Can spores survive in interstellar space?

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Inactivation of spores (Bacillus subtilis) *has been investigated for the first time in the laboratory by vacuum ultraviolet radiation in simulated interstellar conditions. Remarkably, damage produced at the normal interstellar particle temperature of 10 K is less than at higher temperatures, the major damage being produced by radiation in the 2,000–3,000 Å range. Our results place constraints on the panspermia hypothesis.*

THERE is good evidence that life existed on Earth as early as 3,800 Myr ago[1], which leaves only ~200–400 Myr after the Earth's crust cooled[2] for terrestrial chemical evolution to lead to primitive, yet fully developed, life forms. That this might be too short a timescale for such an evolution has led to speculation that either cosmochemistry[3,4] or even panspermia[5,6] is involved in the origin of life on Earth.

We see no reason to reject the hypothesis that several hundred million years is adequate to produce the required prebiotic chemical precursors on Earth. No answer can be given to this question until the basic mechanisms of this transformation are understood. If the probability were quite small that life could start in 300 Myr, does extending this time by a factor of only 50, which takes us to the probable beginning of the Universe (some 15,000 Myr ago[7]), increase the probability sufficiently? Inverting this argument, we deduce that if life is certain to have evolved in 15,000 Myr, the probability cannot be negligible in 300 Myr.

We do not seek to address such questions here; they have been discussed elsewhere[8,9]. If we accept the hypothesis that life began from primordial prebiotic molecules, whether formed first on the Earth[2] or coming from outer space[4], does this belief exclude the possibility that life, once formed, can be transported from one source to another?

We reconsider below some of the ideas of Arrhenius[10] by placing them in a modern astrophysical context. The four fundamental phases of panspermia are: (1) removal to space of biological material which has survived being lifted from the surface to high altitudes; (2) transport from one solar system to another; (3) survival of the biological material over timescales comparable with the interstellar passage time; (4) deposition of the biological material onto a new host planet's atmosphere and surface. We argue that phases (2) and (3) are amenable to quantitative treatment and defer consideration of phases (1) and (4), with phase (1) presenting more problems than phase (4).

Arrhenius[10] suggested that solar radiation pressure could provide a mechanism for driving microorganisms into interstellar space with enough speed to reach another star in a 'reasonable' time which he considered to be 3,000 yr. He was not aware that microorganisms are killed almost as soon as they are exposed to the full solar ultraviolet (UV) on leaving the Earth's atmosphere. Furthermore, he ignored the reverse effect of the radiation pressure which acts to prevent a small particle from entering the environs of another star.

Insofar interstellar transport is concerned, there is an obvious mechanism through the random motion of molecular clouds. The gaseous and particulate matter between the stars is unevenly distributed into concentrations or clouds, with densities up to 10^4 hydrogen atoms cm^{-3} ($n_H = 10^4\ cm^{-3}$) and higher. These clouds move about at random speeds of $10\ km\ s^{-1}$ (ref. 11). Thus, should a bacterial spore be captured into such a cloud it will be swept along with the gas. Given the distance between neighbouring stars of ~0.1–1 pc (0.3–3 light yr)[12], this corresponds to a passage time of 10^5–10^6 yr. Thus, if one star in 1,000 possesses a solar system, a survival time of 10^6–10^7 yr is required. The question we address is can a spore survive as long as 1–10 Myr in a molecular cloud?

A major destructive mechanism for spores in interstellar space is the photolysis caused by photons from starlight. The flux of UV photons with energy ≥ 4 eV is such that each molecular bond in a 0.5-μm radius particle will have been subjected to such a photon every 1,000 yr in the less dense regions of the space between the stars. In the densest regions, this timescale may be increased to 10^6–10^7 yr. Because the small particles in space are normally at temperatures of 10–15 K (refs 13, 14) at first it seems difficult to imagine the maintenance of the chemical integrity required to preserve a living organism.

