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REVIEW ARTICLE

“Saddle-shaped” dose-survival effect, is it a general and valuable phenomenon in microbes in response to heavy ion beam irradiation?

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Abstract

We aimed to verify the “saddle-shaped” dose-survival effect of microbes in response to heavy ion beam irradiation (HI), and further determine the radiation parameter that affects saddle shape formation, and the relationship between the saddle region and the positive mutation rate. A bibliometric analysis was performed based on literature containing the dose-survival effect of microbes in response to HI, from which the data on the particle energies, ionic types, irradiated microbes, survival curves, and maximum positive mutation rates were assembled. Articles reporting a “saddle-shaped” survival curve accounted for 64% of the total relevant articles and possessed a high cited frequency. The predominant articles, authors, and institutions that reported the dose-survival effect of microbes in response to HI proposed the “saddle-shaped” survival curve. It was customarily low-energy (but not moderate- or high-energy) HI that induced the “saddle-shaped” dose-survival effect. In addition, the “saddle-shaped” dose-survival effect was general among ~ 30-genera microbes. More importantly, most of the saddle regions contained the survival fractions within 10–30%, which are customarily used to screen mutants due to a high positive mutation rate. Further, 87% of the maximum positive mutation rates were associated with the saddle region, and 58% were located in the peak of the saddle region. “Saddle-shaped” dose-survival effect is a reliable and general phenomenon among varieties of microbes customarily in response to low-energy HI. Meanwhile, saddle region is always accompanied with high positive mutation rates. Thus, this study will aid in microbial mutation breeding practices.

Keywords Bibliometric analysis · Heavy ion beam irradiation · Microbial mutation breeding · “Saddle-shaped” survival curve · Positive mutation rate

Introduction

Mutagenesis is still one of the most widely used methods to obtain high-yield microbial strains due to difficulties in generating transformants with desired traits via genetically engineered changes in one or two genes. Heavy ion mutagenesis is an effective technique in the field of microbial breeding. Many varieties of microbes with advantageous properties have been developed by ion implantation (Li et al. 2013; Yang et al. 2013b; Fu et al. 2016; Hu et al. 2017b). Mutation breeding using heavy ion beam irradiation (represented as HI) has unique advantages. Compared with traditional mutagenesis methods, diverse types of DNA damage and substantial damage sites can be induced by HI due to greater effects on the organism including energy deposition, momentum transfer, mass deposition, and charge exchange (Feng et al. 2006; Tanaka et al. 2010). Mutations are induced by misrepaired damage. Thus, a high mutation rate and wide mutation spectrum are easier to obtain from mutation breeding using HI (Song et al. 2017).

In contrast with mammalian cells, the biological effects induced by HI have not been investigated in detail in microbial cell models with respect to the physical properties of ion beams and the biological properties of irradiated microbes. However, further detailed studies on the

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physical properties of HI and the resulting biological effects are necessary to promote its application in the field of life sciences. For example, based on the Bragg peak of dose-depth distribution, HI can effectively kill cancerous tissues with slight damage to normal tissues. This property renders the HI as one with most potential in radiotherapy (Guo et al. 2007; Imai et al. 2017; Minohara et al. 2018). Additionally, many studies have investigated the biological effects induced by HI in different types of mammalian cells with different radiation qualities (Guo et al. 2016). These studies have provided valuable references for the selection of dose, dose rate, energy, and other parameters in radiotherapy with complex clinical symptoms. Therefore, more systemic studies on the biological effects induced by HI in microbial cell models are required for meeting the demands of efficiency and effectiveness of mutation breeding. A clear understanding of the microbial responses to HI will guide the selection and control of radiation parameters in microbial mutation breeding practices.

A unique survival curve of microbes was proposed as a “saddle-shaped” survival curve in many previous studies focusing on dose-survival effect of microbes in response to HI. Further, the saddle region in the “saddle-shaped” survival curve was found to accompany high positive mutation rates (Ning and Long 2009; Li et al. 2013). However, there is a lack of quantitative data to clarify this phenomenon. Here, we focused on the “saddle-shaped” dose-survival effect in microbes in response to HI. A bibliometric analysis was performed on studies reporting the dose-survival effect of microbes in response to HI. The patterns of yearly outputs, important articles, top authors, and top institutions were determined both for records with the “saddle-shaped” survival curve and the exponential survival curve. In addition, microbes showing a “saddle-shaped” survival curve in response to HI were listed and analyzed based on the local literature set. The radiation parameter affecting the formation of the “saddle-shaped” dose-survival effect, and the detailed relationships among saddle region, survival fraction, and positive mutation rate were displayed in quantitative results. The reasons for this biological effect were discussed in detail.

Materials and methods

Establishment of local literature set

The Web of Science Core Collection database was employed to establish a local literature set, in which every article contained experimental data on the survival curve of microbes in response to HI. A retrieval term was constructed as (“ion implantation” or “heavy ion” or “ion beam” or “ion irradiation”) and (mutation or mutant or mutagenesis or mutagenic or breed or breeding) to narrow down the search range. The detailed search steps and strategies are shown in Fig. 1. The detailed information on all articles involved in the local literature set is provided in Supplemental Table S1. The time of establishing the local literature was December 2018.

