MINIREVIEW

Broad host range plasmids

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Article written in memory of the late Prof. J.K. Deb who always used to inspire a lot.

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Abstract

Plasmids are and will remain important cloning vehicles for biotechnology. They have also been associated with the spread of a number of diseases and therefore are a subject of environmental concern. With the advent of sequencing technologies, the database of plasmids is increasing. It will be of immense importance to identify the various bacterial hosts in which the plasmid can replicate. The present review article describes the features that confer broad host range to the plasmids, the molecular basis of plasmid host range evolution, and applications in recombinant DNA technology and environment.

Introduction

Plasmids since their discovery have been detected in many different genera. Various microorganisms in which a plasmid can replicate and be maintained is called its host range. Accordingly, plasmids can be classified into narrow and broad host ranges (BHR). The first classification into these two groups was made in 1972 by Datta and Hedges (Datta & Hedges, 1972), who defined BHR plasmids as those which are able to transfer among *Enterobacteria* and *Pseudomonas* spp. However, according to the current understanding, BHR plasmids transfer and maintain among bacteria belonging to different phylogenetic subgroups (Top *et al.*, 1998).

Broad host range plasmids are of considerable interest because they not only play an important role in horizontal gene transfer but also their replicons can serve as good sources for vector construction. Several barriers limit plasmid transfer between unrelated bacteria: interactions at the cell surface may prevent effective mating contact, restriction systems may degrade foreign DNA, or the plasmid may not replicate in the new host. There are several reviews published on BHR plasmids (Kues & Stahl, 1989; del Solar *et al.*, 1993, 1996; Sakai & Komano, 1996). However, in the era of genomics and mobile metagenomics, it would be of immense importance to predict the host range of the plasmid based on the sequence information. For this, it is worth listing the general features that confer broad host range properties to the plasmid. In the present review, we describe the possible reasons behind the unique capability of plasmids to replicate and maintain in varied hosts, molecular basis of host range evolution, and their application in recombinant DNA technology.

Factors affecting broad host range (BHR) plasmid replication

Some well-characterized naturally occurring BHR plasmids and their host range determined so far are shown in Table 1. There are a number of features, which determine the host range of plasmids.

Presence of multiple origins

Some plasmids have multiple origins and one origin functions in one type of host and the others are functional in

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Table 1. Naturally occurring broad host range plasmids described according to their sizes