The three basic factors in interstellar space which are hostile to microbes are: (1) vacuum. In interstellar space the densities are much lower than on Earth. Even so-called dense interstellar clouds with 10^6 hydrogen atoms cm^{-3} ($n_H = 10^6\ cm^{-3}$) have a pressure of only 5×10^{-13} mbar, which would be considered a very good vacuum. (2) Energetic photons and cosmic rays. Even though the average point in space is very distant from the nearest star, the UV radiation is still a factor. Although individual cosmic-ray protons, X rays or γ rays may be more lethal than UV photons, their total energy input is generally orders of magnitude lower than that by the UV. (3) Temperature. Although the temperature of the gas in interstellar space is generally in the range 10–100 K, the mean temperature of a small particle, for example, a bacterial spore or a virus , is generally ≤ 10 K. Thus, we have to consider what happens to a complex biological system in conditions of high-vacuum, low-temperature and vacuum UV irradiation.

Experimental method

We performed the work described here at a laboratory facility routinely used in the Leiden Astrophysics Laboratory to simulate interstellar conditions for the study of chemical evolution of interstellar grains by photoprocessing simple molecules[15,16].

There is both a similarity and an essential difference between interstellar grain evolution and interstellar effects on living organisms. In the former, we are interested in the formation of complex organic molecules from simple molecules, whereas in the latter, we are interested in the destruction and rearrangement of already existing complex molecules. In either case, the basic process starts with the breaking of a molecular bond or the ionization or excitation by a UV photon. The experiment is illustrated in Fig. 1. To relate our new data to earlier investigations, we selected organisms that have been studied previously in various conditions of irradiation, temperature and vacuum, but never in conditions that simulate the interstellar medium.

Bacillus subtilis spores are suitable for viability tests in simulated space environments relevant to the Solar System[17]. They are resistant to vacuum exposure; even in ultra-high vacuum ($\leq 10^{-10}$ torr) ~90% of spores maintain their colony-forming ability compared with dessicated spores. By means of high-vacuum chambers it is possible, using simulated interstellar conditions, to study which cell components are affected either by vacuum or photolysis, or both.

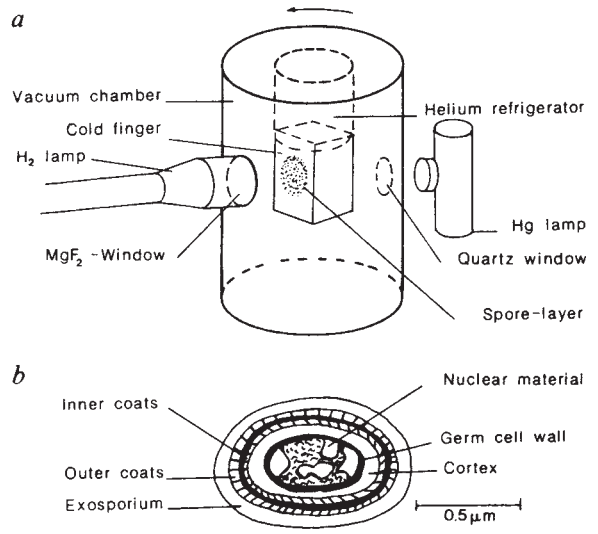

Fig. 1 *a*, Apparatus used to irradiate spores. The cold finger on which the spores are deposited is maintained at either 10 K or room temperature. The quartz window is also used in conjunction with the H_2 lamp. *b*, Cross-section of the spore; scale bar, 0.5 μm.

We limited our experiments to two strains of the *Bacillus subtilis* spore, one which is relatively irradiation-resistant, wild-type 168 (Nester, Marburg), and the other, TKJ 6323 (provided by N. Munakata), which is sensitized to irradiation as a result of repair deficiencies. The spores used in our experiments are freed of all vegetative cells and other debris by enzymatic treatment and density gradient sedimentation.