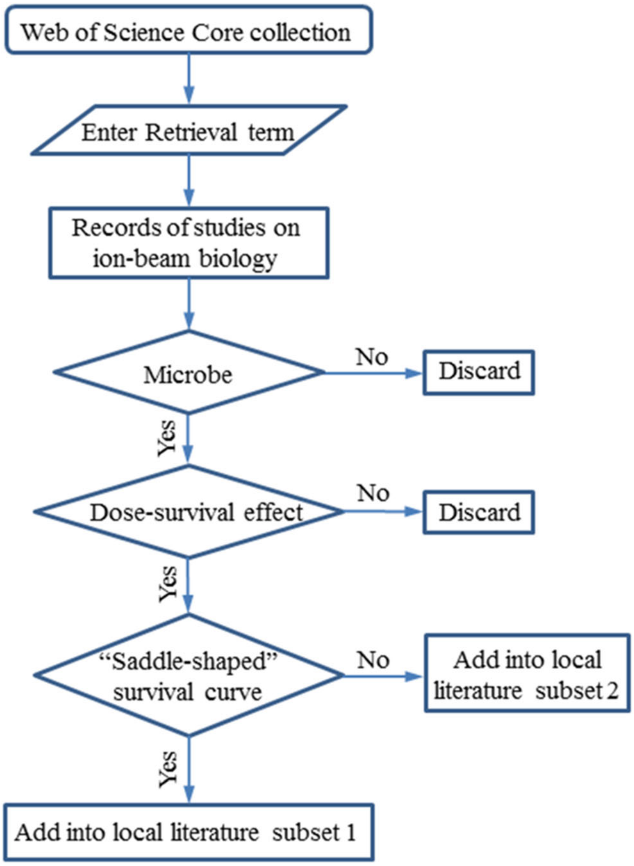


Fig. 1 Search strategy for local literature set

Bibliometric analysis of local literature

The local literature files were downloaded from the Web of Science Core Collection database in tab and plain text formats, respectively. Information on the time-dependent distribution of articles and the number of articles by authors were obtained by analyzing the tab files using Excel. The text file was input into HistCite and CiteSpace (Chen 2006; Garfield 2009). Both these programs have been specifically developed for visual analysis of citations. The citation analysis of articles was performed using HistCite. It is worth mentioning that the relative importance of every article included in the local literature set was indicated by a newly proposed conception named as Relative Importance Index of Article (RIIA). The RIIA was determined by the formula: RIIA = Local Citation Score (LCS)/Records after Published Year (RAPY), where LCS indicates the cited frequency of an article in the local literature set and RAPY indicates the number of articles after the published year of the analyzed article. Higher value of RAPY indicates that the article had more opportunities to be cited. This index takes account of cited frequency and timeliness of the article because articles published later had few opportunities to be cited by other articles in the local literature set. The threshold of important authors was set as publishing more than two papers as corresponding author. The relative importance of the institution was evaluated by the number of published articles and the centrality in the collaboration network. The collaboration network was drawn by CiteSpaceII, in which the area size of the node indicates the number of published articles, the line indicates two institutions appeared in the same articles, the thickness of the line indicates the co-occurrence frequency of two institutions, the color of the line or node indicates the time of the occurrence, and the outer ring filled with magenta expresses the degree of centrality.

Construction of a molecular evolutionary tree of microbes with the "saddle-shaped" dose-survival effect

All microbes with a "saddle-shaped" survival curve were listed based on the local literature set. All these microbes were classified at the genus level. The genetic markers (16s rRNA for prokaryotic microbes and 18s rRNA for eukaryotic microbes) were obtained from NCBI based on several typical species included in every genus. A preliminary version of the molecular evolutionary tree was constructed using MEGA software with all obtained genes. Based on comprehensive consideration of preliminary molecular evolutionary tree, and the microbial classification system on the basis of kingdom, division, class, order, family, genus, and species, only one appropriate sequence of 16s rRNA or 18s rRNA for every genus was selected to construct the final molecular evolutionary tree.

Statistical analysis of previous data

The energies of accelerated particles in the local literature set were classified as low energy (from ~ KeV/u to 10 MeV/u) and moderate or high energy (> 10 MeV/u) according to previous literature (Li et al. 2000). The accelerated ions used to irradiate microbes and obtain corresponding survival curves were sorted by the ionic types. All "saddle-shaped" survival curves of microbes in response to HI were collected based on the local literature set. Details on the survival fractions and positive mutation rates associated with the saddle region in the "saddle-shaped" survival curve were present.

Results and discussion

Significant support for a saddle-shaped survival curve of microbes in response to HI

In total, about 140 records deposited in the Web of Science Core Collection database were related to the contents of irradiating microbes with HI, wherein 96 records containing the dose-survival effect were fitted into the local literature set. Moreover, most of them can be categorized into the subject of HI-based microbial mutation breeding. This information indicates that determining the dose-survival effect is of great significance for the HI-based microbial mutation breeding practice. In fact, it is prerequisite to obtain a survival curve in most of relevant studies both on mechanism and application (Ning and Long 2008; Li et al. 2013, 2018; Fu et al. 2016). Meanwhile, it is notable that the variation in survival fractions of microbes with varying doses of HI presented as a "saddle-shaped" curve in many studies included in the local literature set. The survival fraction first decreased in a dose-dependent manner within a dose range. However, this trajectory changed when the irradiation dose exceeded a dose threshold, beyond which the survival curve presented a convex peak in a certain dose range and the survival fraction increased, and then decreased. The survival curve was thus in the shape of a "saddle" as reported for many types of microbes in previous studies (Liu et al. 2012; Li et al. 2013; Nie et al. 2013; Yang et al. 2013; Yan et al. 2014; Fu et al. 2016; Lin et al. 2016; Zhang et al. 2018). This phenomenon is distinct from that caused by other types of radiations, such as UV, X-rays, and γ-rays (Ning and Long 2009; Yan et al. 2014; Lin et al. 2016; Zhang et al. 2018). The survival fractions of microbes only decreased in a dose-dependent manner after UV, X-rays, and γ-rays.

Figure 2a shows the time distribution of articles reporting the dose-survival effect in response to HI and articles with a "saddle-shaped" survival curve therein, respectively. Overall, there are consistent reports on the "saddle-shaped" survival curve of microbes. Meanwhile, articles with a "saddle-shaped" survival curve accounted for a major proportion (64%) of the total articles reporting the dose-survival effect in microbes in response to HI (Fig. 2b). Based on the local literature set, the "saddle-shaped" survival curve of microbes was first reported by researchers from the Chinese Academy of Science in 2004 (Ge et al. 2004; Liu and Yao 2004; Xu et al. 2004). Additional relevant studies were published in a relatively active manner after 2004, which was also the active phase of studies reporting the dose-survival effect of microbes in response to HI.