Plasmid	Size (kb)	Antibiotic resistance markers	Microorganism from which isolated	Host range*	Reference
pBC1	1.6	Cryptic	*Bacillus coagulans* Zu1961	*E. coli, B. subtilis, B. amyloliquefaciens, S.aureus, S. carnosus, and Lactobacillus reuteri*	De Rossi *et al.* (1992)
pEP2	1.85	Cryptic	*Corynebacterium diphtheriae*	*Corynebacteria*, *Mycobacteria*, and *E. coli*	Zhang *et al.* (1994)
pWVO1	2.2	Cryptic	*Lactococcus lactis* subsp. *cremoris*	*Bacilli*, *Lactococci*, *Streptococci*, *Clostridia* and *Staphylococci*, *E. coli*	Leenhouts *et al.* (1991)
pLF1311	2.38	Cryptic	*Lactobacillus fermentum* VKM1311	*E. coli, Lactobacillus, Lactococcus, Enterococcus, Bacillus*	Aleshin *et al.* (1999)
pAP1	2.4	Cryptic	*Arcanobacterium* (*Actinomyces*) *pyogenes*	*E. coli, Corynebacterium pseudotuberculosis, Arcanobacterium*	Billington *et al.* (1998)
pBBR1	2.6	Cryptic	*Bordetella bronchiseptica*	*E. coli, Bordetella pertussis, B. bronchiseptica, Vibrio cholerae, Rhizobium meliloti, Pseudomonas putida*	Szpirer *et al.* (2001)
pWKS1	2.69	Cryptic	*Paracoccus pantotrophus* DSM 11072	*Paracoccus*, *Agrobacterium tumefaciens*, *Rhizobium leguminosarum,* and *Rhodobacter sphaeroides*	Bartosik *et al.* (2002)
pLS1	4.40	Tc	*Streptococcus agalactiae*	*Streptococcus pneumoniae, Bacillus subtilis, E. coli*	Lacks *et al.* (1986)
pUB6060	5.8	Cryptic	*Plesiomonas shigelloides*	*Aeromonas*, *Pseudomonas*, *Stenotrophomonas*, *Plesiomonas*	Avison *et al.* (2001)
pJD4	7.4	Ap	*Neisseria gonorrhoeae*	*N. gonorrhoeae*, *E. coli*, *Salmonella enterica* serotype Minnesota, *Haemophilus influenzae*	Pagotto & Dillon (2001)
RSF1010/ R1162/R300B	8.68	Sm, sulfonamides	*E. coli/P. aeruginosa/ Samonella enterica* serovar Typhimurium	*Mycobacterium smegmatis*, *S. lividans*, *E.coli*, *S. cerevisiae*, *Schizosaccharomyces pombe*, *Kluyveromyces lactis*, *Agrobacterium*, *Cyanobacteria*, *Pichia angusta* (*Hansenula polymorpha*), and *Pachysolen tannophilus*	Scherzinger *et al.* (1984) and Meyer *et al.* (1982)
pIJ101	8.9	Cryptic	*Streptomyces lividans*	Several *Streptomyces* spp.	Kieser *et al.* (1982)
pSN22	11	Cryptic	*Streptomyces nigrifaciens*	*Streptomyces*, *Staphylococci*, *Actinomadura*, etc.	Kataoka *et al.* (1991)
pAMβ1	26.5	Em, Lincomycin	*Streptococococcus faecalis*	*Streptococcus lactis*, *Lactobacilli*, *Bacilli*, *Listeria monocytogenes*	Clewell *et al.* (1974)
pIP501	30.2	Em, Cm	*Streptococcus agalactiae*	*Streptococci*, *Staphylococci*, *Enterococci*, *Listeria*, *Streptomyces lividans*, *E. coli*	Horodniceanu *et al.* (1976)
ZM6100(Sa)	39	Km, Sp, Cm	*Zymomonas mobilis* ZM6100(Sa)	*Z. mobilis, E. coli*	Strzelecki *et al.* (1987)
pCU1	39	Sp, Sm, Ap	Purple bacteria	*Agrobacterium*, *Bradyrhizobium*, and *Rhizobium*	Krishnan & Iyer (1988)
RA3	45.9	Sp, Sm, Cm	*Aeromonas* spp.	Alpha-, Beta-, and *Gammaproteobacteria*	Kulinska *et al.* (2008)
pMOL98	55.5	Cryptic	Polluted soil captured in *C. metallidurans*	β-proteobacteria (predicted)	Van der Auwera *et al.* (2009)
RK2/RP4/ RP1/R68	60	Km, Tc, Ap	*Pseudomonas aeruginosa*	Many Gram-negative bacteria *Escherichia coli, Pseudomonas aeruginosa, Pseudomonas putida, Azotobacter vinelandii, Erwinia crysanthemi*, *Erwinia amylovora*, *Erwinia herbicola*, *Ochrobactrum anthropi*	Thomas *et al.* (1982) and Pansegrau *et al.* (1994)
pB10	64.5	Tc, Sm, Amoxicillin and sulfonamides	Waste water treatment plant	Alpha-, Beta-, and *Gammaproteobacteria*	De Gelder *et al.* (2005)

*Experimentally determined and reported host range.

other hosts. Examples of such plasmids are pJD4 (IncW) and pCU1 (IncN). The BHR plasmid pJD4 (7.4 kb) isolated from *Neisseria gonorrhoeae* was found to contain three clustered but distinguishable origins of replication, namely ori1, ori2, ori3, and two genes for replication initiation proteins, RepB and RepA, necessary for the functioning of ori2 or ori3 and ori1, respectively. Plasmids containing ori1 require DNA polymerase I (Pol I) for replication, and those carrying ori2 and ori3 do not require Pol I. Plasmid pJD4 is the smallest plasmid characterized containing three origins of replication and two unique Rep proteins (Pagotto & Dillon, 2001).

