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Antimutagenic and reactivative activities of dairy propionibacteria

LI Vorobjeva, E Yu Khodjaev, TA Cherdinceva

Department of Microbiology, Biology Faculty, Moscow State University, Moscow 119899, Russia

Summary — Cells of *Propionibacterium freudenreichii* subsp *shermanii* lower the mutagenicity of 4-nitroquinoline-1-oxide in the tester strain *Salmonella typhimurium* TA100 in the modified Ames test. The bacterial adaptation to the mutagen presence in the medium is demonstrated. It is possibly linked to induction of an antimutagenic activity. A dialysed fraction of soluble proteins of the extract (dialysate) lowers the mutagenicity of UV-C light in *Salmonella typhimurium* and manifests protective and reactivative effects on UV-B- and UV-C-irradiated cells of *S typhimurium, Escherichia coli* and the yeast *Candida guilliermondii*. The reactivation does not occur after the cell irradiation with a light of total optical spectrum and visible light. (The last was studied with the yeasts.) These data serve as an indirect indication of the dialysate proteins participation in the repair of DNA injured by UV-B and UV-C irradiation. The reactivation by dialysate occurs in *E coli* and the yeasts *Saccharomyces cerevisiae* and *C guilliermondii*, inactivated by heating. The reactivative activity of the dialysate was localized in its 2 subfractions that manifests in *E coli* inactivated not only by UV light but also by heating.

antimutagen / UV reactivation / 4-nitroquinoline-1-oxide / propionibacteria

Résumé — L'activité antimutagène et réactivatrice des bactéries propioniques laitières. *Les cellules et les composés de l'extrait cellulaire de* Propionibacterium freudenreichii *subsp* shermanii *diminuent l'effet mutagène du 4-nitroquinoline-1-oxide chez le microorganisme-test,* Salmonella typhimurium *TA100, utilisé dans le test modifié de Ames. L'adaptation des bactéries à la présence des composés mutagènes dans le milieu a été observée. Elle est liée probablement à l'induction de l'activité antimutagène. La fraction dialysée des protéines solubles de l'extrait cellulaire (le dialysat) diminue l'effet mutagène des rayons UV-C sur* Salmonella typhimurium *et manifeste l'activité de protection et de réactivation (protectrice et réactivatrice) envers les cellules de* Escherichia coli *et des levures* Candida guilliermondii *traitées par les rayons UV-B et UV-C. La réactivation n'est pas observée dans les cellules de bactéries et de levures traitées par les rayons de spectre optique complet ou par la lumière visible (dans le cas des levures). Ces résultats indiquent indirectement la participation des protéines de dialysat à la réparation de l'ADN endommagé par les rayons UV-B et UV-C. La réactivation par le dialysat est observée aussi chez les levures* Saccharomyces cerevisiae *et* C guilliermondii, *inactivées par le chauffage. L'activité de réactivation du dialysat était localisée dans ses deux subfractions qui possédaient une activité réactivatrice pour les cellules de* E coli *AB1157 inactivées non seulement par UV, mais aussi par le chauffage.*

antimutagène / UV réactivation / 4-nitroquinoline-1-oxide / bactérie propionique

INTRODUCTION

An antimutagenicity phenomenon was discovered in the 1950s (Novick and Czillard, 1952; Glass and Novick, 1959), when the study of the purine nucleotides revealed their ability to decrease the levels of spontaneous and induced mutability of *Escherichia coli* cells. The term *antimutagen* (AM) has been adopted for factors that reduce the frequency of spontaneous and induced mutagenesis by different modes of action. It was suggested by Kada *et al* (1981) to subdivide the AMs into desmutagens (DSMs) and bioantimutagens (BioAMs). DSMs diminish the frequency of induced mutations by mutagen inactivation outside the cell *via* chemical or biochemical modification of mutagens. BioAMs modulate mutagenesis at the cellular level *in vivo*. AMs and their mode of action were reviewed by Kada *et al* (1986).

AMs are produced by all living creatures on Earth from bacteria to humans, sensing a pressure of both the exogenous and endogenous mutagens. The list of endogenous mutagens includes such bacterial metabolites as H_2O_2, nitrosamines, some antibiotics, formaldehyde and H_2S. In the course of normal metabolism of humans some substances with mutagenic activity appear: humoral mutagens, originating after stress; O_2^- and OH• radicals; phagocytes, generating H_2O_2, O_2^-, OCl^-, chloramine during consumption of alive and dead bacteria and cells; viral infection. Some components of food, products damaged by mould (by mycotoxins, aflatoxins), natural pesticides, nitrates, nitrosamine are the sources of mutagens. Almost all natural food consumed by humans contains carcinogens, mutagens, teratogens and clastogenes (Ames and Gold, 1991).

Today, the frequency of natural mutations increases significantly and is affected by some abiotic and biotic factors (Alekperov *et al*, 1977). Mutagens, teratogens and carcinogens are generated by natural radiation, randon, radionuclides in the Earth crust, pesticides, synthetic dyes and so on (Dubinin, 1976).