In addition, the top authors for articles reporting the dose-survival effect and articles with a "saddle-shaped" survival curve therein were respectively selected by sequential consideration of the number of papers published

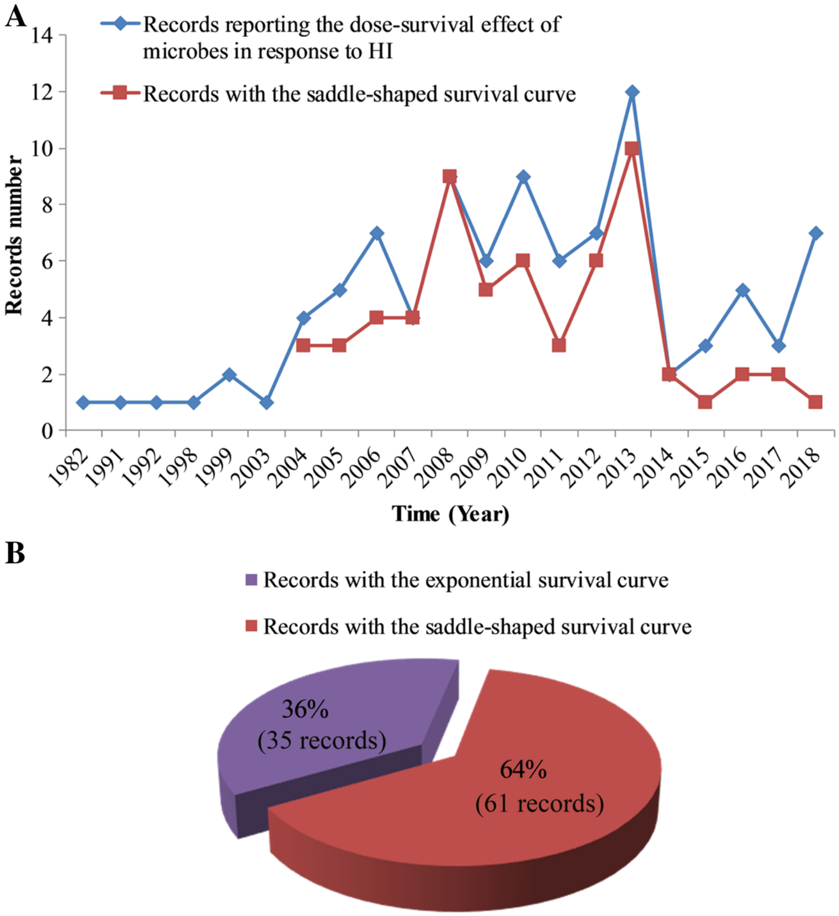


Fig. 2 Total articles reporting a survival curve of microbes in response to HI vs. articles with a "saddle-shaped" survival curve therein. Yearly outputs (**a**) and the relative ratio (**b**). Articles with a "saddle-shaped" survival curve were continuously reported since 2004 and accounted for a major proportion of the total articles reporting the dose-survival effect of microbes in response to HI

as the corresponding author, first author, and any author (Fig. 3A). All the authors considered to be important for articles with a "saddle-shaped" survival curve were also important authors for articles reporting the dose-survival effect. It means that the prominent authors who reported the dose-survival effect of microbes in response to HI proposed the "saddle-shaped" survival curve. The collaboration networks among institutions reporting the microbial dose-survival effect or "saddle-shaped" dose-survival effect are shown in Fig. 3B. Chinese Academy of Sciences was the predominant institution for reporting both. The important institutions for articles with a "saddle-shaped" survival curve were in accordance with the important institutions for articles reporting the dose-survival effect. These results suggested that the important institutions carrying out these relevant studies proposed the "saddle-shaped" dose-survival effect of microbes in response to HI. The low-energy HI occurred more frequently than moderate- or high- energy HI both for literature showing the dose-survival effect and the "saddle-shaped" dose-survival effect (Fig. 3C). Similarly, the main ionic types used to irradiate microbes were nitrogen ion and carbon ion, which were also the main ionic types for articles showing a "saddle-shaped" survival curve (Fig. 3D). Thus, the radiation parameters used in studies reporting the "saddle-shaped" survival curve were the main radiation parameters selected in studies relating to the dose-survival effect of microbes in response to HI.

The top ten articles with high RIIA are listed in Table 1. It can be seen that ~70% of them reported the "saddle-shaped" survival curve. Thus, articles with a "saddle-shaped" survival curve were some of the most important articles reporting the dose-survival effect of microbes in response to HI. For these articles, the total global citation score (GCS) was 168, and the average GCS was 21 in the Web of Science Core Collection database. The relatively high GCS suggests that articles containing a "saddle-shaped" curve are widely approved. All top ten articles focused on mutation breeding of industrial microbes by HI, supporting that studies on dose-survival effects play an important role in microbial mutation breeding by HI. Additionally, these articles were published in some influential journals, including *Annals of Microbiology*, *Applied Biochemistry and Biotechnology*, *Bioresource Technology*, *Journal of Applied Microbiology*, *Journal of Industrial Microbiology and Biotechnology*, *Journal of Microbiology and Biotechnology*, and *World Journal of Microbiology and Biotechnology*. Combining with the

