophilus* KU-1 is used by prolyl hydroxylase, an obligatory enzyme in the formation of connective tissue. Therefore, stabilization of metastatic growth can be also explained by increased development of connective tissue, in agreement with Tokin's theory that stimulation of normal form-building processes results in inhibition of cancer growth.

In conclusion, *B. oligonitrophilus* KU-1 cells increase the organism's resistance to tumor cells without the use of any inhibitor agents. This anticancer activity is evident but its precise mechanisms should be explored in the future. A balance of integrating bacteria and specific inhibitors of rejuvenation systems of tumor cells exists. We believe that the search for strains with more integrating capacity is needed as well as broad clinical investigations. Finally, we are seeking the test systems that would permit us to find silicate bacteria with sharply defined integrating features.

References

1. Benner SE, Winn RJ, Lippman SM, Poland J, Hansen KS, Luna MA, et al. Regression of oral leukoplakia with alpha-tocopherol: a community clinical oncology program chemoprevention study. J Natl Cancer Inst 1993;85:44-7.
2. Robbins KT, Kumar P, Regine WF, Wong FS, Weir AB 3rd, Flick P, et al. Efficacy of targeted supradose cisplatin and concomitant radiation therapy for advanced head and neck cancer: the Memphis experience. Int J Radiat Oncol Biol Phys 1997;38:263-71.
3. Brizel DM, Albers ME, Fisher SR, Scher RL, Richtsmeier WJ, Hars V, et al. Hyperfractionated irradiation with or without concurrent chemotherapy for locally advanced head and neck cancer. N Engl J Med 1998;338:1798-804.
4. Alheit H, Saran FH, Warrington AP, Rosenberg I, Perks J, Jalali R, et al. Stereotactically guided conformal radiotherapy for meningiomas. Radiother Oncol 1999;50:145-50.
5. Flickinger JC, Kondziolka D, Maitz AH, Lunsford LD. Gamma-knife radiosurgery of imaging-diagnosed intracranial meningioma. Int J Radiat Oncol Biol Phys 2003;56:801-6.
6. Bolsi A, Fogliata A, Cozzi L. Radiotherapy of small intracranial tumours with different advanced techniques using photon and proton beams: a treatment planning study. Radiother Oncol 2003;68:1-14.
7. Krasilnikov NA, ed. Opredelitel bakterii i actinomycetov (Manual for identification of bacteria and actinomycete). Moscow-Leningrad: USSR Academy of Sciences Press; 1949.
8. Alexandrov VG, Zak GA. Aluminosilicate-destroying bacteria [in Russian]. Mikrobiologia 1950;19:97-108.
9. Ames BN, McCann J, Yamasaki E. Methods for detecting carcinogens and mutagens with the *Salmonella*/mammalian microsome mutagenicity test. Mutat Res 1975;31:347-64.
10. Fiskesjö G. *Allium* Test for screening chemicals; evaluation of cytological parameters. In: Wang W, Gorsuch JW, Hughes JS, eds. Plants for environmental studies. Boca Raton-New York: CRC Press-Lewis Publishers; 1997:313-33.