Antimutagenesis is a natural process and most of the AMs are naturally occurring compounds. Antimutagenic properties are inherent to antioxidants, superoxide dismutase (SOD), catalase, GSH-peroxidase, β-carotin, α-tocopherol, vitamin C, uric acid (major antioxidant in saliva) and many other substances, some of which are mentioned in the reviews by Hocman (1988) and Hartman and Shankel (1990). Antimutagenicity is detected for more than 300 chemicals, mostly of vegetable and animal origin and some synthesized chemically.

Bacteria only recently became the target of investigations into antimutagenicity. There are strong indications of desmutagenic activity of milk, fermented by *Lactobacillus bulgaricus*, *Streptococcus thermophilus* (Bodana and Rao, 1990) and *Streptococcus faecalis* (Hosono *et al*, 1986). The activity was directed against 4-nitroquinoline-1-oxide (4NQO) and 2-aminofluorine (2-AF). Lactic acid bacteria are the components of natural microflora of humans and some animals.

Antitumour qualities of cultured milk were demonstrated by Reddy *et al* (1973, 1983) and Shackelford *et al* (1983). Peptides with antimutagenic effect against induced mutagenesis by N-methyl-N'-nitro-N-nitrosoguanidine (MNNG) and UV light in *E coli* MP-1 and spontaneous mutagenesis in *Bacillus subtilis* N16 1125, were isolated from the cultural broth of streptomycetes (Osawa *et al*, 1986). Ethanol extracts from the fungus *Craterellus cornucopiodes* and 6 other fungi inhibit mutagenicity of aflatoxin B, benzo[a]pyrene, acridine half mustard (ICR-191) and 2-nitrofluorine. The conceivable mechanisms include direct chemical interaction with the mutagen and/or the inhibition of the activation process in the case of promutagens (Gruter *et al*, 1990). It was

first demonstrated by Vorobjeva *et al* (1991) that propionibacteria produce some peptides with an antimutagenic effect against mutagenesis induced by MNNG and NaN_3. Antimutagenicity was partly due to SOD activity of the cells (Vorobjeva *et al,* 1993b). It was also shown (Vorobjeva *et al,* 1993a) that some strains of skin and dairy propionibacteria excrete in the medium the compounds with desmutagenic properties against 4NQO. The obtained data support the suggestion that desmutagenesis is caused by the excreted reducing, possibly, SH compounds that inactivate electrophilic groups of mutagen.

UV light is one of the common mutagens and its effect has significantly increased with depletion of the ozone layer. There are numerous substances, both individual and complex, that possess protective properties against UV light and X rays. Some are produced by bacteria. In the course of evolution, bacteria were affected by UV light and acquired the mechanism of lowering the mutagenic and inactivative effects of UV radiation. The first investigations concerning this problem were performed in the 1960s to the early 1970s on filament strains of *E coli* as a target for irradiation and different strains of *E coli* as a source of AM substances. Partial reactivation of *E coli* wild-type (filament) cells was possible by the treatment with cell-free extract or its nucleoprotein fraction obtained from nonirradiated isogenic cells (Korgaonkar and Raut, 1967). X-irradiated *E coli* B, a filament-forming strain, showed an improved survival of X rays after treatment with its own extract. The soluble protein was the active principle (Mukherjee and Bhattacharjee, 1971). It was shown that extract from *E coli* AB 1899NM (filament strain) promotes cell division and colony formation of the same cells exposed to X ray or UV irradiation (Adler *et al,* 1966). The active factor was associated with a heat labile particulate (membrane-associated) fraction (Fisher *et al,* 1969). Perhaps, the reactivative effect of different fractions of cell extracts is at least partly caused by its AM activity. Taking into consideration this possibility, we studied the reactivative effect of some fractions of cell extracts from propionibacteria that possess AM activity as it was earlier demonstrated in our laboratory (Vorobjeva *et al,* 1991).

Cell extracts from different strains of propionibacteria cause a reactivation of UV-inactivated nonfilament strain *E coli* AB 1157 (Vorobjeva *et al,* 1993c). The reactivative factor has a peptide nature (Vorobjeva *et al,* 1994).

In this study we investigated 1) the antimutagenic effect of the compounds generated by *Propionibacterium freudenreichii* subsp *shermanii* against mutagenesis induced by UV mimetic mutagen - 4NQO and UV light in *Salmonella typhimurium*; 2) the reactivative effect of dialysed fraction of soluble proteins on *E coli* and the yeast *Candida guilliermondii* irradiated by UV light (UV-B, UV-C), visible light, the light of total optical spectrum and by heating. Thus, the targets were both of procaryotic and eucaryotic origin, and the reactivation was studied after the cell inactivation by various stress factors. The data obtained revealed previously unknown properties (activities) of dairy propionibacteria. These observations may broaden the field for their practical use.

MATERIALS AND METHODS

Strains and growth media

Cells of *Propionibacterium freudenreichii* subsp *shermanii* VKM-103 (from the collection of Microorganisms of Russian Academy of Science) were used as a source of AM and reactivative substances. *Salmonella typhimurium* TA 100 strain (his^-, $uvr\ B^-$, base-pair substitution tester) was a kind gift of Professor BN Ames (University of California, CA, USA). *Escherichia coli* AB1157 (from the collection of the Department of Genetics, Moscow State University), the yeasts

Candida guilliermondii VSB-656 (from the collection of "VNII Synthez belok", Moscow) and *Saccharomyces cerevisiae* KM-231 (from the collection of the Department of Microbiology, Moscow State University) served as test cultures.

