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MINI-REVIEW

Extrachromosomal genetic elements in *Micrococcus*

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Abstract *Micrococci* are Gram-positive G+C-rich, nonmotile, nonspore-forming actinomycetous bacteria. *Micrococcus* comprises ten members, with *Micrococcus luteus* being the type species. Representatives of the genus play important roles in the biodegradation of xenobiotics, bioremediation processes, production of biotechnologically important enzymes or bioactive compounds, as test strains in biological assays for lysozyme and antibiotics, and as infective agents in immunocompromised humans. The first description of plasmids dates back approximately 28 years, when several extrachromosomal elements ranging in size from 1.5 to 30.2 kb were found in *Micrococcus luteus*. Up to the present, a number of circular plasmids conferring antibiotic resistance, the ability to degrade aromatic compounds, and osmotolerance are known, as well as cryptic elements with unidentified functions. Here, we review the *Micrococcus* extrachromosomal traits reported thus far including phages and the only quite recently described large linear extrachromosomal genetic elements, termed linear plasmids, which range in size from 75 kb (pJD12) to 110 kb (pLMA1) and which confer putative advantageous capabilities, such as antibiotic or heavy metal resistances (inferred from sequence analyses and curing experiments). The role of the extrachromosomal elements for the frequently proven ecological and biotechnological versatility of the genus will be addressed as well as their potential for the development and use as genetic tools.

Keywords *Micrococcus* · Extrachromosomal elements · Plasmid · Linear plasmid · Phage

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Introduction

Micrococcus is the type genus of Micrococcaceae, a family of the order Actinomycetales. The genus comprises aerobic high G+C (66.4–75.5 %) Gram-positive bacteria, which are nonmotile and do not form spores (Baird-Parker 1974). The spherical cells, usually arranged as tetrads, are catalase- and oxidase-positive (Ohama et al. 1990). Since the first description of the taxon by Cohn in 1872, it has repeatedly been revised (Kokur et al. 1975; Stackebrandt et al. 1995) with the last amended and still valid classification done by Wieser et al. (2002). Accordingly, the ten *Micrococcus* species are: *M. luteus* (the type species of the genus, with three biovars; Cohn 1872; Wieser et al. 2002), *M. lylae* (Kloos et al. 1974; Wieser et al. 2002), *M. antarcticus* (Liu et al. 2000), *M. flavus* (Liu et al. 2007), *M. endophyticus* (Chen et al. 2009), *M. yunnanensis* (Zhao et al. 2009), *M. terreus* (Zhang et al. 2010), *M. lactis* (Chittpurna et al. 2011), *M. niistensis* (Dastager et al. 2010a), and *M. cohnii* (Rieser et al. 2012).

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M. luteus is considered to belong to the normal flora of the mammalian skin. However, a number of *Micrococcus* strains were routinely isolated from diverse habitats such as soil, dust, water, air, polar ice, mucous membranes, plants, cheese, sausages, activated sludge, industrial effluents, and sponges (Altalhi 2009; Antony et al. 2012; Chen et al. 2009; Bultel-Poncé et al. 1998; Dib et al. 2008, 2009a; García Fontán et al. 2007; Liu et al. 2000, 2007; Manikandan et al. 2011; Martins et al. 2012; Ordonez et al. 2009; Robertson and Perry 1961; Stierle et al. 1988; Wieser et al. 2002; Zhang et al. 2010; Zhao et al. 2009). *Micrococcus* members are capable of surviving in oligotrophic environments (Antony et al. 2012; Dib et al. 2008; Greenblatt et al. 2004); *M. luteus* was even detected in microbial surveys of the MIR and the ISS stations (Gu 2007). Though the *Micrococcus* species do not form spores, after prolonged incubation of stationary phase cultures, *M. luteus* is able to enter a latent state, forming morphologically differentiated nonspore dormant structures (Kaprelyants and Kell 1993; Kaprelyants et al. 1993, 1996; Mukamolova et al. 1995; 1998; 2006; Votyakova et al. 1994). Such state of low metabolic activity (Kaprelyants et al. 1993) apparently permits survival for long periods of time under environmental conditions being adverse for growth. The isolation of a *M. luteus* strain from a 120-million-year-old amber sample (Greenblatt et al. 2004) is suited to underscore the bacterium's adaptiveness to nutrient-lacking extreme environments (Dib et al. 2008; Ordonez et al. 2009).