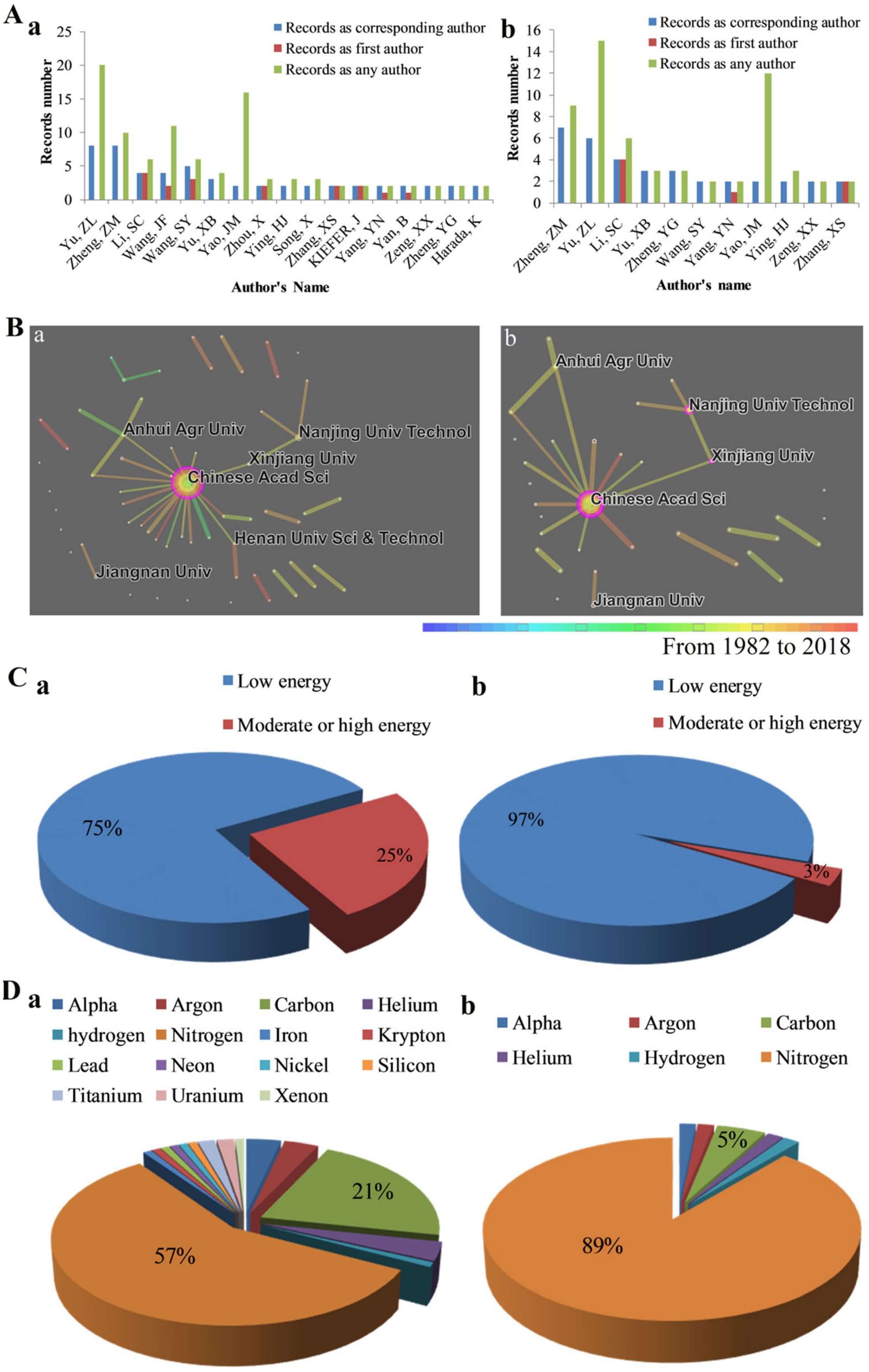


Fig. 3 Important authors (**A**), top institutions (**B**), energy classification (**C**), and main ionic types (**D**) for all articles reporting the dose-survival effect of microbes in response to HI (**a**) and articles showing a "saddle-shaped" therein (**b**), respectively. The predominant authors and institutions reporting the dose-survival effect of microbes in response to HI proposed the "saddle-shaped" dose-survival effect. Low-energy HI occurred more frequently in the local literature set. Nitrogen ion and carbon ion were the main ions used to irradiate microbes in studies on ion-beam microbiology, which were also the main ionic types in the articles with a "saddle-shaped" survival curve

analyses on yearly outputs, top authors, and important institutions, it can be seen that the "saddle-shaped" dose-survival effect is one of noteworthy radiobiological effects in microbes induced by HI. Also, this phenomenon is relatively reliable and approved.

"Saddle-shaped" dose-survival effect customarily in response to low-energy HI

The energy of HI is an important radiation parameter. In most studies, energy optimization is regarded to be as essential as

Table 1 Top ten articles reporting the dose-survival effect of microbes in response to HI