The propionic acid bacteria were grown at 30°C in anaerobic conditions in a minimal glucose medium of the following composition (%): glucose 1.5, trypton (Difco) 0.1, yeast extract (Difco) 0.05, $(NH_4)_2SO_4$ 0.3, KH_2PO_4 0.2, $CaCl_2$ 0.002, $MgSO_4$ 0.002, NaCl 0.002, $CoCl_2$ x 6 H_2O 0.001, distilled water (pH 6.8–7.0). In special experiments, yeast extract was replaced by 3 vitamins (μg/l): Ca-panthothenate,1 000; biotin, 0.1; thiamin,100. *E coli* was grown in nutritive broth (oxoid N2) and on the surface of the plates (oxoid-agar).

Vogel-Bonner glucose agar medium (VBM) was used for reverse mutation assays with *S typhimurium* TA100. The VMB consisted of Vogel-Bonner E medium (2 g citric acid monohydrate, 13.1 g K_2HPO_4 x $3H_2O$, 3.5 g Na $(NH_4)_2HPO_4$ x $4H_2O$, 0.2 g $MgSO_4$ x $7H_2O$) in 200 ml of distilled water, neutralized with NaOH, 4 g glucose and 15 g Difco agar/l.

C guilliermondii and *S cerevisiae* were grown in nutrient wort with aeration (230 rot/min) at 32°C.

Preparation of induced cells

P freudenreichii subsp *shermanii* was grown in the previously mentioned medium with the addition of 4NQO (0.3 μg/ml). After 24 h, the biomass was harvested by centrifugation at 10 000 *g* for 20 min and added repeatedly as inoculate to the same medium with the mutagen and cultivated for 24 h. The biomass was then isolated by centrifugation, washed twice with 0.05 mol/l sodium phosphate buffer (pH 7.4) and resuspended in the same buffer.

Preparation of cell suspension and dialysate of P freudenreichii *subsp* shermanii *cells: modulators for antimutagenic assay and a source of reactivative substances*

The bacteria were harvested by centrifugation at 10 000 *g* for 20 min, washed twice with 0.05 mol/l sodium phosphate buffer (pH 7.4) and resuspended in the same buffer to the exact optical density, approximately equal to a comparative experiment on the antimutagenesis.

A cell suspension in 0.05 mol/l sodium phosphate buffer (pH 7.4) in a ratio of 1:2 (w/v) was sonicated (40 s x 5 at 4°C) and then centrifuged at 144 00 *g* for 1 h at 4°C. The concentrated extracts were then dialysed against 0.05 mol/l sodium phosphate buffer (pH 7.4) at 10°C to remove the low molecular mass constituents. Cell dialysates (modulators) were applied to the antimutagenesis and reactivation assays after sterilizing by filtration through a Millipore filter (0.22 mm).

Sources of irradiation, the irradiation procedure and estimation of the bacteria viability

A high pressure mercurial lamp (DPS-1000) combined with light filters UVC-2 and ZS-3 (290–320 nm), ZS-10 and C3C-21 (400–600 nm) or BC (> 290 nm) served as a source of UV-B light (290–320 nm), visible light (400–600 nm) and a light of total optical spectrum (> 290 nm). The irradiation was focused on a quartz tube with the pattern located in the closed thermostated holder at 15 cm from the lamp. The intensities of the pattern were the following: for UV-B, 5 W/m^2; for visible light, 60 W/m^2 and for light of total optical spectrum, 85 W/m^2.

The overnight cultured cells of *S typhimurium* TA100 or *E coli* AB1157 were washed, resuspended in 5 ml of 0.05 mol/l phosphate buffer (pH 7.4) and UV-C irradiated (254 nm) in a Petri dish by a germicidal lamp BUV-30. The intensity of the pattern irradiation was 10 W/m^2.

The reactivative effect of the dialysate on the bacteria inactivated by irradiation or heating was estimated by the viability value (measured in percentages) by the number of grown colonies on Petri dishes.

C guilliermondii cells in logarithmic growth phase were washed twice with distilled water with centrifugation (1 500 *g* for 5 min) and resuspended in mineral medium (Strachovskaya *et al*, 1993) containing 1% of sucrose to the 10^5 cells/ml. Five ml of the suspension were irradiated by UV-C light in Petri dishes (diameter: 5 cm) or by the irradiation from DPS-1000 lamp in quartz

tubes (diameter: 1 cm). The irradiation was performed at 22°C with mixing of the suspension. The cell viability was estimated by the microcolony method (Corogodin, 1958).

Assay for antimutagenicity

The protocol used for the determination of the antimutagenic activity is the same as that used for the determination of the mutagenic potential of a substance as proposed by Maron and Ames (1984). Studying the desmutagenesis, the modulator (the cells) was incubated with the mutagen at 37°C. After incubation with the mutagen, the cells were separated by centrifugation and thrown off. The cell suspension of the test culture was then added and plated.