Even though *Micrococcus* spp. are considered to be harmless, nonpathogenic, commensal organisms (risk class 1, Approved List of Biological Agents 2004. Advisory Committee on Dangerous Pathogens. Health and Safety Executive, UK, http://www.hse.gov.uk/pubns/misc208.pdf), some may cause valve endocarditis, folliculitis, bacteremia, meningitis, pneumonia (Adang et al. 1992; Miltiadous and Elisaf 2011; Yap and Mermel 2003; Souhami et al. 1979), and even fatal infections (Shanks et al. 2001) as nosocomial pathogens particularly in immunocompromised people such as HIV patients. The genus proved to be a source of useful compounds for biotechnological applications. Industrially relevant enzymes have been purified and characterized, such as glutaminases (Chantawannakul et al. 2003; Nandakumar et al. 1999; Yano et al. 2006; Yoshimune et al. 2006), esterases (Akita et al. 2001; Imura et al. 1999; Maruyama et al. 2005), proteases (Clark et al. 2000; Manikandan et al. 2011; Mohedano et al. 1997), and amylases (Fan et al. 2009). In different varieties of soft and hard cheeses, *Micrococcus* strains appeared to be main components of the secondary microflora, playing an important role during ripening and the development of the final organoleptic qualities (Addis et al. 2001; Prado et al. 2001; Robertson and Perry 1961). *Micrococcus* strains are important for degradation and bioremediation processes; they can catabolize a wide range of uncommon and harmful compounds such as pyridines, insecticides, herbicides, dyes, polycyclic aromatic hydrocarbons, polyacrylonitrile polymers, phthalate esters, and chlorinated biphenyls (Bevinakatti and Ninnekar 1993; Doddamani and Ninnekar 2001; Du et al. 2011; Eaton and Ribbons 1982; El-Sayeda et al. 2005; Fischer-Colbrie et al. 2007; Mulla et al. 2011; Rajee and Patterson 2011; Saratale et al. 2009; Sims et al. 1986; Tallur et al. 2008; Zheng et al. 2009; Zhuang et al. 2003a, b). *M. luteus* cells are capable of accumulating heavy metals from poor ore (Levchenko et al. 1997; Levchenko et al. 2002) and they can remove strontium and copper from aqueous solutions (Faison et al. 1990; Wong et al. 2001).

A *M. luteus* strain with antagonizing activities for *Aeromonas hydrophila*, a pathogen for *Oreochromis niloticus*, displayed probiotic effects in vitro and in vivo for the Nile tilapias as it enhanced the growth performance and resistance of the fish against *A. hydrophila* infections (Abd El-Rahman and El-Bana 2006). *M. luteus* strains are used as test strains to assay the presence of antibacterial compounds such as lysozyme and antibiotics (Stelzner et al. 1982; https://www.dsmz.de/catalogues/details/culture/dsm-20030.html; http://www.dsmz.de/catalogues/details/culture/DSM-1790.html). On the other hand, there are *Micrococcus* strains capable of producing antibiotics or other antibacterial compounds like neoberninamycin, 2,4,4′-trichloro-2′-hydroxydiphenylether, limazepines, and micrococcin (Biskupiak et al. 1988; Bultel-Poncé et al. 1998; Fotso et al. 2009; Jayaprakash et al. 2005; Kim et al. 2006) as well as biofuel value products such as long-chain alkenes (Albro and Dittmer 1970; Beller et al. 2010; Young et al. 2010). A soil-borne strain (NII-0909) was shown to produce multiple plant growth enhancers and indeed proved its potential as a biofertilizer (Dastager et al. 2010b). Only quite recently, the genome of *M. luteus* (the Fleming strain, NCTC 2665, DSM 20030T, and ATCC 4698), was sequenced and annotated; it consists of a single circular chromosome spanning 2,501,097 bp (G+C content, 73 %) encoding 2,403 predicted proteins (Young et al. 2010).

Extrachromosomal genetic elements may well contribute to the successful colonization, adaptation, and persistence in extreme habitats as well as to the tolerance to heavy metals and/or toxic organic compounds as known for other bacteria such as *Streptococcus thermophilus* (Solow and Somkuti 2001), *Streptomyces* spp. (Ravel et al. 1998), or *Pseudomonas putida* (Molina et al. 2011). Here, we summarize current knowledge on the extrachromosomal elements (including circular plasmids, phages, and linear plasmids) found in this ecologically and biotechnologically important genus.