Order	Details on paper	LCS	GCS	RIIA	Energy	Excellent mutants screened from saddle region
1	A new strategy for strain improvement of *Aurantiochytrium* sp. based on heavy-ions mutagenesis and synergistic effects of cold stress and inhibitors of enoyl-ACP reductase. Enzyme and Microbial Technology. 2016, 93–94: 182–190.	2	12	0.2	80 MeV	N/A
1	Strain improvement of *Trichoderma viride* for increased cellulase production by irradiation of electron and C-12(6+)-ion beams. Biotechnology Letters. 2016, 38 (6): 983–989.	2	5	0.2	270 MeV	N/A
3*	Mutation breeding of chitosanase-producing strain *Bacillus* sp. S65 by low-energy ion implantation. Journal of Industrial Microbiology & Biotechnology. 2006, 33 (12): 1037–1042.	13	32	0.178	15 keV	Yes
4*	Breeding of d(−)-lactic acid high producing strain by low-energy ion implantation and preliminary analysis of related metabolism. Applied Biochemistry and Biotechnology. 2010, 160 (2): 314–321.	8	21	0.177	10 keV	Yes
5*	A novel approach for improving the productivity of ubiquinone-10 producing strain by low-energy ion beam irradiation. Applied Biochemistry and Biotechnology. 2006, 72 (3): 456–461.	10	28	0.137	10 keV	Yes
6*	Mutation of *Gluconobacter oxydans* and *Bacillus megaterium* in a two-step process of L-ascorbic acid manufacture by ion beam. Journal of Applied Microbiology. 2004, 96 (6): 1317–1323.	11	40	0.129	20 keV	No mention
7	High yield antibiotic producing mutants of *Streptomyces erythreus* induced by low energy ion implantation. Nuclear Instruments & Methods in Physics Research Section B—Beam Interactions with Materials and Atoms. 1998, 140 (3–4): 341–348.	10	20	0.109	40 and 60 keV	N/A
8*	Breeding of L(+)-lactic acid producing strain by low-energy ion implantation. Journal of Microbiology and Biotechnology. 2004, 14 (2): 363–366.	9	18	0.106	15 keV	Yes
9*	Mutant of a xylanase-producing strain of *Aspergillus niger* in solid state fermentation by low energy ion implantation. World Journal of Microbiology and Biotechnology. 2005, 21 (6–7): 1045–1049.	8	18	0.1	10 keV	Yes
10*	N+ ion beam implantation of tannase-producing *Aspergillus niger* and optimization of its process parameters under submerged fermentation. Annals of Microbiology. 2013, 63 (1): 279–287.	2	4	0.1	10 keV	Yes
10*	Enhancement of docosahexaenoic acid production by low-energy ion implantation coupled with screening method based on Sudan Black B staining in *Schizochytrium* sp. Bioresource Technology. 2016, 221: 405–411	1	7	0.1	5, 10, and 15 keV	Yes

Most of them showed a "saddle-shaped" survival curve of microbes in response to low-energy ion beam irradiation. Moreover, most of the studies with a "saddle-shaped" survival curve screened excellent mutants from the saddle region

*Indicates articles containing a "saddle-shaped" survival curve. N/A indicates that the particular article did not show a "saddle-shaped" dose-survival effect

dose optimization (Wang et al. 2012; Nie et al. 2013; Zhang et al. 2013; Song et al. 2014; Fu et al. 2016). Usually, energies of HI are divided into low energy (from ~ KeV/u to 10 MeV/u) and moderate or high energy (> 10 MeV/u) (Li et al. 2000). Overall, low-energy HI-based microbial mutagenesis research just underwent the development of ~ 30 years (Feng et al. 2006). The moderate- or high-energy HI-based microbial mutagenesis was mainly carried out after 2000 (Hu et al. 2017b). There was a delay for microbial mutagenesis using moderate or high energy relative to that based on low-energy HI. This fact may be one of the main reasons of low proportion of studies using moderate or high energy (Fig. 3c). Different energies correspond to distinct dose–depth distribution curves. For the same sample, moderate- or high-energy HI caused a longer penetration depth than low-energy HI (Li et al. 2000). Further, the relative proportions among energy deposition, momentum transfer, mass deposition, and charge exchange are different between low energy and moderate or high energy. Low-energy radiation has more mass deposition, charge deposition, and the resultant nuclear reaction than moderate- or high-energy radiation (Shao and Yu 1997; Li et al. 2000). Based on these considerations, the effect of particle energy on the formation of the "saddle-shaped" survival curve was revealed by statistical analysis of relative proportions of low-energy HI and moderate- or high-energy HI used in studies containing the dose-survival effect and studies showing a "saddle-shaped" survival curve therein (Fig. 4). It can be seen that records with the exponential survival curve accounted for a vast majority (92%) of total records in studies using moderate- or high-energy HI (Fig. 4a). However, it is the records with the "saddle-shaped" survival curve that accounted for a vast majority (82%) of total records in studies using low-energy HI (Fig. 4b). Moreover, the relative proportion of studies using moderate and high energy was further less than that using low energy (Fig. 3c). Thus, there were very few records with "saddle-shaped" survival curve in the moderate- or high-energy group. It can be inferred that "saddle-shaped" dose-survival effect of microbes was customarily induced by low-energy (but not moderate- or high-energy) HI. We specially paid attention to relevant studies without a "saddle-shaped" survival curve of microbes in response to low-energy HI. A wider interval between adjoining doses or a narrow range of irradiation doses may be one of the reasons for missing the phenomenon (Tang et al. 2006; Wang et al. 2011; Cheng et al. 2016; Xie et al. 2018). For a minority of studies reporting the "saddle-shaped" dose-survival curve in response to moderate- or high-energy HI (Jiang et al. 2017; Hu et al. 2017a), the combination of the particular sample conditions (containing thickness, density, ingredient, etc.) and radiation parameters (containing the dose, energy, ionic type, etc.) may result in the "saddle-shaped" dose-survival effect.

Various microbes showing a "saddle-shaped" survival curve

The microbes previously reported to show a "saddle-shaped" survival curve in the local literature set were classified at the genus level. Various types of microbes presented with a "saddle-shaped" survival curve. Part of them included in local literatures (based on the Web of Science Core Collection database) could be classified into 24 genera (Fig. 5a). There were 13 genera based on articles included in the Chinese Scientific Citation Database. Seven genera were shared by two databases. In total, 30 microbial genera were reported to present with a "saddle-shaped" survival curve within the scope of our investigation (Fig. 5b). Bacteria, actinomycetes, and fungi were included. Microbes belonging to the *Aspergillus* or *Bacillus* genus accounted for about 10–15% of the total number of microbes. The other genera accounted for single-digit percentages. In addition, a molecular evolutionary tree was constructed for most of these microbial genera (28 genera), which genetic markers were available in the NCBI database (Fig. 5c). The separate branches and nodes of the molecular evolutionary tree were rich and irregular. It means that the evolutionary statuses of these genera reflect a great degree of diversity. These data suggested the "saddle-shaped" survival curve customarily in response to low-energy HI is one of the general biological effects across many microbes.