Results

In the first experiment, we investigated the variability of susceptibility of spores of different genotype to UV (wavelength 2,537 Å, from a low-pressure mercury lamp). This wavelength is termed RUV (reference UV). The parameters of our experiments are summarized in Tables 1 and 2. The inactivation kinetics for the sensitive and wild-type strains at room temperature and atmospheric pressure are compared in Fig. 2*a* to show the strongly enhanced sensitivity of the repair-deficient strain TKJ 6323 to RUV. Because of mutations in the excision-, recombination- and sporephotoproduct repair, strain 6323 is ~50 times more sensitive to RUV than wild-type strain 168 in aqueous suspensions and ≥10 times more sensitive when irradiated on a surface.

The effect of low temperature (10 K) and high vacuum ($<10^{-6}$ torr) on the amount of lethal damage to spores is compared with results at room temperature, whether at atmospheric pressure or high vacuum, in Fig. 2*b*, *c*. We find that strain 168 irradiated at room temperature is at least four times more sensitive in vacuum than at 1 bar. This is the same effect as found by Horneck[17]. If, however, the sample is cooled to 10 K before irradiation, spores are not inactivated with fluences which would have been lethal dose rates at room temperature.

Although an increase in sensitivity is often observed with decreasing temperature to ~−80 °C, decrease in sensitivity to RUV is observed below 130 K (ref. 18). Extrapolating the data of Ashwood-Smith *et al.* we predict that a dose rate of 2×10^4 J m^{-2} is sufficient to inactivate samples to F_{10}, where F_{10} is defined as the fluence required to inactivate 90% of the spores. We confirm this trend to less sensitivity at extremely low temperature by noting that irradiating a sample of wild-type (WT 168) at 10 K and vacuum with a dose of 1×10^4 J m^{-2} of RUV allowed 25% survival.

An experiment performed with TKJ 6323 (Fig. 2*c*) indicates that this RUV-susceptible spore is as strongly resistant as WT 168. The amount of lethal lesions is significantly reduced at 10 K because TKJ 6323 is unable to repair damages during germination and development to the vegetative cell.

To simulate the UV flux in interstellar space, we use a microwave-powered H_2-discharge lamp. The emission of this source is peaked, in the vacuum UV (1,000 $<\lambda\leq$ 1,900 Å), at 1,215 Å (Lyman α) and 1,600 Å and has an increasing continuum in the far UV (2,000-3,000 Å).

Table 1 Comparison between laboratory and interstellar conditions

	Laboratory	ISM
Pressure	8×10^{-8} mbar	3×10^{-15} mbar
Temperature	≥10 K	≥10 K
UV flux ($E>6$ eV)	10^{15} quanta cm^{-2} s^{-1}	10^8 quanta cm^{-2} s^{-1}
Fluence ratio: Far UV (2,000-3,000 Å) / Vacuum UV (1,000-2,000 Å)	5	1.1

The mean flux of the lamp's spectrum, in the vacuum UV, is of the order of 1.5×10^{15} quanta cm^{-2} s^{-1}. The energy flux of the lamp above 2,000 Å is roughly five times as high as in the vacuum UV portion. We thus have to use two different scales when demonstrating the vacuum UV irradiation and results with full spectrum irradiations (see Tables 1 and 2). Estimating a mean flux of vacuum UV photons in the average interstellar medium of 1×10^8 cm^{-2} s^{-1}, 1 h of irradiation by vacuum UV in the laboratory corresponds to ~10^3 yr in space. The first question then is whether vacuum UV with photon energies >6 eV ($\lambda<$ 2,000 Å) has an effect on the survival of spores similar to that observed with RUV.

The lamp is equipped with a shutter to study the effects at relatively short exposure times. Figure 2*d* shows an inactivation curve with samples of strain WT 168 irradiated at 10 K and vacuum. Almost no cells can survive fluences ≥60 kJ m^{-2} using the full spectrum (vacuum UV+far UV) of the H_2 lamp which corresponds in Fig. 2*d* to ≥10 kJ m^{-2} with the vacuum UV.