Circular resistance plasmids

Although *Micrococcus* species are considered to be harmless for healthy individuals, antibiotic resistance determinants can well be of clinical importance as these traits may

be transferred to only distantly related harmful bacteria. In fact, there are *Micrococcus* plasmids that were demonstrated to be transferable and replicable in *Streptomyces* as well as in *Escherichia coli*, concomitantly suggesting a natural intrinsic potential as shuttle vectors in gene technology; see below for details (Dhanarani et al. 2009; Verma et al. 1989, 1993). Moreover, a *M. luteus* strain isolated from the skin of a patient suffering from acne was found to host a mobile genetic element conferring resistance to erythromycin (encoded by *mefE*) that could be transferred via conjugation to *Enterococcus faecalis* (Luna et al. 1999).

Several strains isolated from high-altitude Andean lakes in Argentina displayed a multiple antibiotic resistance phenotype (including macrolides) along with the resistance to arsenate and arsenite (Dib et al. 2008). Eady et al. (2000) described *M. luteus* strains with multiple antibiotic resistance, and all of the 32 isolates were reported to be erythromycin resistant. In a phenotypic analysis of 14 *Micrococcus* strains from a strain collection in Venezuela (Atencio et al. 2009), it was found that all of them exhibited a multiresistance phenotype (three or more antibiotics, including erythromycin, rifampicin, vancomycin, and carbenicillin) linked to the presence of a plasmid of approximately 23 kb in size. A number of cells of another isolate that was shown to tolerate high concentrations of cadmium, lead, mercury, benzylpenicillin, ampicillin, tetracycline, erythromcycin, carbenicillin, and sulfadiazine lost part of their resistance (cadmium, ampicillin erythromycin, carbenicillin, and tetracycline) when cultivated in rich liquid medium at 44 °C (Bhattacharyya et al. 1988), which agrees with the assumption that extrachromosomal genetic elements conferred the latter capabilities.

The 10-kb plasmid pMQV10 of *M. luteus* RJ6 (Table 1) was proven to confer streptomycin resistance to the bacterium as well as the ability to degrade cholesterol and phytosterols (Verma et al. 1989, 1993). The same authors were able to transform *E. coli* and *Streptomyces ambofaciens* with the unmodified pMQV10. In *E. coli*, the plasmid was stably maintained at low copy number, and in *S. ambofaciens*, it was apparently integrated into the chromosome. However, irrespective of its location, the element conferred the antibiotic resistance to both heterologous hosts (Verma et al. 1989, 1993). Expectedly, retransformation of a cured derivative of *M. luteus* RJ6 with pMQV10, restored the cholesterol degradation ability as well as the streptomycin resistance (Dogra and Qazi 1999). Based on some physiological properties, strain RJ6 was considered to belong to *Micrococcus roseus* (Dogra and Qazi 2001). Thus, it might happen that upon thorough classification, the strain must indeed be excluded from the genus. Further evidence for plasmid-borne resistance exists for *Micrococcus* sp. (M-36) which became nalidixic acid sensitive upon elimination of a plasmid that was cured by SDS treatment (Guha et al. 1997).

With respect to the antibiotic resistance, the rather small pMEC2 (Fig. 1) is the most thoroughly studied micrococcal plasmid. The circular molecule, only 4.2 kb in size, was originally detected in *M. luteus* MAW843 that was isolated from human skin (Liebl et al. 2002). As for other erythromycin-resistant bacteria, the strain also displayed resistance to other macrolides and lincosamin antibiotics. Ethidium bromide-mediated curing of pMEC2 resulted in erythromycin sensitivity and, consistently, introduction of pMEC2 into another *M. luteus* strain conferred the antibiotic resistance to the new host. The erythromycin resistance was found to be inducible and it was possible to express the gene heterologously in *Corynebacterium glutamicum* using a shuttle plasmid construction (Liebl et al. 2002). The resistance determinant spans a region of about 1 kb. Sequence analyses identified an open reading frame (ORF) designated as *erm*(*36*) encoding a predicted protein with similarities to the 23S rRNA adenine N^6-methyltransferases from other high-G+C Gram-positive bacteria such as *Corynebacterium*, *Bifidobacterium*, *Mycobacterium*, and *Streptomyces*. As for the erythromycin-resistance determinant of *Staphylococcus aureus* (*ermC*) (Weisblum 1984, 1985), its expression is inducible. However, the regulation mechanism of *erm*(*36*) differs and remains to be elucidated. Because of its small size and its already proven versatility as part of a shuttle plasmid, pMCE2 appears to be a rather promising candidate as a cloning device and gene vehicle.