Saddle region of positive significance

The survival fractions of microbes in response to physical or chemical mutagens play an important role in studies on mutation breeding. It is customary to think that survival fractions from 10% to 30% were associated with high positive mutation rates (Yuan et al. 2007; Xu et al. 2010; Song et al. 2014). Particularly, it was reported that the peak range of "saddle-shaped" survival curves is usually accompanied with a high

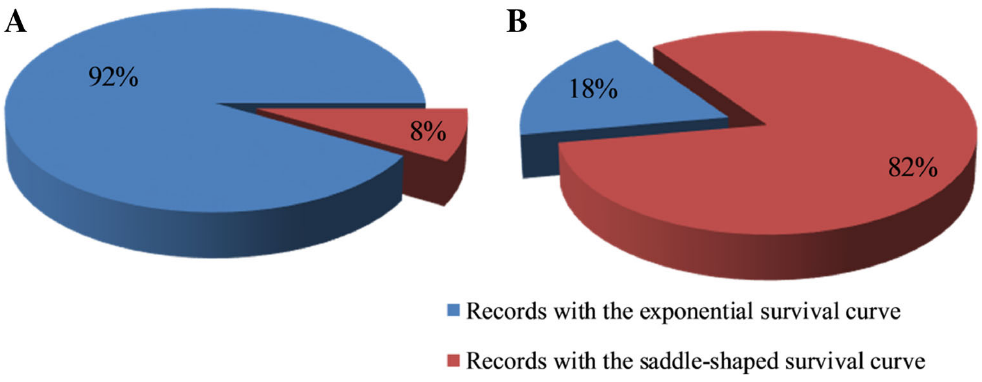
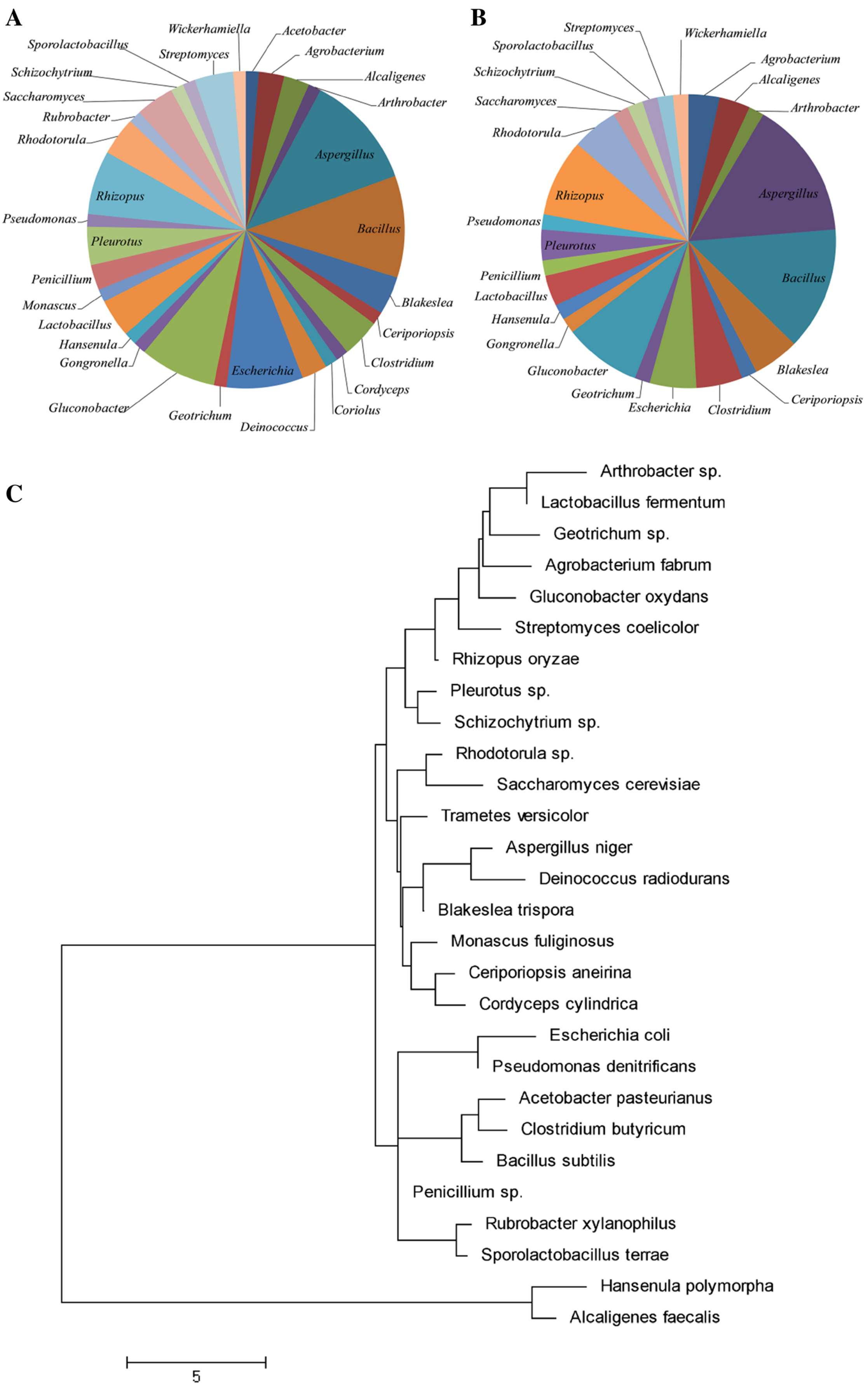


Fig. 4 Relative proportions of records reporting an exponential dose-survival curve and the records with a "saddle-shaped" survival curve for moderate- or high-energy group (**a**) and low-energy HI group (**b**)