To explain the inactivation profiles induced by vacuum UV irradiation alone, we checked for the biological effectiveness of this source at several wavelengths in the vacuum UV using interference filters of centre wavelengths 1,600, 1,400 and 1,200 Å (half-width of filters ~200 Å).

The radiation transmitted through these three filters was measured by CO_2 dosimetry. Note that the hydrogen lamp spectral output, particularly at the very short wavelengths, is unstable so that the relative fluxes at 1,200 and 1,600 Å may vary by as much as a factor of 2 from run to run, however, this does not affect our results significantly. We examined survival of our samples at doses between 1 and 120 kJ m^{-2}. There was no significant destruction of spores when irradiating with wavelenths λ = 1,400 and 1,215 Å, within our chosen dose rates, at both room temperature and 10 K. On the other hand, we observed significant inactivation of spores when irradiating with λ = 1,600 Å light in room-temperature conditions.

Irradiation with the total spectrum (vacuum UV+far UV) shows that spores are killed very effectively but significantly less at 10 K than at room temperature (Fig. 2*e*, *f*). Substitution of the MgF_2 window (which is transparent down to 1,100 Å) by a quartz window (which only transmits radiation longward of 2,000 Å) leads to the result that the remaining far UV spectrum of the lamp is as effective as the total spectrum at both temperature extremes (Fig. 3). We find that irradiation with wavelengths λ > 2,000 Å accounts for essentially all the inactivation when comparison is made with the inactivation curve of the H_2 lamp's full spectrum.

Thus, we conclude that: (1) wavelengths ≤1,400 Å do not affect the spores within our chosen dose rates; (2) 1,600-Å light inactivates spores but it takes doses delivered at room temperature, which are significantly higher than applying the lamp full spectrum; (3) the total flux of the lamp inactivates at doses ≥60 kJ m^{-2} (vacuum UV > 10 kJ m^{-2}). It is evident that photons of energies (E) < 6 eV are the more effective ones in spore killing than those with $E>6$ eV.

One factor in the interstellar medium that may provide a mechanism for the protection of spores is that of absorption of