By acridine orange-mediated curing, Thavasi et al. (2007) reported that the loss of a plasmid of approximately 2.8 kb from a marine *M. luteus* was accompanied by the loss of penicillin, ampicillin, tetracycline, amoxicillin, kanamycin, and chloramphenicol resistance (Table 1). Similarly, a 5.1-kb plasmid from a *Micrococcus* strain isolated from poultry litter conferred multiple antibiotic resistance (kanamycin, tetracycline, erythromycin, ampicillin, tobramycin, streptomycin, rifampicin, and chloramphenicol) that was transferable. Curing approaches and transformation experiments with the isolated plasmid (or conjugation with *E. coli*) resulted in the loss of the antibiotic resistance phenotypes in the plasmid-free cured strains and the expression of such determinants in the new hosts, respectively (Dhanarani et al. 2009).

Circular degradative plasmids

Degradative plasmids carry genes that confer on their host bacteria the ability to degrade xenobiotics, compounds not routinely found in nature (definition according to the plasmid biology book, Ogawa et al. 2004). Earliest reports dealing with prokaryotic degradative plasmids were about diverse *P. putida* strains (Dunn and Gunsalus 1973; Rheinwald et al. 1973; Williams and Murray 1974). It took more than 10 years

Table 1 Circular plasmids in *Micrococcus*

Strain[a]	Isolation source	Designation	Size (kb)	GenBank accession number	Associated phenotype(s)	HGT[g] recipients	Reference(s)
M. luteus strains	n.d.	NN (9 plasmids)	1 to 30.2	–	Cryptic	n.d.	Mathis and Kloos (1984)
Micrococcus sp.[b]	n.d.	NN	n.d.	–	Multiple antibiotic resistance, heavy metals resistance	n.d.	Bhattacharyya et al. (1988)
M. luteus RJ6	n.d.	pMQV10	10	–	Streptomycin resistance. Cholesterol degradation	*E. coli* and *Streptomyces ambofaciens* (ATCC 23877)	Verma et al. (1989, 1993)
Micrococcus sp. M-36	Soil	NN	n.d.	–	Degradation of malathion and chlorpyrphos. Nalidix acid resistance	n.d.	Guha et al. (1997)
Micrococcus sp. AG-43	Soil	NN	n.d.	–	Degradation of malathion and chlorpyrphos	n.d.	Guha et al. (1997)
M. luteus 28	Costal marine sediments	pSD10	50.7	AY034092	Transposases, replication genes	n.d.	Zhong et al. (2002)
M. luteus MAW843	Human skin	pMEC2	4.2	AF462611[d]	Macrolide and lincosamide resistance	n.d.	Liebl et al. (2002)
M. luteus NCIMB 13267[c]	Soil	pMLU1	2.3	AJ439695	Cryptic	n.d.	Mukamolova et al. (2002)
Micrococcus sp. 9	Lake water	NN	2.7	–	Osmotolerance	n.d.	Lobova et al. (2005)
M. luteus	Estuary water and sediments	NN	~2.8	–	Multiple antibiotic resistance[e]	n.d.	Thavasi et al. (2007)
Micrococcus sp.	Poultry litter	NN	5.1	–	Multiple antibiotic resistance[f]	*E. coli* DH5α	Dhanarani et al. (2009)
Micrococcus sp. 11	Stems and leaves from grapevine	NN	>50	–	n.d.	n.d.	Altalhi (2009)
Micrococcus sp.	Petroleum-contaminated soils	NN	~64	–	Hydrocarbon biodegration	n.d.	Mirdamadian et al. (2010)

n.d. not described, *NN* unnamed

[a] Mathis and Kloos (1984) and Labuzek et al. (1994) have described plasmid-carrying *Micrococcus* species but as the genus *Micrococcus* has been emended (Stackebrandt et al. 1995; Wieser et al. 2002), they have not been included in this review