Similarly, another plasmid pCU1 (39 kb) belonging to IncN family contains three replication origins, two of which, called oriB and oriS, function in both PolA+ and PolA- hosts, and a third called oriV functions only in PolA+ hosts. It also encodes RepA, which is required by oriB. The oriS can function without RepA and polymerase I, but the iteron region should also be deleted. The three replication origins and RepA protein are localized in a 2053-bp region (Kim *et al.*, 1994). Thus, functionally distinguishable origins in small replicons may be a way of endowing such replicons with a broad host range.

However, there are certain narrow host range plasmids such as F and R6K (IncX), which contain multiple origins. Thus, this feature is not exclusive to BHR plasmids. Plasmid R6K (38 kb) contains three replication origins (α, β, and γ) and encodes for two replication proteins, pir (encoding the π protein for origins α and γ) and bis (Bis for origin β; Mukhopadhyay *et al.*, 1986). Plasmid F (94.5 kb) contains three independent replication regions, RepFIC, RepFIA, and RepFIB. A 9-kb mini-F plasmid that contains the RepFIA region has two origins oriV and oriS and a single replication initiation protein Rep. Replication from oriV when both oriV and oriS are present is bidirectional, whereas replication from oriS when oriV is deleted is unidirectional (Keasling *et al.*, 1992).

Structure of origin

The structure of the origin also plays an important role in governing the host range. The best example is plasmid RK2, which belongs to IncP family. The IncP plasmids (classified in *Escherichia coli* as IncP and in *Pseudomonas* as IncP-1) were divided into two subclasses, designated as IncP alpha and IncP beta (Yakobson & Guiney, 1983). The IncP alpha subgroup consists of indistinguishable plasmids R18, R68, RK2, RP1, and RP4 (Pansegrau *et al.*, 1994). IncP-1beta plasmids, example pB10, pKJK10, etc., are known to be highly promiscuous. They have the ability to transfer between and replicate in nearly all species of the *Alpha-*, *Beta-* and *Gamma proteobacteria*.

Broad host range plasmid RK2 (60 kb) consists of a replication origin (oriV) and a TrfA (trans-acting replication function)-encoding gene (Durland & Helinski, 1990). The minimal origin (oriV) possesses five iterons and is functional in *E. coli*. However, the presence of three additional iterons stabilizes RK2 maintenance in *Pseudomonas putida* (Schmidhauser *et al.*, 1983). In addition, the region with four DnaA boxes is essential for RK2 replication in *E. coli*, but is dispensable for replication of the plasmid in *P. aeruginosa* (Shah *et al.*, 1995; Doran *et al.*, 1999). This suggests that structural elements of origin are employed for BHR plasmid replication and maintenance in different bacterial hosts.

The plasmids isolated from Gram-positive bacteria usually replicate via rolling circle (RC) method and contain a single-stranded origin (sso) and a double-stranded origin (dso). The plasmid-encoded Rep protein makes a site-specific nick at dso and becomes covalently attached to the 5′ phosphate at the nick site. The 3′ OH end acts as a primer for the synthesis of leading strand. The parental strand is converted into double-stranded DNA by replication initiated at sso. Four types of ssos have been identified in RCR plasmids: (i) ssoA, present in several staphylococcal plasmids such as pT181 and pE194 (Gruss *et al.*, 1987; del Solar *et al.*, 1993) (ii) ssoU, described for pUB110 (van der Lelie *et al.*, 1989); (iii) ssoT, commonly found in *Bacillus* plasmids (Bron *et al.*, 1987), and (iv) ssoW, described for lactococcal plasmid pWV01 (Seegers *et al.*, 1995). The ssoA- and ssoW-type origins are fully active only in their native hosts (te Riele *et al.*, 1986; Gruss *et al.*, 1987). On the other hand, lagging strand replication from the ssoU type origins is very efficient in various Gram-positive bacteria such as *Bacillus subtilis*, *Staphylococcus aureus*, *Streptococcus pneumoniae,* and *Lactococcus lactis* (Boe *et al.*, 1989; van der Lelie *et al.*, 1989; Kramer *et al.*, 1995; Meijer *et al.*, 1995; Seegers *et al.*, 1995).