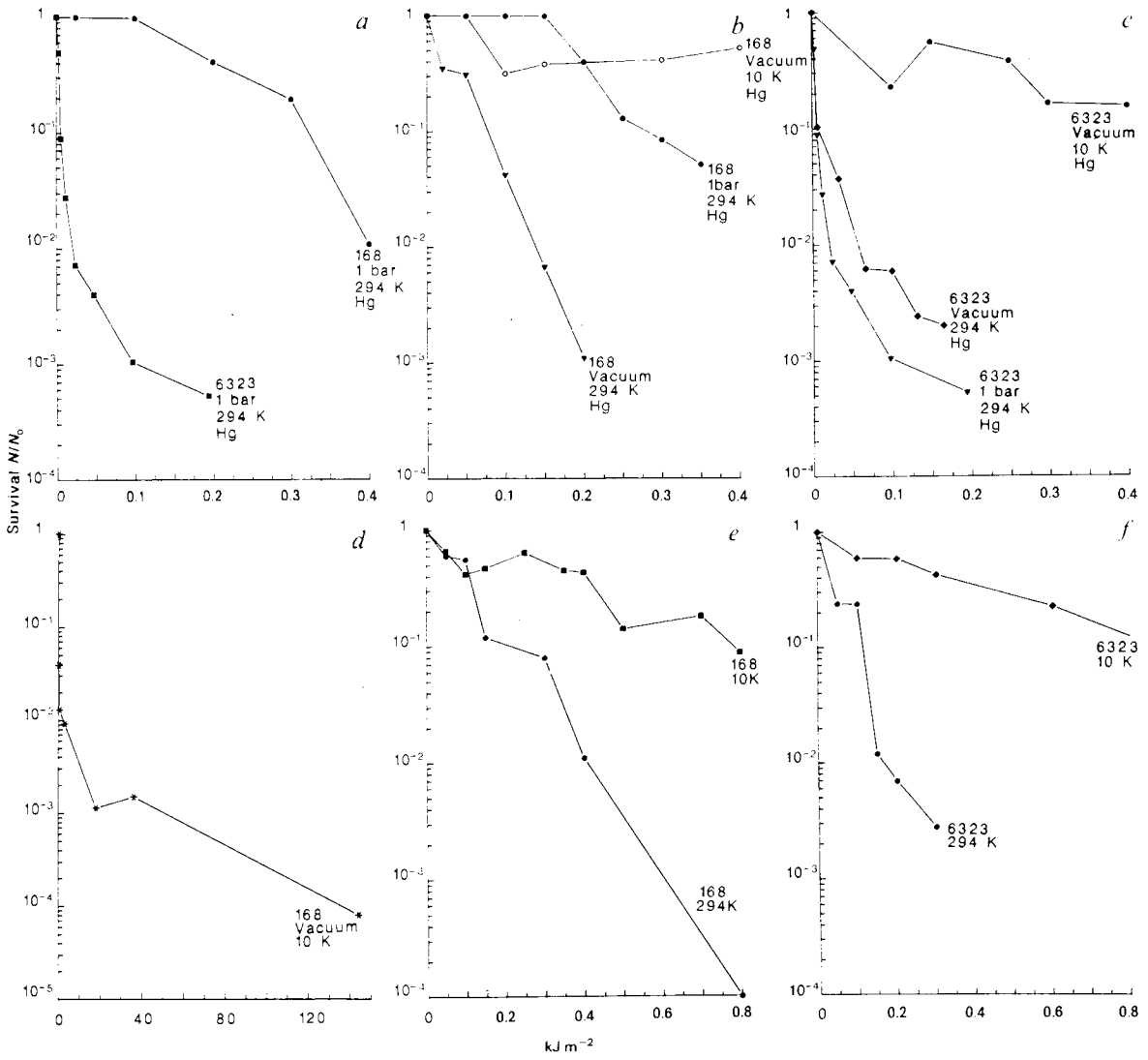

Fig. 2 Semilogarithmic plots of inactivation curves for *Bacillus subtilis* spores. *a*, WT 168 and TKJ 6323 spores irradiated at room temperature and 1 bar with a mercury low-pressure lamp. *b*, WT 168 spores irradiated with a mercury lamp. Irradiations in vacuum are performed at room temperature and 10 K. Air-dried spores are irradiated at 1 bar and room temperature. *c*, TKJ 6323 spores irradiated in vacuum at room temperature and 10 K. For comparison air-dried spores are irradiated at 1 bar and room temperature. *d*, WT 168 spores irradiated with a H_2 lamp at 10 K in vacuum with high doses. Full spectrum vacuum UV+far UV. Abscissa scale is vacuum UV alone. *e*, WT 168 spores irradiated in vacuum at 10 K and room temperature with a H_2 lamp. Full spectrum vacuum UV+far UV. Abscissa scale is vacuum UV alone. *f*, TKJ 6323 spores, irradiations as in *e*.

UV light within mantles of molecules which may grow to a thickness of 1 μm within the mean lifetime of a molecular cloud.

We therefore created an artificial mantle on spores to check whether this influences the survival in simulated interstellar conditions. We deposited a mixture of $H_2O:CH_4:NH_3:CO$ (1:1:1:1) on top of the spores on a cold finger at 10 K and vacuum ($<1\times10^{-6}$ torr). The mantle thickness is measured by monitoring the interference pattern of light of a He/Ne-laser by means of a photomultiplier tube. Mantles of various thicknesses were deposited and the system irradiated with the H_2 lamp.