The protocol was as follows: 0.1 ml of fresh *Salmonella* culture was added to 2 ml of top agar containing 0.5 mmol/l histidine/biotin and mixed thoroughly before pouring on a minimal glucose agar plate. According to the dose-response effects, we used 4NQO at a concentration of 0.125 μg per plate; 500–1 000 revertants were grown on Petri dish at this concentration and no increase of the 4NQO inhibitory effect was observed. Revertant colonies were counted in each case. The experiments were performed in triplicate and relevant statistical tests were applied. The antimutagenic effect was expressed as a percentage of the decrease of reverse mutations as follows:

$$\text{Antimutagenic effect (\%)} = \frac{(a-b) \times 100}{a-c}$$

where a = the number of histidine revertants induced by mutagen (positive control), b = the number of histidine revertants induced by mutagen in the presence of a modulator, c = the number of revertants induced in the presence of a modulator alone and solvent (negative control). For survival counts, 0.1 ml of 10^{-5} dilution was mixed with 2 ml of top agar, containing 0.1 mg L-histidine and 1.5 g D-biotin and the mixture was poured on the Vogel-Bonner agar medium.

Reconstruction experiments

While studying a possible influence of the dialysate on start time of the first division and the cell division rate, 2 irradiated cell suspensions of *E coli* AB1157 (1 of which was incubated with the dialysate before the irradiation) were plated without harvesting or after harvesting for certain periods (from 0 to 225 min) in the nutrient broth with aeration (200 rot/min).

Analysis

The bacteria growth was followed by optical density measurements (540 nm, Ultrospec II, Pharmacia). The protein content was estimated according to Lowry *et al* (1951).

Chemicals

4-Nitroquinoline-1-oxide (Sigma, USA) was dissolved in dimethyl sulfoxide (DNSO) at a concentration of 1 μg/ml (stock solution) and diluted with 0.05 mol/l sodium phosphate buffer (pH 7.4) just before use.

RESULTS

Antimutagenic activity of Propionibacterium freudenreichii *subsp* shermanii. *Antimutagenesis against mutagenesis induced by 4-nitroquinoline-1-oxide*

4-Nitroquinoline-1-oxide (4NQO) is a stable compound that can be used in long physiological experiments. Accumulation of the bacterial biomass after 24-h growth depends on the concentration of the mutagen (fig 1). The most significant effect is manifested at relatively low concentrations of 4NQO in the medium (below 2 μg/ml). At a further gradual increase of its concentration (up to 10 μg/ml), the growth inhibition proceeds more smoothly. This response of *P freudenreichii* subsp *shermanii* to the increasing quantities of 4NQO in the medium is different in comparison with *E coli* AB 1157, where accumulation of

biomass is inversely proportional to the mutagen concentration in intervals of 0–25 μg/ml (fig 1).

Successive (every 24 h) passages of propionibacteria in a medium containing 0.3 μg/ml of 4NQO were performed for the study of possible bacterial adaptation to the mutagen presence. The data shown in figure 2 indicate that at the first 2 passages the bacterial growth was lower than the growth of control culture (in the absence of 4NQO), but after the third passage the levels almost equalized. This adaptive reaction may be caused by induction of the desmutagenic activity of the cells. The data on the cell desmutagenesis are presented in table I. Ten min after a start of preincubation of the propionibacteria cells with 4NQO, its mutagenicity against test strain *S typhimurium* TA 100 decreased to 17% for the control cells and 34% for the induced cells. If the preincubation period for 4NQO had been prolonged to 4 h, the 4NQO antimutagenicity against *S typhimurium* would have completely disappeared (data not shown). The growth of the induced cells in the medium containing large amounts of 4NQO (3 and 5 μg/ml) was inhibited to a much lesser extent than the control cells (data not shown).

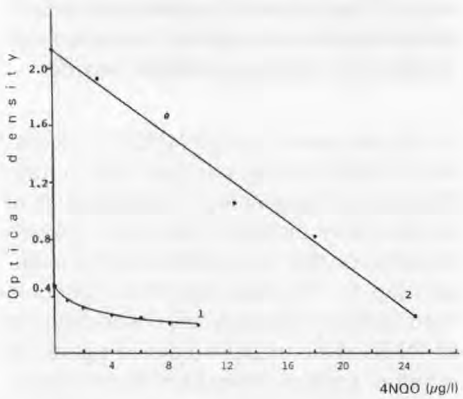

Fig 1. Dependence of the accumulation of *P freudenreichii* subsp *shermanii* (1) and *E coli* (2) biomass on 4NQO concentration in the medium. *Accumulation de biomasse de* P freudenreichii *subsp* shermanii *(1) et* E coli *(2) en fonction de la concentration de 4NQO dans le milieu.*

Antimutagenesis against mutagenesis induced by UV-C light

It was shown earlier that the dialysed fraction of soluble proteins from *P freudenreichii* subsp *shermanii* possesses higher protective activity in UV-C-irradiated *E coli* AB 1157 cells than the nondialysed fractions. Thus, the dialysates were used for a study of antimutagenesis in *S typhimurium.* Typical data, presented in table II, indicate that pre- and postincubation with the dialysate resulted in a 2-fold viability increase and in a decrease of the reversion frequency to 80 and 60%, respectively.

A special control experiment established that the AM effect of the dialysate is not linked to a delay of cell division after its addition to the test-strain suspension (fig 3).