The presence of ssoU is an important factor in determining the promiscuity of RC plasmids. It is important to note that the ssoT of pBAA1, which is fully active in *B. subtilis* and *S. aureus* (Seery & Devine, 1993), has 69% homology with the ssoU sequence. On the other hand, the extent of homology between ssoU and ssoAs of plasmids pE194 and pLS1 and the ssoW of pWV01, which function efficiently only in their native hosts, is 52%, 50%, and 59%, respectively. Therefore, it is likely that regions that are conserved between ssoU and ssoT but absent in the ssoAs and ssoW are critical for broad host range replication and plasmid promiscuity. Such sequences may be important for interaction with various RNA polymerases, and possibly other host proteins.

The rate of conversion of ssDNA intermediates into double-stranded DNA forms depends upon the efficiency

with which the host proteins recognize a given sso. Kramer *et al.* (1999) determined the molecular basis of the broad host range function of ssoU type origins. They suggested that a strong interaction between the ssoU and RNA polymerase from different bacterial hosts is an important factor in determining the broad host range replication of ssoU-containing RC plasmids.(Kramer *et al.*, 1999).

It is generally believed that inefficient ssDNA conversion rather than lack of expression of the Rep protein is the major factor preventing establishment of plasmids from Gram-positive bacteria in *E. coli*. Goze and Ehrlich (Goze & Ehrlich, 1980) showed that a hybrid between pC194 and pBR322, the latter containing an efficient lagging strand initiation site for *E. coli*, is able to replicate in this bacterium under conditions in which pBR322 replication is prevented. The *B. thuringiensis* plasmid pTX14-3 cannot be established in *E. coli*, although its Rep protein is expressed in this bacterium from its own promoter (Andrup *et al.*, 1994). The Rep protein of the RC plasmid pKYM, isolated from the Gram-negative bacterium *Shigella sonnei*, shows a strong homology with Rep proteins of Gram-positive plasmids (Yasukawa *et al.*, 1991). However, instead of an sso which is characteristic for Gram-positive plasmids, pKYM contains a specific Gram-negative lagging strand initiation signal showing 74% identity with the ssDNA conversion signals of the filamentous phages fd, f1, and M13 (Kodaira *et al.*, 1995).

A limited number of Gram-positive plasmids are exceptional in that they are able to replicate in *E. coli* also. Apparently, in these cases, Rep is functionally expressed and ssDNA molecules are converted to double-stranded plasmid molecules. Their SSOs are probably functional in *E. coli*.

Replication initiation independent of host initiation factors

Plasmids that do not require host proteins for replication can maintain themselves in many different bacteria. For example, the IncQ plasmids have a broader host range than any other known replicating element in bacteria. The features responsible for this are as follows: initiation of replication, involving DnaA-independent activation of the origin, and a dedicated primase, which is strictly host independent (Meyer, 2009).These plasmids are usually nonconjugative but are mobilizable by a variety of type IV transporters. Moreover, they have high copy number and exhibit reduced metabolic load. The studies have been carried out mostly on RSF1010, R300B, and R1162, which are nearly identical plasmids, isolated from *E. coli* strain 3, *Salmonella enterica* serovar Typhimurium, and *Pseudomonas aeruginosa,* respectively.