11. Trahenberg IM, Sova RE, Shevtel VO. Problema normy v toksikologii (The problem of norm in toxicology) [in Russian]. Moscow: Medicine; 1991.
12. Jemal A, Clegg LX, Ward E, Ries LA, Wu X, Jamison PM, et al. Annual report to the nation on the status of cancer, 1975-2001, with a special feature regarding survival. Cancer 2004; 101:3-27.
13. Guarner F, Schaafsma G. Probiotics. Int J Food Microbiol 1998; 39:237-48.
14. D'Souza AL, Rajkumar C, Cooke J, Bulpitt CJ. Probiotics in prevention of antibiotic associated diarrhoea: meta–analysis. BMJ 2002;324:1361-6.
15. Guandalini S, Pensabene L, Zikri MA, Dias JA, Casali LG, Hoekstra H, et al. *Lactobacillus GG* administered in oral rehydration solution to children with acute diarrhea: a multicenter European trial. J Pediatr Gastroenterol Nutr 2000; 30:54-60.
16. Schiffrin EJ, Rochat F, Link-Amster H, Aeschlimann JM, Donnet-Hughes A. Immunomodulation of blood cells following the ingestion of lactic acid bacteria. J Dairy Sci 1995;78: 491-7.
17. Majamaa H, Isolauri E, Saxelin M, Vesikari T. Lactic acid bacteria in the treatment of acute rotavirus gastroenteritis. J Pediatr Gastroenterol Nutr 1995;20:333-8.
18. Felley CP, Corthesy-Theulaz I, Rivero JL, Sipponen P, Kaufmann M, Bauerfeind P, et al. Favourable effect of an acidified milk (LC-1) on *Helicobacter pylori* gastritis in man. Eur J Gastroenterol Hepatol 2001;13:25-9.
19. Gupta P, Andrew H, Kirschner BS, Guandalini S. Is *Lactobacillus* GG helpful in children with Crohn's disease? Results of a preliminary, open-label study. J Ped Gastroenterol Nutr 2000;31:453-7.
20. Mattila-Sandholm T, Blum S, Collins JK. Probiotics: towards demonstrating efficacy. Trends Food Sci Technol 1999;10: 393-9.
21. Brander WT, Clark DA. Inhibition of anaerobic bacteria as a screen for anti-tumor agents. Cancer Res 1958;2:299-304.
22. Folmar G, Knel G. Influence of bacterial drugs and bacteriophages on tumor and normal cell cultures. In: Neumann IM, ed. Biological bases of malignant growth. Moscow: Nauka; 1950:451-60.
23. Roberts J, Prager M, Bachynsky N: The antitumor activity of *Escherichia coli L*-asparaginase. Cancer Res 1966;26: 2213-27.
24. Carswell EA, Old LJ, Kassel RL, Green S, Fiore N, Williamson B. An endotoxin-induced serum factor that causes necrosis tumors. Proc Natl Acad Sci USA 1975;72:3666-70.
25. Reddy GV, Friend BA, Shahani KM. Antitumour activity of yogurt components. J Food Protect 1983;46:8-11.
26. de Moreno de Leblanc A, Perdigon G. Yogurt feeding inhibits promotion and progression of experimental colorectal cancer. Med Sci Monit 2004;10:BR96-104.
27. Aso Y, Akaza H, Kotake T, Tsukamoto T, Imai K, Naito S. Preventive effect of *Lactobacillus casei* preparation on the recurrence of superficial bladder cancer in a double-blind trial. Eur Urol 1995;27:104-9.
28. Okawa T, Niibe H, Arai T, Sekiba K, Noda K, Takeuchi S, et al. Effect of LC9018 combined with radiation therapy on carcinoma of the uterine cervix. A phase III, multicenter, randomized, controlled study. Cancer 1993;72:1949-54.
29. Ouwehand AC, Kirjavainen PV, Shortt C. Probiotics: mechanisms and established effect. Int Dairy J 1999;9:43-52.
30. Hirayama K, Rafter J. The role of probiotic bacteria in cancer prevention. Microbes Infect 2000;2:681-6.
31. Ouwehand AC, Salminen S, Isolauri E. Probiotics: an overview of beneficial effects. Antonie Van Leeuwenhoek 2002;82: 279-89.
32. Tokin BP, ed. Obschaya embryologia (General embryology). Moscow: High School; 1977.
33. Reffitt DM, Ogston N, Jugdaohsingh R, Cheung HF, Evans BA, Thompson RP, et al. Orthosilicic acid stimulates collagen type 1 synthesis and osteoblastic differentiation in human osteoblast-like cells in vitro. Bone 2003;32:127-35.
34. Carlisle EM. Silicon as an essential element for the chick. Science 1972;178:619-21.
35. Voronkov MG, Kuznetzov IG, eds. Silicon in living organism. Novosibirsk: Nauka; 1984.
36. Voronkov MG. Biological activity of silatranes. Top Curr Chem 1979;84:77-135.
37. Voronkov MG, Skorobogatova VI, Vugmeister EK, Makarskii VV. Silicon in nucleic acids [in Russian]. Dokl Akad Nauk SSSR 1975;220;723-5.
38. Voronkov MG. Biologically effective organo-silicious compounds—advances, problems, prospects. Cesk Farm 1973; 22:406-9.
39. Malkov SV, Barabanschikov BI. Influence of silicon metabolism on genetic transformation in *Bacillus subtilis* [in Russian]. Mol Gen Microbiol Virol 1991;2:9-11.
40. Prozorov AA, eds. Transformation in bacteria. Moscow: Nauka; 1988.
41. Malkov SV, Polosov GY, Zakharova NG. The decrease in allergenic activity of plants [in Russian]. Bull RASHN 2004; 1:63-4.
42. Paganelli R, Ciuffreda S, Verna N, Cavallucci E, Paolini F, Ramondo S, et al. Probiotics and food-allergic diseases. Allergy 2002;57 (Suppl 72):97-9.
43. Voronkov MG, Grigalinovich GA, Zelchan GI. Inhibitor action of some silicon compounds on the growth of malignant tumor cells [in Russian]. Dokl Akad Nauk SSSR 1971;200: 967-9.