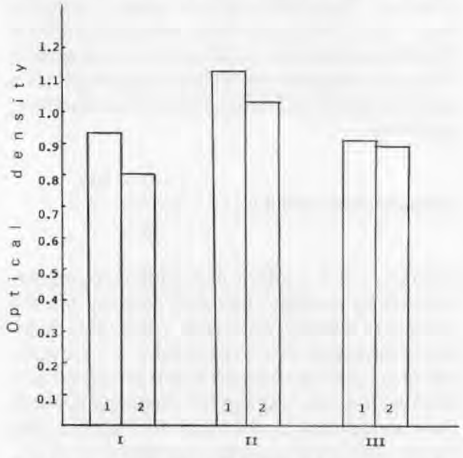

Fig 2. Accumulation of *P freudenrichii* subsp *shermanii* biomass after regular 24 h passages in the medium free of (1) and containing 4NQO (0.3 μg/ml) (2). I, II, III = number of reinoculations. *Accumulation de biomasse par la culture de* P freudenreichii *subsp* shermanii *après des passages de 24 h dans le milieu sans (1) ou contenant (2) 4NQO (0,3 μg/ml). I, II, III = nombre de réinoculations.*

Mitogenic effect of the cell extract was also not detected. Antimutagenic activity of dialysate of both the induced and control propionibacteria cells against UV-C-induced mutagenesis almost did not differ (data not shown).

In an earlier study (Vorobjeva *et al*, 1993a), we demonstrated that cultural broth obtained as the result of the propionic acid fermentation manifests AM effect against the 4NQO-induced mutagenesis (4NQO is a UV mimetic agent). However, in the pre-

Table I. The desmutagenic effect of the induced and noninduced cells of *P freudenreichii* subsp *shermanii* on the mutagenesis of 4-nitroquinoline-1-oxide.
Activité desmutagène des cellules de P freundreichii *subsp* shermanii *contre l'action mutagène du 4-nitroquinoline-1-oxyde.*

Source of desmutagens	*Average no of colonies plate (X ± SE)*	*Lowering of the inhibition (%)*
Noninduced cells	1 063.0 ± 35.0	17
Induced cells	850.0 ± 21.0	34
Positive control	1 281.0 ± 42.0	0

Spontaneous: 36.0 ± 6.0 revertants.
Spontanés : 36,0 ± 6,0 revertants.

Table II. Antimutagenic and reactivative effect of the dialysate from *P freudenreichii* subsp *shermanii* on UV-C-irradiated *S typhimurium* TA 100.
Action antimutagène et réactivatrice du dialysat de P freundreichii *subsp* shermanii *sur les cellules de* S typhimurium *TA100 traitées par les rayons UV-C.*

Condition of irradiation, cell protection	*Average cell number/ml x 10^5 (X ± SE)*	*Viability (%)*	*Average no of revertants/ml (X ± SE)*	*Revertant/ 10^5 cells*
Nonirradiated cells	453.0 ± 7.8	100	750.0 ± 40.6	0.16
Nonprotected irradiated cells	0.004 ± 0.0002	0.0009	4 720.0 ± 240.2	$1 \cdot 10^5$
Preincubation (5 min)	0.010 ± 0.0002	0.002	1 970.0 ± 81.9	$1.9 \cdot 10^4$
Postincubation (5 min)	0.0086 ± 0.0003	0.0019	2 960.0 ± 1185	$3.4 \cdot 10^4$

Irradiation dose = 8 J/m^2. The dialysate addition did not change the number of colonies. Concentration of the added dialysate = 20 μg protein/ml.
Dose de radiation = 8 J/m^2. L'addition de dialysat ne change pas le nombre de colonies. La concentration de dialysat = 20 μg protéine/ml.

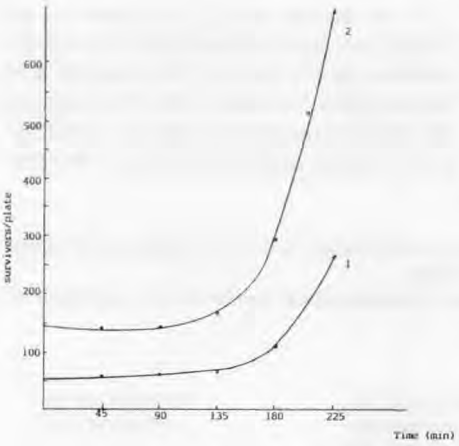

Fig 3. Effect of dialysate of *P freudenreichii* subsp *shermanii* (20 μg protein/ml) on the time of first division of *S typhimurium* TA 100. (1) Without dialysate, (2) with added dialysate.
Influence du dialysat de P freudenreichii *subsp* shermanii *(20 μg protein/ml) sur le temps de génération (première croissance) des cellules de* S typhimurium *TA 100. (1) Sans dialysat, (2) avec dialysat.*

sent study it was established that preincubation of the irradiated cells with the cultural broth (initial synthetic medium with 3 added vitamins) did not lead to a decrease of the revertant number and to an increase of the cell viability (data not shown).

Protection and reactivation of the UV-irradiated E coli *AB 1157 cells*

The previous-mentioned data indicate that 5 min incubation of *S typhimurium* suspension with the dialysate of propionibacteria (20 μg protein/ml) results not only in the decrease of the revertant number, but also in the increase of the viable cell number. It was shown earlier in our laboratory that protection or reactivation of UV-C-inactivated *E coli* AB 1157 occurs after pre- or postincubation of cell suspension with the dialysate (Vorobjeva *et al*, 1993c). The lesser the viability of the irradiated cells, the higher the reactivative or protective effect of the dialysate. This effect takes place both after the immediate and the delayed (up to 15 min) addition of the preparation. The further delay of the addition up to 30 min leads to a partial loss of the preparation efficiency (table III).