A
Wickerhamiella
Acetobacter
Sporolactobacillus
Streptomyces
Agrobacterium
Schizochytrium
Alcaligenes
Saccharomyces
Arthrobacter
Rubrobacter
Rhodotorula
Aspergillus
Rhizopus
Pseudomonas
Bacillus
Pleurotus
Penicillium
Blakeslea
Monascus
Lactobacillus
Ceriporiopsis
Hansenula
Gongronella
Clostridium
Escherichia
Cordyceps
Gluconobacter
Coriolus
Geotrichum
Deinococcus
B
Streptomyces
Wickerhamiella
Sporolactobacillus
Agrobacterium
Schizochytrium
Alcaligenes
Saccharomyces
Arthrobacter
Rhodotorula
Aspergillus
Rhizopus
Pseudomonas
Pleurotus
Bacillus
Penicillium
Lactobacillus
Hansenula
Gongronella
Blakeslea
Gluconobacter
Geotrichum
Ceriporiopsis
Escherichia
Clostridium
C
Arthrobacter sp.
Lactobacillus fermentum
Geotrichum sp.
Agrobacterium fabrum
Gluconobacter oxydans
Streptomyces coelicolor
Rhizopus oryzae
Pleurotus sp.
Schizochytrium sp.
Rhodotorula sp.
Saccharomyces cerevisiae
Trametes versicolor
Aspergillus niger
Deinococcus radiodurans
Blakeslea trispora
Monascus fuliginosus
Ceriporiopsis aneirina
Cordyceps cylindrica
Escherichia coli
Pseudomonas denitrificans
Acetobacter pasteurianus
Clostridium butyricum
Bacillus subtilis
Penicillium sp.
Rubrobacter xylanophilus
Sporolactobacillus terrae
Hansenula polymorpha
Alcaligenes faecalis
5

◀ **Fig. 5** Microbes with a "saddle-shaped" survival curve in the local literature set and the diversity of them. Classification of microbes (**a**) (included in Web of Science core collection (**b**) and Chinese Science Citation Databases) showing the "saddle-shaped" dose-survival effect. The genus-level molecular evolutionary tree of microbes with saddle-shaped survival curve in response to HI (**c**)

positive mutation rate (Song et al. 2010; Xu et al. 2010; Zhao et al. 2012). To show the quantitative results on the relationships among saddle region, survival fraction, and positive mutation rate, survival fractions and positive mutation rates were analyzed based on the "saddle-shaped" survival curve. Information on the survival fraction range within the saddle region is shown in Fig. 6a, b. The distributions of the maximum positive mutation rates in different regions of the "saddle-shaped" survival curve are shown in Fig. 6c and d. It is seen that most of the saddle regions contained survival fractions within 10–30%. In addition, 58% of the maximum positive mutation rates were located in the peak regions, and 87% were associated with the saddle regions. Meanwhile, for important studies reporting the "saddle-shaped" dose-survival effect, most of them screened excellent mutants from the saddle region (Table 1). These data confirmed that saddle region of the "saddle-shaped" survival curve is usually related to a high positive mutation rate.

Mechanism of "saddle-shaped" dose-survival effect in microbes customarily in response to low-energy HI

Some researchers proposed the possible mechanisms for the "saddle-shaped" survival curve of microbes, which was mainly aimed at low-energy HI. It can be summed up in three points as follows. One is based on the interaction between the accelerated ions and biological system involving energy deposition, mass deposition, momentum exchange, and electric charge (Shao and Yu 1997). When the irradiation dose is limited to a certain range, the damage just increases monotonically. When it exceeds a threshold with an increase in dosage, the ions deposited in cells may form new chemical substances by combining with the secondary molecular fragments induced by HI. These reactions will neutralize the secondary