We find that such mantles do indeed protect spores from vacuum UV irradiation but not from far UV. The reason for this is that these ices consist of simple molecules which absorb strongly in the vacuum UV but are essentially transparent in the far UV. Thus using the full H_2-lamp spectrum on $\geq$0.5-μm mantle-coated spores, at 10 K, we find, as expected, that the sensitivity of TKJ 6323 is not noticeably decreased whereas that of WT 168 is decreased by a factor of 10. This demonstrates again the fact that the vacuum UV damages are distinguishably different from those due to far UV.

Note that the mantles which accrete in interstellar space have been themselves irradiated by the UV and consequently consist of complex rather than simple molecules. Such photo-processed mantle materials should absorb strongly at wavelengths <3,000 Å and consequently provide effective shielding in the far as well as in the vacuum UV.

Discussion

It seems possible to distinguish between the types of damage occurring within and outside the core of a spore, as a function of wavelength. The outer layers of the spore protect the core by attenuating UV light (see ref. 19 for review). Within the core, damage may be divided into several types: (1) cyclobutane-type dimers; (2) 5-thyminyl-5,6-dihydrothymine spore photoproduct (TDHT); (3) DNA-protein crosslinks; (4) DNA strand breaks; (5) DNA adducts. Outside the core, damages are produced within proteins rather than in DNA.

In general, there is an energy discrimination between the various types of damage such that, for example, TDHT forms as a result of low-energy photons (<6 eV) whereas the DNA-protein crosslink and strand break cross-sections are peaked at ~10 eV (ref. 20).

Because at extremely low temperatures and ultra-high vacuum we are dealing with very dry states of the system, irradiation mainly induces radicals leading to TDHT through a well-estab-

Table 2 Stain exposure conditions

	P	T (K)	UV	F_{10}* ($J\,m^{-2}$)	F_{10} (sample) / F_{10} (reference)	D_{37} (linear regression) ($J\,m^{-2}$)	Absorption cross-section $\sigma(m^2\ photon^{-1})$
168	1 bar	294	Hg lamp	325	1(ref)	170	4.58×10^{-21}
168	vacuum	294	Hg lamp	75	0.23	39	2.0×10^{-20}
168	vacuum	10	Hg lamp	—	$\gg1$	—	$\leqslant2\times10^{-23}$
168	vacuum	294	H_2 lamp	200	0.615	105	1.18×10^{-20}
168	vacuum	10	H_2 lamp	800	2.46	220	5.65×10^{-21}
6323	1 bar	294	Hg lamp	35	0.1	12.7	6.14×10^{-20}
6323	vacuum	294	Hg lamp	32	0.09	28.7	2.71×10^{-20}
6323	vacuum	10	Hg lamp	—	$\gg1$	—	$\leqslant1\times10^{-22}$
6323	vacuum	294	H_2 lamp	200	0.285	100	1.24×10^{-20}
6323	vacuum	10	H_2 lamp	950	2.71	180	6.9×10^{-21}

* These fluences for the H_2 lamp are calculated using only the range 1,200–2,000 Å. The fluences including the far UV are six times larger.

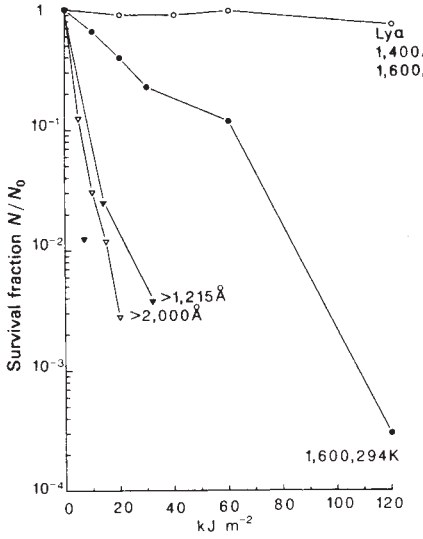

Fig. 3 WT 168 spores irradiated with a H_2 lamp in vacuum at various wavelengths. The fluences, in each case, are determined by the specified wavelength region of irradiation.

lished mechanism[21]. Note, therefore, that we are not inducing singlet or triplet structure. At 10 K we observed no discrimination between spores of strains WT 168 and TKJ 6323 irradiated with RUV. This immediately suggests that the UV photolysis induced at low temperatures is repair independent or, in other words, that other photoproducts which are not amenable to any known cellular repair mechanism are produced.