Table IV presents the data on reactivation of UV-B-irradiated *E coli* cells by the dialysate at different viability values. Both types of irradiation (UV-B and UV-C) have some common features: inversely proportional dependence of the reactivative (protective) efficiency on cell viability, the effect manifests after pre- or postincubation with the dialysate or after its delayed (up to 10 min) addition to the suspension.

Reactivative and protective properties of the dialysate were also demonstrated in UV-C-irradiated mutant strains *E coli* AB 2463 rec A^-, AB 1886 uvr A^- and P 3478 pol A^- with various defects in DNA reparation systems (data not shown). However, the reactivation of *E coli* AB 1157 was not observed after the irradiation with a light of total optical spectrum (> 290 nm) (table V).

Reactivation of UV-irradiated yeast Candida guilliermondii

The reactivation was detected also with eukaryotic organisms, the yeast *C guilliermondii* after the addition of the dialysate to UV-C-irradiated cell suspension (table VI). Note that the reactivation efficiency is higher in dialysed than in nondialysed preparations and increases while the yeast viability decreases. Preliminary experiments showed that an increase of the dialysate concentration from 60 to 400 μg protein/ml leads to an increase in its efficiency.

Protection and reactivation of UV-C-irradiated yeasts take place after pre- or postincubation with the dialysate, respectively.

Table III. The effect of the addition time of the dialysate of *P freudenreichii* subsp *shermanii* on viability of UV-C-(254 nm) irradiated *E coli* AB 1157.
Influence du temps d'addition du dialysat de cellules de P freudenreichii *subsp* shermanii *sur la survie des cellules de* E coli *AB1157 traitées par les rayons UV-C.*

Condition of irradiation	*Time of the dialysate addition (min)*	*Average cell number/ml x 10^6 (X ± SE)*	*Viability (%)*
Nonirradiated cells (control 1)	–	16.5 ± 0.84	–
Nonirradiated cells with added dialysate (control 2)	0	17.2 ± 0.75	–
Irradiated cells	–	0.077 ± 0.004	0.47
Preincubation	5	0.573 ± 0.007	3.47
Postincubation	0	0.318 ± 0.008	1.93
Postincubation	15	0.314 ± 0.007	1.9
Postincubation	30	0.154 ± 0.005	0.93

Irradiation dose = 57 J/m^2. Concentration of the added dialysate = 20 μg protein/ml.
Dose de radiation = 57 J/m^2. Concentration de dialysat = 20 μg protéine/ml.

Table IV. Reactivation of UV-B (290–320 nm) irradiated *E coli* cells by the dialysate of *P freudenreichii* subsp *shermanii.*
Réactivation des cellules de E coli *traitées par les rayons UV-B (290–320 nm) par le dialysat de P* freudenreichii *subsp* shermanii.

Condition of irradiation	*Time of the dialysate addition (min)*	*Dose of irradiation (kJ/m^2)*	*Average cell number/ml x 10^6 (X ± SE)*	*Viability (%)*
Nonirradiated cells	–	–	79.1 ± 1.7	
Irradiated cells	–	3.9	0.105 ± 0.004	0.13
Irradiated cells	–	4.1	0.059 ± 0.002	0.07
Preincubation	5	3.9	0.245 ± 0.007	0.31
Preincubation	5	4.1	0.217 ± 0.005	0.27
Postincubation	0	4.1	0.144 ± 0.004	0.18
Postincubation	5	4.1	0.151 ± 0.003	0.19
Postincubation	10	4.1	0.138 ± 0.005	0.17

Concentration of the added dialysate = 20 μg protein/ml.
Concentration de dialysat = 20 μg protéine/ml.

Table V. The effect of the dialysate of *P freudenreichii* subsp *shermanii* on viability of *E coli* irradiated by the light of total optical spectrum.
Influence du dialysat de P freudenreichii *subsp* shermanii *sur la survie de cellules de* E coli, *traitées par la lumière de spectre optique complet.*

Condition of irradiation	*Time of the dialysate addition (min)*	*Dose of irradiation (kJ/m²)*	*Average cell number/ml x 10^6 (X ± SE)*	*Viability (%)*
I				
Nonirradiated cells	–	–	153.0 ± 2.9	
Irradiated cells	–	6.4	3.12 ± 0.08	2.0
Irradiated cells	–	7.7	0.78 ± 0.02	0.5
Preincubation	5	6.4	3.18 ± 0.09	2.1
Preincubation	5	7.7	0.78 ± 0.01	0.5
Postincubation	5	6.4	3.03 ± 0.06	2.0
Postincubation	5	7.7	0.76 ± 0.02	0.5
II				
Nonirradiated cells	–	–	95.8 ± 1.6	
Irradiated cells	–	7.7	0.72 ± 0.014	0.75
Irradiated cells	–	8.9	0.41 ± 0.02	0.43
Irradiated cells	–	10.2	0.08 ± 0.003	0.08
Preincubation	5	8.9	0.39 ± 0.016	0.4
Preincubation	5	10.2	0.09 ± 0.002	0.09
Postincubation	5	8.9	0.39 ± 0.015	0.4
Postincubation	5	10.2	0.09 ± 0.003	0.09

Concentration of the added dialysate = 20 μg protein/ml. I and II = 2 different experiments.
Concentration de dialysat = 20 μg protéine/ml. I et II = 2 essais différents.