[b] Although plasmid screenings were not performed for this strain, results suggest the presence of plasmids conferring resistance to cadmium, ampicillin, erythromycin, carbenicillin, and tetracycline

[c] The Fleming strain 2665

[d] Partial sequence available

[e] Kanamycin, tetracycline, erythromycin, ampicillin, tobramycin, streptomycin, rifampicin, and chloramphenicol

[f] Penicillin, ampicillin, tetracycline, amoxycillin, kanamycin, and chloramphenicol

[g] Horizontal gene transfer

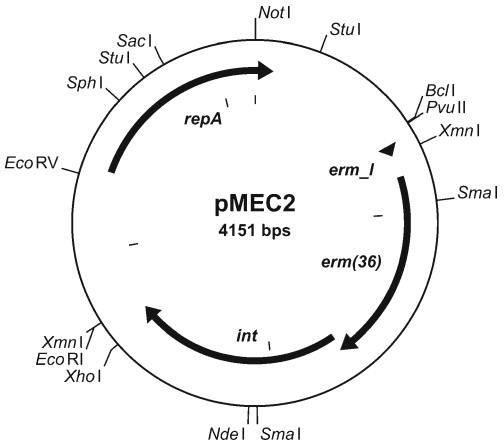

Fig. 1 Map of the micrococcal circular plasmid pMEC2, GenBank Accession Number: KC005723. Four ORFs are indicated as *arrows*: the gene *erm*(*36*) encodes a protein with similarity to 23S rRNA adenine N^6-methyltransferases and confers resistance to macrolides and lincosamin. The short ORF *erm_l* may be involved in the regulation of *erm*(*36*) expression (Liebl et al. 2002). The translation product of the 1.0 kb ORF *int* is similar to integrase family proteins and putative transposases of *M. luteus* and other high G+C Gram-positive bacteria. The product of *repA* can be assigned to the replicase protein family and is proposed to be the DNA replication initiator protein of pMEC2

until *Micrococcus* species were described with similar capabilities to metabolize a broad range of such substrates, including pyridines, insecticides, herbicides, dyes, and biphenyls (Bevinakatti and Ninnekar 1993; Doddamani and Ninnekar 2001; Du et al. 2011; El-Sayeda et al. 2005; Mulla et al. 2011; Saratale et al. 2009; Sims et al. 1986; Tallur et al. 2008; Zheng et al. 2009). Degradation of the insecticides malathion and chlorpyriphos by two strains (M-36 and AG-43) was attributed to plasmids as cured derivates (by acridine orange or SDS) concomitantly lost such quality and, in addition, *Micrococcus* sp. M-36 lost its nalidixic acid resistance (Guha et al. 1997).

As mentioned, *Micrococcus* sp. RJ6 possesses the plasmid borne capacity to degrade cholesterol. Side chain degradation yields androsta-1,4-diene-3,17-dione and androstenedione serves as an important starting material for manufacturing steroidal drugs. The strain was found to carry the 10-kb low copy number plasmid pMQV10 (Verma et al. 1989). Curing experiments revealed that besides the antibiotic resistance, the plasmid is also involved in cholesterol degradation. However, transformation of *E. coli* or *Streptomyces* species, though it established the antibiotic resistance, failed to transfer the cholesterol degradation capability, but a cured derivative (RJC6) regained the attribute when the plasmid was reintroduced (Dogra and Qazi 1999).

Besides the fact that *E. coli* lacks a cholesterol transport system, the degradation of the lateral chain is rather complex (Harder and Probian 1997; Kieslich 1985). Considering also the small size of pMQV10, it is hardly imaginable that the plasmid confers such capability. Plasmid pMQV10 might encode one step of the cholesterol degradation only, such as the cholesterol oxidase, the first enzyme in cholesterol catabolism (Kieslich 1985). In addition, the adaptation of translational signals (such as the Shine/Dalgarno sequence) and/or codon usage or transcriptional signal sequences (such as the −35 and −10 regions and spacing to enhance the promoter strength) may help to solve expression issues and clear the way to develop a more versatile shuttle vector for *Micrococcus* and *E. coli*.