DNA–protein crosslinking is increased when spores are RUV-irradiated in vacuum[22]. The enhancement for the formation of this photoproduct in vacuum is about a factor of 12 greater than irradiations at 1 bar and correlates with an increase in inactivation[23].

The increase of non-DNA damage has been described by Smith and O'Leary[24]. They point out that UV-induced photoproducts which are not amenable to photoreactivation and dark repair may be formed at considerably lower rates than other types of photochemical lesions but may, nevertheless, be more deleterious to cells[25].

The chemical analysis of DNA photoproducts induced in spores by vacuum irradiation of solar UV (to be published elsewhere) indicates that TDHT is the major photoproduct of DNA formed at both 10 K and room temperature. Thus, in accordance with our survival data, TDHT does not contribute very much to inactivation of spores when irradiated at 10 K.

This observation is consistent with a result of Ashwood-Smith *et al.*[26] who found that the lack of supersensitivity of purified DNA to RUV irradiation at moderately low temperatures (−79 °C) strongly suggests that the lesions responsible for the low-temperature supersensitivity are not primarily associated with changes in DNA. For phages, it has been shown with dose rates comparable to those in our experiment, that crosslinks between DNA and proteins are induced through long-lived radicals. In the dry spore, this mechanism might also be plausible and is probably suppressed at extremely low temperatures because of inhibited diffusion[27,28].

When using the H_2 lamp we expect a shift in the target chromophore. This light source emits photons of much higher energies than the RUV source. Although these energies may split intramolecular bonds of DNA and peptides[29,30], and the Lyman α of the lamp coincides with the maximum efficiency for DNA strand breaking[20], nevertheless, we observe that most of the effective damage arises from the far UV rather than the vacuum UV.

Thus, at 10 K, we found, surprisingly, that the vacuum UV and RUV kill much fewer cells than the residual spectrum, which is that between 2,000 and 3,000 Å exclusive of the 2,537-Å Hg line. Further spectral studies are needed to determine the precise region of the susceptibilities at 10 K.

Astrophysical implications

According to our survival data, a dose of ~6 $kJ\,m^{-2}$ of vacuum and far UV inactivates spores to F_{10} at 10 K. Noting the fact that the H_2 lamp emits five times as much energy in the far UV relative to the vacuum UV as compared with the interstellar medium (ISM) implies that F_{10} for the diffuse interstellar medium occurs in ~150 yr.

To inactivate spores to $F_{0.1}$ (99.9% inactivated) requires a dose of 120 $kJ\,m^{-2}$ (20 $kJ\,m^{-2}$ of vacuum UV in Fig. 2*d*), which corresponds to 2,500 yr of irradiation in the diffuse ISM where the UV radiation is not attenuated. Spores exposed to such a dose can be regarded as dead if we accept the theory that the levelling off of the inactivation curve is an artefact of our experimental method[31].

Two possibilities exist, which may lead to the prolongation of the mean lifetime of a bacillus in the ISM: (1) spores are deposited within dense clouds in which the UV radiation is attentuated by several orders of magnitude; (2) spores accrete mantles of condensable matter which reduces the penetration of UV to the outer layer of the spore by a factor as large as 10 or more.