The maximum efficiency was revealed after 10 min incubation (table VII). These conditions were performed in further experiments. We also demonstrated the reactivative effect of the dialysate on the yeast cells inactivated by UV-B light but not in the case of irradiation by visible light (400–600 nm) or by the light of total optical spectrum (> 290 nm) (fig 4).

Reactivation of E coli *and yeasts inactivated by heating*

The data presented in table VIII indicate an increase of viability of the bacteria and yeasts inactivated by heating after postincubation with the dialysate. The efficiency of this increase was inversely proportional to the viability of the inactivated cells, thus repeating the regularity earlier detected for UV-irradiated bacteria and yeasts. Moreover, 2 fractions of soluble proteins (sedimented by ammonia sulfate at 20–40% and 60–80%, respectively) that contain the reactivative and protective activity in UV-irradiated *E coli* also reveal a reactivative effect in bacterial cells, inactivated by heating.

DISCUSSION

Dairy propionibacteria have been employed in cheesemaking since antiquity: today they are also used in fodder ensilage, bread-

making, processing of some sour milk production, vitamin B_{12} production (Vorobjeva, 1984), contained in probiotics (Gilliland, 1988). The regions of use (except for the latter) are based on the formation and excretion of metabolites of propionic acid bacteria.

Our work has revealed the previously unknown property of propionibacteria to produce compounds inactivating some chemical mutagens *via* the desmutagenesis process. Desmutagens may be excreted in the cultural broth and/or may be localized in the bacterial cells (Vorobjeva *et al,* 1993a,b). We used 4NQO, a typical mutagen causing transition mutations. In this study, it was shown that a desmutagenic effect against 4NQO is manifested by the cells and is apparently caused by inactivation of its electrophilic groups (Vorobjeva *et al,* 1995). Since mutagenicity of many compounds is linked to their electrophilicity (Ketterer,

Table VI. The effect of dialysate and nondialysed extracts of *P freudenreichii* subsp *shermanii* on viability of *Candida guilliermondii* irradiated by UV-C (254 nm) light.
Influence des extraits dialysés et non dialysés de cellules de P freudenreichii *subsp* shermanii *sur la survie des cellules des levures* C guilliermondii, *traitées par les rayons UV-C (254 nm).*

Dose of UV-C irradiation (J/m²)	*Viability (%) (X ± SE)*		
	Without extract	*100 g protein/ml added*	
		Dialysed extract	*Nondialysed extract*
600	10.0 ± 1.0	36.0 ± 1.8	22.0 ± 1.2
900	0.4 ± 0.01	4.0 ± 0.7	1.0 ± 0.3

Concentration of the added preparations = 20 μg protein/ml.
Concentration d'extraits cellulaires = 20 μg protéine/ml.

Table VII. The influence of preliminary and postradiational effect of the dialysate on viability of *C guilliermondii* inactivated by UV-C irradiation.
Influence du dialysat (pré- et post-traitement) sur la survie des levures C guilliermondii, *inactivées par les rayons UV-C.*

Time of incubation (min)	*Viability (%) (X ± SE)*		
	Without dialysate	*Preincubation*	*Postincubation*
5	0.5 ± 0.03	5.0 ± 0.6	9.0 ± 0.8
10	0.5 ± 0.02	7.0 ± 0.8	17.0 ± 1.2
15	0.5 ± 0.01	2.0 ± 0.5	10.0 ± 0.9

Irradiation dose = 900 J/m². Concentration of the added dialysate = 200 μg protein/ml.
Dose de radiation 900 J/m². Concentration de dialysat = 200 μg protéine/ml.

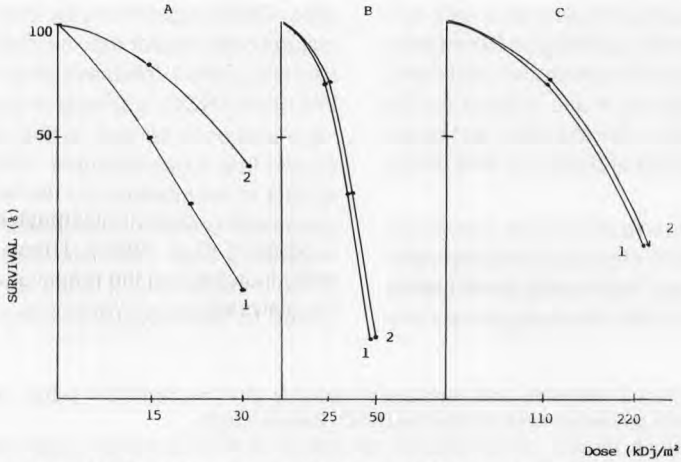

Fig 4. Viability curves of *C guilliermondii* irradiated by UV-B light (A), by light of total optical spectrum (> 290 nm) (B) or visible light (C) without (1) or after 10 min postincubation with the dialysate from *P freudenreichii* subsp *shermanii* (200 μg protein/ml) (2).
Courbes de survie de cellules de C guilliermondii *traitées par les rayons UV-B (A), les rayons de spectre (> 290 nm) optique complet (B) ou la lumière visible (C) sans (1) ou après incubation 10 min avec le dialysat de* P freudenreichii *subsp* shermanii *(200 μg protéine/ml) (2).*

Table VIII. Reactivation of *E coli, S cerevisiae* and *C guilliermondii* inactivated by heating after 15 min postincubation with the dialysate of *P freudenreichii* subsp *shermanii.*
Réactivation de cellules de E coli, S cerevisiae *et* C guilliermondii *inactivées par chauffage après 15 min d'incubation avec le dialysat de* P freudenreichii *subsp* shermanii.