Plasmid RSF1010 (8684 bp) contains three novel genes: *repA*, *repB*, and *repC*. The product of the *repA* gene has ssDNA-dependent ATPase and DNA helicase activity (Scherzinger *et al.*, 1997), *repC* product binds to the iterons and opens the origin region, creating the entry site for the RepA helicase (Scherzinger *et al.*, 1991), and the *repB* product encodes a primase. *In vivo*, a 2.1-kilobase segment of the plasmid, bearing the replication origin, can establish itself as an autonomous replicon if the DNA region carrying the three rep genes is present in the same cell on an independent plasmid (Scherzinger *et al.*, 1984). Thus, BHR plasmids belonging to the IncQ incompatibility group encode three replication proteins that obviate the need for host proteins.

Another example of initiator protein, DnaA, independent replication is shown by IncP plasmids. The initiation events of replication of RK2 in different unrelated bacteria were examined by cloning the genes encoding for the replicative helicase, DnaB, of *Pseudomonas putida* and *Pseudomonas aeruginosa*. The respective purified proteins were tested for activity along with *E. coli* DnaB at RK2 oriV. It was found that all three helicases could be recruited and activated at the RK2 origin in the presence of the host-specific DnaA protein and the TrfA protein. *Escherichia coli* or *P. putida* DnaB was active with either TrfA-33 or TrfA-44 (TrfA is expressed as 33 and 44 kDa forms), while *P. aeruginosa* DnaB required TrfA-44 for activation, suggesting that the two forms are used variably in different hosts (Caspi *et al.*, 2001). The homologs of TrfA44 and TrfA33 identified in other IncP1 plasmids are referred as TrfA1 and TrfA2, respectively. It was demonstrated that TrfA2 and origin were sufficient to confer broad host range to IncP1 plasmids. TrfA1 on the other hand was found to have no role on the long-term persistence, but its presence led to enhanced transformation efficiency and plasmid copy number (Yano *et al.*, 2012). Moreover, for the *Pseudomonas* spp. helicases, the host DnaA protein was found to be essential for helicase complex formation and activity at oriV (Konieczny & Liberek, 2002). Thus, the helicase loading mechanism is adapted to the genetic background of the specific host bacterium (Caspi *et al.*, 2001).

Plasmid-encoded helicase called PcrA have been reported from Gram-positive bacteria also. They are essential helicases and are required for the RC replication of small multicopy plasmids (Anand *et al.*, 2004). The *pcrA* gene originally identified as being required for plasmid pT181 replication has been identified in all the Gram-positive bacteria whose genomes have been sequenced so far with an exception of plasmid pC194. The latter has been shown to replicate although at a lower copy number, in *E. coli* and in this host, its replication is supported by DNA helicase II (UvrD) that has 40%

identity to PcrA. It has been reported that interaction between the plasmid initiator proteins and heterologous PcrA helicases may be critical in establishing RC plasmids in different hosts.

Anand *et al.* (2004) have demonstrated that heterologous PcrA helicases from *Bacillus anthracis* and *Bacillus cereus* are capable of unwinding *Staphylococcus aureus* plasmid pT181 from the initiation-generated nick and promoting *in vitro* replication of the plasmid. These helicases interact with the RepC initiator protein of pT181. The ability of PcrA helicase to unwind noncognate RC plasmids may contribute to the broad host range replication and dissemination of RC plasmids in Gram-positive bacteria.