In a screening/enrichment procedure for dibutylphthalate degradation, a *Micrococcus* strain (12B) capable of growing with a number of phthalate esters as the sole carbon and energy source was identified (Eaton and Ribbons 1982). Cultivation at elevated temperatures yielded at high frequency nonrevertible mutants that lost their capability to grow on phthalate esters. Though there was no analysis in terms of extrachromosomal elements, it is conceivable that plasmids were eliminated by the high temperature as for other plasmid-carrying *Micrococcus* strains (Bhattacharyya et al. 1988; Dib et al. 2010a). Similarly, a *Micrococcus* strain isolated from petroleum-contaminated soil carrying a plasmid of approximately 64 kb in size was cured by serial subcultivations in nutrient broth, concomitantly leading to the loss of its hydrocarbon degradation capability, thus demonstrating such capacity to be plasmid-associated (Mirdamadian et al. 2010).

Since there are a number of *Micrococcus* strains metabolizing unusual substrates, such as malachite green (Du et al. 2011), polyacrylonitrile polymers (Fischer-Colbrie et al. 2007), naphthalene (Zhuang et al. 2003a), benzoic acid (Muthukumar et al. 2009), and cypermetrin (Tallur et al. 2008), it would be worth checking whether such qualities are conferred by extrachromosomal elements.

Circular osmotolerance and transposase-rich plasmids

In the moderately halotolerant *Micrococcus* sp. 9, isolated from a brackish lake in Russia (Lobova et al. 2005), the approximately 2.7-kb plasmid pSH1 was found. Elevated NaCl concentrations led to an increase in the copy number of pSH1 in a way that it was directly correlated to the actual NaCl concentration, eventually facilitating growth in the presence of 20 % NaCl, thereby proving the plasmid's role for the host's osmotolerance. In fact, there are only few reports concerning extrachromosomal elements associated with osmotolerance, as for pSY10 (similar in size to pSH1) from the marine cyanobacterium *Synechococcus* sp. NKBG 042902. The copy number increased fivefold when the salinity of the growth medium increased from 0 to 3 % NaCl (Takeyama et al. 2000). While the latter example was definite for NaCl, the use, in a broad host range plasmid (pMJ101), of a specific mutant allele of *proBA* from *E. coli*

known to confer osmoprotection by overproducing proline as a compatible solute, conferred general osmotolerance to its host (Jakowec et al. 1985).

Another halophilic *Micrococcus* strain isolated from marine sediments (*M. luteus* 28) was found to harbor the 50,709-bp plasmid pSD10 (accession number: AY034092), the largest circular micrococcal plasmid sequenced thus far (Zhong et al. 2002). The G+C content of 68 % resembles that of its host and, in general, agrees with actinobacteria. The annotated sequence of pSD10 displays 51 ORFs. The plasmid is currently only known to encode a replication system and a remarkable number of transposable elements. In fact, the latter represent 25 % of the total sequence (approximately 13 kb), suggesting a high degree of genetic mobility. However, pSD10 apparently does not encode functions for self-transfer (conjugation) nor does it encode apparent valuable functions for its host.

Cryptic circular plasmids

The first report providing evidence for extrachromosomal traits in *Micrococcus* dealt with more than 50 elements ranging in size from 1.5 to 135.9 kb (Mathis and Kloos 1984). However, upon the mentioned repeated phylogenetic amendments of the genus (Wieser et al. 2002), only *M. luteus* and *M. lylae* were left behind as genuine species. Thus, nine plasmids with sizes from 1.5 to 30.2 kb were left over, found in only 20 % of the screened *M. luteus* representatives. Since antibiotic and heavy metal resistance profiles of the wild type strains could not be distinguished from plasmid-free cured derivatives, such elements still remain cryptic.

pMLU1 (G+C 62.3 %) is apparently a cryptic plasmid of *M. luteus* NCIMB 13267 (the Fleming strain 2665) that closely matches (96 % nucleotide sequence identity) with plasmid pMBCP (accession number: AF144733) from *Ralstonia picketti*, a Gram-negative bacterium isolated from bovine duodenum. In the latter bacterium, the plasmid conferred a low-level cadmium resistance (Bruins et al. 2003). Surprisingly, however, when the *M. luteus* NCTC 2665 genome sequence became available (Young et al. 2010), no plasmid was reported, though the strain was previously reported to host pMLU1 (Mukamolova et al. 2002). Anyway, the chromosome contains type IV secretion genes encoding VirB4- and VirD4-like proteins (Young et al. 2010) known to be involved in plasmid replication or transfer.