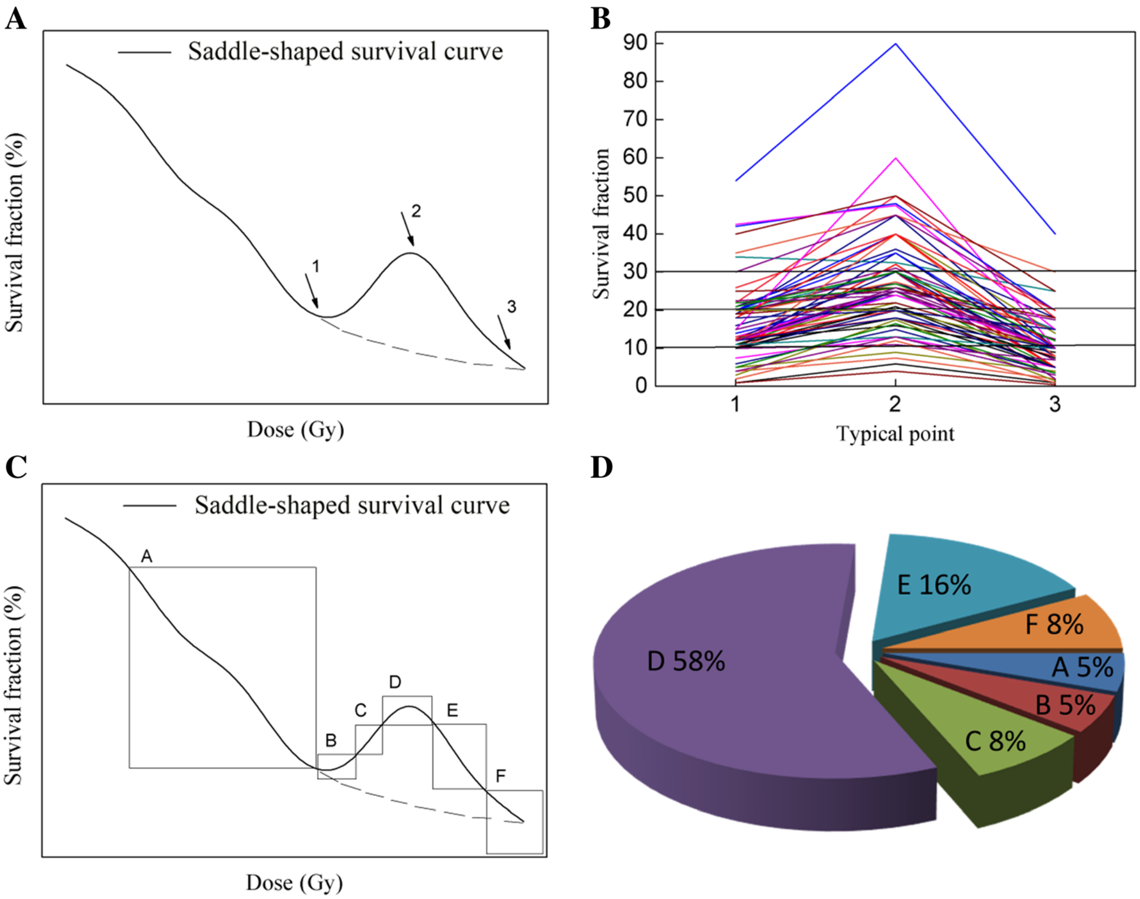


Fig. 6 Relationships among saddle region, survival fraction, and positive mutation rate. Three points in the saddle region were employed to indicate the survival fraction range of the saddle region (**a**). Most saddle regions largely contained survival fractions within 10–30% (**b**). The "saddle-shaped" survival curve was separated into six regions (A, B, C, D, E, and F) (**c**). B, C, D, or E was regarded as the saddle region. The frequencies (the number after letter) of the maximum positive mutation rates were counted in each region (**d**). The saddle regions (especially the peak region) were related to a higher positive mutation rate

molecules, such as ROS and RNS, thereby alleviating the corresponding damage induced by secondary molecules. The second is attributed to the protective action of the mass deposition and charge deposition (Du et al. 1999; Song et al. 1999), reckoning that the considerable ions deposited in the sample may act as a physical barrier due to the effect of mass deposition and charge deposition. This point is in accordance with the fact that low-energy radiation has more mass deposition and charge deposition than moderate- or high-energy radiation (Shao and Yu 1997; Li et al. 2000). The third is related to the repair system. It takes into account that the repair system may be further activated when the irradiation dose exceeds a threshold (Song et al. 1999; Ning and Long 2008; Fu et al. 2016). The further activation of the repair system can be regarded as an adaptive response. However, as the irradiation dose increases continuously, the repair system will gradually collapse. Thus, the survival fraction first increases, and then decreases within a certain dose range. Preliminary studies suggested that the variation in activities of superoxidase dismutase, catalase, and peroxidase and the productions of ROS and RNS also showed a "saddle-shaped" trajectory corresponding to the "saddle-shaped" survival curve in response to low-energy HI in *Escherichia coli* and *Deinococcus radiodurans* (Ning and Long 2008), supporting the mechanism on repair system activation. Additionally, some mathematical models based on possible mechanisms were constructed to describe the "saddle-shaped" survival curve of microbes in response to low-energy HI. One of the most classical models is the EMC model (Shao and Yu 1997), where E, M, and C indicate energy, mass, and charge, respectively. It means that the distinctive survival effect in microbes is based on the unique physical properties of HI. The detailed function is described as $S = \exp(-P \times (aD + UD^2 - VD^3 \times \exp(-kD)))$, wherein D represents the irradiation dose, P represents the average probability of causing death for a DSB, "$aD + UD^2$" indicates the DSB number caused by a particular dose of ion beam irradiation, "$VD^3 \times \exp(-\mathrm{kD})$" indicates the decreased DSB number due to the effect of mass deposition and charge deposition. Further, the relevant experimental data fitted the EMC function well.

Conclusion and future prospect

The determination of dose-survival fractions plays an important role in microbial mutation breeding. In this study, we focused on the "saddle-shaped" survival curve of microbes induced by HI. This distinctive survival effect in microbes induced by HI should not be ignored, which is general across many microbes. The corresponding literature accounted for a major proportion of the total articles reporting the dose-survival effect in microbes in response to HI. Moreover, our analyses on previous studies suggested that "saddle-shaped" dose-survival effect of microbes was customarily presented in response to the low-energy HI, but not the moderate- or high-energy HI. Meanwhile, the peak range of "saddle-shaped" survival curves is usually related to a high positive mutation rate. However, the mechanism underlying the saddle-shaped survival curve of microbes customarily in response to low-energy HI was less studied through specific experiments. Further, institutions that reported these results were included in relatively closed groups. This may be caused by the fact that studies on microbes irradiated by HI depend on the presence of heavy ion accelerators. These large-scale scientific facilities are possessed by few institutions, of which some do not conduct studies on microbial irradiation by HI. In addition, the data on the "saddle-shaped" survival curve of microbes were always included in studies on microbial mutation breeding by HI. Therefore, additional and special experiments are needed to reveal the detailed mechanism underlying the formation of a "saddle-shaped" curve customarily in response to low-energy HI.

At present, the mechanism for the saddle-shaped survival curve of microbes may be revealed through the combined omics technologies in detail. The essence to reveal this mechanism is to compare damage, repair, and mutagenesis between the saddle region and other regions based on the "saddle-shaped" survival curve. This may consist of three important works: detailed investigation on damage and repair with varying irradiation dose, high-throughput screening of mutant strains with positive traits, and effective identification of mutations in mutant strains at the genome level. In addition, research on HI-based microbial mutagenesis just underwent a short-phase development. So, it is still in a developing stage. We believe HI-based mutation breeding will be more popular due to its many verified advantages and resultant achievements. Meanwhile, the particular scientific device required by HI-based mutagenesis may be established in more institutes. Our paper can thus provide references for further studies on the "saddle-shaped" dose-survival effect in microbes with ion implantation to further promote microbial mutation breeding practices.

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Compliance with ethical standards

Conflicts of interest The authors declare that there are no conflicts of interest.

Research involving human participants and/or animals N/A

Informed consent Informed consent was obtained from all individual participants included in the study.

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