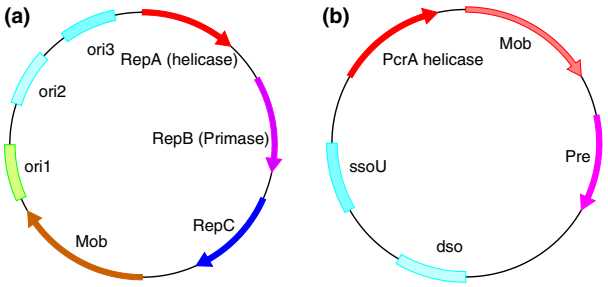

Fig. 1. Idealized depiction of a broad host range plasmid originating from a Gram-negative bacteria (a) and Gram-positive bacteria (b). The genes *repA*, *repB,* and *repC* encode for the helicase, primase. and initiator protein. The single-stranded origin ssoU and the PcrA helicase is highlighted, which confers broad host range to Gram-positive bacteria.

Other features

It was observed that BHR IncP plasmids contain few sites for restriction enzymes when compared with narrow host range plasmids, and this has been suggested as a benefit as they can easily overcome the restriction barrier of the host cells (Meyer *et al.*, 1977). Results from the BHR IncQ and IncPα plasmids indicate that increase in copy number can be permitted in some hosts but not in others (Haugan *et al.*, 1995). The topology of the plasmids is also not the same in different hosts. For example, the supercoiling density of plasmids replicating in *Bacillus subtilis* seems to be lower than in other Gram-positive bacteria (Novick *et al.*, 1986).

Conjugation also plays an important role in the transfer of plasmids. Conjugative plasmids are self-transmissible. They carry the genes necessary for transfer initiation at origin of transfer (*OriT*) called MOB genes (also called Dtr genes, for DNA transfer replication) and mating-pair apparatus formation (Zatyka and Thomas, 1998). However, a large group of plasmids are nonself-transmissible, but they can be mobilized via a mating apparatus provided by a self-transmissible plasmid (Smillie *et al.*, 2010). An extensive review article from the group of de la Cruz details the entire process of plasmid transfer via conjugation and host range (Smillie *et al.*, 2010).

Thus, the presence of one or more of the above-listed features in a plasmid is likely to impart broad host range capabilities to it (Fig. 1a and b). However, there are certain plasmids, which have broad host range, but the reasons are not known. Plasmid pCR1 and pCR2 isolated from *Corynebacterium renale* can replicate in *E. coli* (Srivastava *et al.*, 2006; Walia *et al.*, 2007). Similarly, a 640-bp minimal replicon obtained from the plasmid pool from *Acidothiobacillus ferroxidans* has been shown to replicate in a number of bacteria (Kalyaeva *et al.*, 2002).

In an attempt to predict the host range of plasmids, genomic signature method was developed. Trinucleotide composition of the plasmid, which is often similar to the chromosome of the current host, was compared with all completely sequenced bacterial chromosomes. The method was validated by testing on plasmids with known host range. It was found that in the case of IncW, IncP, IncQ, and PromA family of plasmids, the signatures were not similar to any of the chromosomal signatures suggesting that these plasmids have not been ameliorated in any host due to their promiscuous nature (Suzuki *et al.*, 2010).

Molecular basis of broad host range plasmid evolution

Plasmids have been reported to adapt and evolve in an otherwise unfavorable host. The stability of plasmid pB10 (64.5 kb), originally isolated from a wastewater treatment plant in Germany (Schluter *et al.*, 2003), was compared among 19 strains within the *Alpha-*, *Beta-* or *Gammaproteobacteria*. Ten strains showed no detectable plasmid loss over 200 generations, two strains showed plasmid-free clones only sporadically, and three strains exhibited rapid plasmid loss within 80 generations. Mathematical modeling was carried out, and it was suggested that the variations over time could be due to compensatory mutations (De Gelder *et al.*, 2007). To investigate whether the same plasmid can adapt to unfavorable hosts, evolution experiments were performed, and it was suggested that regular switching between distinct hosts hampers adaptive plasmid evolution. The complete genome sequences of four evolved plasmids where true host range expansion was observed revealed a point mutation in *trbC* gene that encodes for a putative prepilin involved in mating-pair formation. Thus, it was shown that a BHR plasmid can adapt to an unfavorable host and thereby expand its long-term host range (De Gelder *et al.*, 2008). Interestingly, the same authors revealed that host range of pB10