Both the far and vacuum UV in the centre of a cloud of density $n_H = 10^4\ cm^{-3}$ and radius of 1 pc are attenuated by a factor of 10^8–10^9 (ref. 32). Because there generally exist internal sources of UV in addition to the attenuated radiation, which comes from distant stars, it is more conservative to consider the mean UV radiation within a molecular cloud region to be reduced to not less than $\sim10^{-4}$ times that of the diffuse ISM radiation. This minimum is internally produced by conversion of cosmic-ray energy and stellar-wind energy[33,34].

The growth of a mantle on a bacterium occurs within a cloud by accretion of molecules at a rate[35]:

$$\frac{da}{dt} = 3.43\times10^{-22}\ n_H\ cm\ s^{-1}$$

Thus, in 1.5×10^5 yr, one should accrete a mantle of ~ 0.15 μm. This material is irradiated by UV during condensation, and, because of the production of new molecules and radicals, absorbs in the UV more than the originally simple molecules. The attentuation of 3,000-Å radiation (and shorter) through such a layer is greater than a factor of 30 as measured in our laboratory.

Combining the effects of accretion with cloud attenuation leads to a biological time (F_{10}) of 4.5-45 Myr, which seems to be long enough for spores to be transported from one solar system to another.

Conclusions

We have presented experimental evidence for the effects of very low temperature and UV radiation, characteristic of the ISM, on the survival of bacteria. In the most general environment in space, 10% survival times are only of the order of hundreds of years, too short for panspermia to work.

However, in a substantial fraction of space within dark clouds, we have shown that, even with conservative figures, survival times as long as millions to tens of millions of years are attainable. In such conditions, clouds could transport organisms from one solar system to another in times significantly shorter than the mean survival time. This occurs with significant probability. However, the problem of survival of bacteria during the ejection and the ultimate deposition on a non-hostile host planet still has not been addressed.

The only way a bacterium can survive above the Earth's atmosphere is if it is coated by a mantle of some material which attenuates the solar UV radiation by a factor of $\sim 10^9$. A thickness of 0.9 μm of material with an imaginary index of refraction of 0.5 would be sufficient, but how a spore can come to be so coated and blown out to space remains completely unknown. A possible suggestion would be the ejection to the upper atmosphere accompanying some explosive event—perhaps a comet or meteorite collision. The ejection process may be non-destructive if the impacting object clears the atmosphere and leaves a channel for the escape of high-speed ejecta[36]. However, one still requires that coincident with the meteorite/comet collision there occurs the passage of the solar system through a dense molecular cloud which can capture the ejecta. The complementary question of deposition is answered affirmatively if, when the spore enters a new solar system, it already has a mantle of absorbing material of the required thickness.

Although we have not completely answered the question of panspermia, we have provided some experimental results which lead to well-defined restrictions on the required processes.

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A peculiar supernova in the spiral galaxy NGC4618

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Optical spectra of a bright stellar object near the nucleus of the spiral galaxy NGC4618 reveal strong, very broad emission lines similar to those in quasars but having the wrong relative wavelengths. Although lines of hydrogen and helium are absent, the most prominent features can be attributed to neutral atoms of oxygen, sodium, and magnesium at the redshift of NGC4618. The object is almost certainly a supernova whose highly unusual spectrum may be indicative of a fundamentally new subclass.

DURING an extensive spectroscopic survey of nearby galactic nuclei[1], we have discovered[2] a bright starlike object in the peculiar SBbc(rs)II.2 spiral NGC4618 (brightness $B_T = 11.20$ mag, velocity $v = 532$ km s^{-1}, $b^{II} = 75°.8$)[3], also known as Arp 23 and VV73. It appeared to be close to one end of a prominent bar (position angle, PA $\approx 64°$) in the central region of the galaxy, and was assumed to be the nucleus. Its spectrum is unlike that of any astronomical object yet published.

Observations

Spectra of NGC4618 were obtained on 28.52 February 1985 UT with the double spectrograph[4] at the Cassegrain focus of the