Micro-organism	*Conditions of heating (t°C, time min)*	*Viability (%)*			
		Without dialysate	*With added dialysate*		
			Dialysate	*Fraction I*	*Fraction II*
E coli	45, 30	74	91	–	–
	45, 90	23	85	–	–
	42, 60	58	–	90	88
S cerevisiae	42, 30	66	91	–	–
	42, 60	5.9	9.5	–	–
	42, 90	4.5	8.4	–	–
C guilliermondii	42, 60	33.8	32.9	–	–
	42, 90	20.5	27.0	–	–

The dialysate fractions were isolated by sedimentation with ammonium sulfate: 20–40% (I) and 60–80% (II). In the case of *E coli,* 20 μg protein/ml and in the case of the yeasts, 400 μg protein/ml of dialysate was added. – : not determined.
Les subfractions de dialysat étaient isolées par la sédimentation avec le sulphate d'ammonium : 20–40% (I) et 60–80% (II). Dans le cas de E coli, *20 μg de protéines/ml et dans le cas des levures, 400 μg protéines/ml, de dialysat étaient additionnés. – : non déterminé.*

1988), it can be assumed that the desmutagenic properties of propionibacteria may be used for detoxification of the other mutagens. It is interesting to note that desmutagenic activities detected in bacterial systems often coincide with the capability to suppress cancer formation in experimental animals (Wattenberg, 1978).

The detected "adaptation" of *P freudenreichii* subsp *shermanii* to 4NQO existence in the medium is at least partly caused by antimugenesis (desmutagenesis) induction. This observation is important since propionibacteria inhabit human intestines (Kornyeva, 1981) and their number in the human large bowel range from 10^8 to 10^{10} cfu/g dry weight of faeces (Macfarlane *et al*, 1986). Both the "native" propionibacteria from intestines and the ones used in food and ensilage processing can manifest a positive effect on the health of humans and animals. Food would be safer to eat if its carcinogenic ingredients were inactivated before consumption by using propionic acid bacteria.

Factor(s) of protein nature localized in the propionibacteria cells reveal antimutagenic and reactivative effects against mutations induced by UV-C light in *S typhimurium* TA 100. Moreover, antimutagenicity of the dialysate in control and induced by low quantities of 4NQO cells almost coincided, thus indicating the existence of different AM mechanisms against UV-C- and 4NQO-induced mutagenesis.

The dialysate and its 2 active fractions possess protective and reactivative effects on UV-C- and UV-B-inactivated prokariotic *(S typhimurium, E coli)* and eukaryotic *(C guilliermondii)* organisms.

UV-B irradiation (290–320 nm) is a potent natural mutagen and carcinogen. Today, its ecological value has become more significant due to an accelerated depletion of the ozone layer (Stolarski *et al*, 1991).

The dialysate did not reactivate bacterial *(E coli)* and yeast cells after irradiation with visible light (400–600 nm) and light of total optical spectrum (290–1 000 nm). The cell inactivation by UV-B and UV-C light, mainly caused by DNA injuries, resulted in the formation of pyrimidine dimers and 6.4-photoproducts (Mitchell, 1988). Lethal action of visible light on yeasts manifests *via* damages of their plasmatic membrane (Pospelov *et al*, 1987). These data indirectly indicate that the reactivative effect of the dialysate protein factors is linked to the repair of damaged DNA regions. The reactivation was observed in prokariotic and eukariotic organisms. In both cases, some common features were established: dialysed extract was more effective than non-dialysed and its efficiency was inversely proportional to cell viability.

Protection and reactivation of both the bacteria and yeasts manifest after pre- or postincubation although optimal concentrations of the dialysate in yeast cell suspensions were nearly 10–20-fold higher than in the bacterial ones.

The dialysate and 2 of its active fractions also reveal reactivative effect in *E coli* cells inactivated by heating. The same fractions have protective and reactivative activity in UV-C-irradiated *E coli* cells. The reactivation by dialysate was also demonstrated in eukaryotic organisms – *S cerevisiae* and *C guilliermondii* – inactivated by heating (table VIII). The observed "universality" of the reactivative factor from propionibacteria may be explained by the data of Van Bogelen *et al* (1987). They demonstrated in bacteria the existence of a general response to some environmental stresses. This response manifests *via* coordinated functioning of SOS system (DNA repair), heat-shock system and systems responsible for neutralization of oxidants and ethanol.

We must now try to isolate the reactivative factor for a detailed study of its physical and chemical properties and for elucidation of the mechanism of its action.

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