within an activated-sludge microbial community was significantly influenced by the type of donor strain (De Gelder *et al.*, 2005). In a study by Sota *et al.* (2010), evolutionary experiments were carried out using IncP1 mini replicon in four different hosts, where the plasmid was reported to be unstable. After 1000 generations, it was found that stability was improved in all the coevolved hosts and in only one case in the ancestral host. Sequencing results showed mutations at the N terminus region of TrfA (Sota *et al.*, 2010). To gain deeper insight, colony PCR and pyrosequencing were carried out on randomly selected colonies up to several generations. Several new mutations in TrfA were observed after 200 generations in novel hosts, but after 1000 generations, only one or two genotypes dominated the population. Thus, clonal interference that is a competition between coexisting hosts with different plasmid genotypes was shown to play an important role in plasmid host adaptation (Hughes *et al.*, 2012). Homologous recombination has been proposed to play an important role in plasmid evolution in the case of IncW (Fernandez-Lopez *et al.*, 2006) and IncP1 (Norberg *et al.*, 2011) and F (Boyd *et al.*, 1996). In yet another study, single amino acid change in RepA of the narrow host range plasmid pPS10 or in *E. coli* DnaA resulted in expansion of plasmid's replication range (Fernandez-Tresguerres *et al.*, 1995; Maestro *et al.*, 2002, 2003). Studies on plasmid host evolution are limited, and more studies are needed in this direction for predicting the expansion or contraction or shift in the host range of plasmids.

Implications of broad host range plasmids to recombinant DNA technology

The recombinant DNA revolution began in *E. coli* and evolved rapidly because the well-studied plasmids (ColE1, p15A, and pSC101) and bacteriophage needed little modification for use as recombinant DNA vectors. The vectors, however, have narrow host range. Using broad host range replicons as the basis of cloning, vector development has the advantage that the cloning may be performed by standard techniques in *E. coli*, which is easy to manipulate, and the recombinant plasmid is subsequently transferred to different experimental hosts, usually by conjugation or electroporation. Historically, three main types of broad

Table 2. Vectors constructed using replicons from broad host range plasmids

Name of replicon	Name of vector constructed	Type of vector	Antibiotic resistance	Size (kb)	Reference
RK2	pRK290	Cloning vector	Tc	20	Ditta *et al.* (1985)
RK2	pLAFR1	Cosmid vector	Tc	21.6	Vanbleu *et al.* (2004)
RK2	pLAFR5	Cosmid vector with two cos sites	Tc	21.5	Keen *et al.* (1988)
RK2	pRS44	Cloning vector	Cm, Km	10.3	Aakvik *et al.* (2009)
RK2	pJB137	Expression vectors	Ap	7.6	Blatny *et al.* (1997)
	pJB653		Ap	7.0	
RK2	pGNS-BAC-1	BAC vector	Cm	11.9	Kakirde *et al.* (2011)
RK2	pFAJ1700	Expression vector	Ap, Tc	10.5	Dombrecht *et al.* (2001)
RSF1010	pDSK509	Cloning vector	Km	9.3	Keen *et al.* (1988)
RSF1010	pKT210	Cloning vector	Cm	11.8	Bagdasarian *et al.* (1981)
RSF1010	pAYC32	Cloning vectors	Ap, Sm	9.7	Chistoserdov & Tsygankov (1986)
	pAYC39		Ap, Sm, Tc		
	pAYC51/52	Cosmid vector	Ap, Sm, cos	11.3	
RSF1010	pJFF224-NX	Expression vector	Cm	7.8	Frey (1992)
RSF1010	pQLacZ1	Cloning vector	Sm, Sp	17.1	O'Sullivan *et al.* (2010)
	pQLacZ2		Sm, Sp	17.9	
	pQLacZ3		Cm	16.1	
RSF1010	pHRP309	Promoter probe vector	Gm	12	Parales & Harwood (1993)
pBBR1	pBHR1	Cloning vector	Cm, Km	5.3	Szpirer *et al.* (2001)
pBBR1	pBBR1MCS2	Cloning vector	Km	5.1	Kovach *et al.* (1995)
	pBBR1MCS3		Tc	5.2	
	pBBR1MCS4		Ap	4.9	
	pBBR1MCS5		Gm	4.7	
R300B	pGSS33	Cloning vector	Ap, Cm, Tc, Sm	13.4	Sharpe (1984)
R300B	pDG105	Cloning vector	Km	9.0	Gambill & Summers (1985)
R300B	pHT128	Cloning vector	Cm, Tc	12.5	Takahashi & Watanabe (2002)
pWVO1	pBAV1K-T5	Expression vector	Km	8.6	Bryksin & Matsumura (2010)

host range replicons have been popular for vector construction: RK2 (IncP), RSF1010 (IncQ), and pSa (IncW). Currently, pBBR1 and pWVO1 which can replicate in a wide variety of Gram-negative hosts and Gram-negative bacteria (Davison, 2002) are also used for the same purpose. A few vectors constructed using the replicons obtained from BHR plasmids are listed in Table 2.

Impact of broad host range plasmids in Environment

Mobile broad host range plasmids significantly contribute to the dissemination of beneficial genetic traits among the population of a certain species and even beyond species boundaries. These include the resistance to antibiotics, metals (Smalla *et al.*, 2006), quaternary ammonium compounds (QACs), and triphenyl methane dyes such as crystal violet, malachite green, and basic fuchsin. (Schluter *et al.*, 2007), degradation of herbicides such as 2,4-dichlorophenoxyacetic acid (2,4-D), atrazine, haloacetate, p-toluene sulfsonate, chlorobenzoic acid etc. (Don & Pemberton, 1981, 1985). It was found that wastewater treatment plants are reservoirs for BHR plasmids and from there, they are introduced into streams (Akiyama *et al.*, 2010). They serve as an important tool for interdomain or intergeneric gene transfer by conjugation. Plasmid RSF1010 has been seen as an example of an interdomain gene transfer agent. It can be transferred from *Agrobacterium tumefaciens* into plant cells in the presence of vir functions in *A. tumefaciens*. The transfer of RP1 between *Erwinia herbiola* or *P. syringae* donors and *Erwinia amylovora* recipient is an example of intergeneric gene transfer. Thus, BHR plasmids exhibit that gene pools of all the domains are interlinked. (Droge *et al.*, 1998).

Conclusions

Broad host range plasmids can replicate and stably maintain the genes they carry in taxonomically distant species. As such, they represent useful vectors for recombinant DNA technology. Here, we have consolidated several features that are likely to confer broad host range replication and maintenance capabilities to the plasmid. This will be helpful in ascertaining host range properties to the new plasmids. However, for some plasmids, the reasons are not so evident suggesting that there could be some unknown mechanisms governing the host range in those cases. The database of BHR plasmids is limited, and more studies are required. New plasmids from metagenomic studies or from individual cells would probably shed light on the conserved features and newer mechanisms operating related to host–plasmid interaction.

Tribute

Professor J.K. Deb (August 1, 1947–March 14, 2010) obtained his PhD in Chemistry from Banaras Hindu University, India. He did his postdoctoral training at Baylor College of Medicine, Houston, USA. He also worked as a postdoctoral fellow in University of Wisconsin Madison, Madison, USA. He returned to India in 1983 when he joined the Department of Biochemical Engineering and Biotechnology, Indian Institute of Technology, Delhi, India. He produced nine PhD students. As a teacher, he was admired by a vast majority of students. His area of interest was plasmid biology and developing corynebacterial expression systems. He was fascinated by the naturally occurring broad host range plasmids and wanted to continue research in that. This review article is dedicated to him for his contributions to science and for his inspiration to students.

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