Though there are several reports of circular micrococcal plasmids able to replicate in and confer, at least, parts of their phenotypes to the Gram-negative *E. coli*, a number of questions remain unsolved such as the function and the origin of pMLU1 that has a very high sequence similarity to pMBCP of the Gram-negative *R. picketti*. Generation of plasmid-free strains, conjugation approaches with different hosts and recipients as well as checking heavy metal resistance as for pMBCP, may contribute to provide answers.

Bacteriophages

First evidence for *Micrococcus* phages date back to 1956 (Burgi and Naylor 1956) and refer to UV-irradiated *M. lysodeikticus* (renamed *M. luteus*) cultures. In general, there are very few reports on micrococcocal phages, the youngest already 33 years ago (Compton et al. 1979). Altogether, only ten phages have been described in some detail. Morphologically, they have isometric and hexagonal heads and long, noncontractile, and flexible tails and belong, therefore, to the morphological group B1 (Ackermann and Eisenstark 1974). They have double-stranded DNA (dsDNA) genomes with high G+C contents (63.3–73.5 %) (Table 2) (Ackermann 1975; Burgi and Naylor 1956; Compton et al. 1979; Field and Naylor 1962; Lee and Davidson 1970; Lovett and Shockman 1970a, b; Peters and Pulverer 1975; Scaletti and Naylor 1959; Sozzi et al. 1973; Wetmur et al. 1966). Currently, phages N1, N3, N4, and N8 are available from the American Type Culture Collection (ATCC) in the host organism *M. luteus* (Schroeter) Cohn ATCC 4698. Regrettably, there is no phage that has been completely or partially sequenced. It seems that *Micrococcus* phages have lost attraction over the years despite the

Table 2 Bacteriophages in *Micrococcus* (modified from Compton et al. 1979)

Bacteriophage designation	Phage group[b]	G+C content, mol% as determined by		ATCC number
		Buoyant density in CsCl[c]	Chemical analysis[d]	
N1[a]	I	65.3	69.9	4698-B1
N2	I	64.3	71.6	n.a.
N3	II	68.4	72.2	4698-B4
N4	I	68.4	68.8	4698-B2
N5[a]	III	71.4	72.5	n.a.
N6	III	65.3	70.9	n.a.
N7	IV	73.5	73.6	n.a.
N8	V	68.4	71.6	4698-B3
W	V	68.4	69.9	n.a.
X	IV	n.d.	n.d.	n.a.

n.d. not determined, *n.a.* not available

[a] Type species

[b] Based on the host range and on the plaque-forming ability, these 10 *Micrococcus* phages were divided into six groups

[c] Methods according to Mandel et al. (1968)

[d] DNA bases separated by chromatography were indentified and quantified by ultraviolet spectroscopy

evident biotechnological relevance of the genus and, linked to it, the need for genetic tools.

Linear plasmids

Plasmids were originally defined as extrachromosomal covalently closed circular dsDNA molecules in prokaryotes (Lederberg 1952) and, indeed, linear extrachromosomal elements have long been unknown. It took about 25 years (with respect to Lederberg's definition) until the first linear plasmid, presumably not by chance, and somewhat contradictory to the above definition, in a eukaryote, was found in *Zea mays* (Pring et al. 1977). Soon after, the first bacterial linear plasmids were described in *Streptomyces rochei* (Hayakawa et al. 1979). The latter belongs to a class of genetic elements occasionally called invertrons (Sakaguchi 1990; Yang et al. 2002), which are characterized by proteins covalently attached to the 5′ ends of the linear DNA molecules (see also Fig. 2 and Meinhardt et al. 1986) and by the presence of inversely oriented sequence repetitions at both ends, the terminal inverted repeats (TIRs). Meanwhile, such genetic elements have been found in bacteria, plants, yeasts, and filamentous fungi (Griffiths 1995; Hinnebusch and Tilly 1993; Meinhardt et al. 1990; Rohe et al. 1992). In eukaryotes, there are mitochondrially localized linear elements, most of which being cryptic, whereas the cytoplasmic virus-like yeast elements (Satwika et al. 2012a), sometimes captured by the host nucleus (Frank and Wolfe 2009; Satwika et al. 2012b), may encode anticodon nucleases acting as killer toxins. Among the Actinomycetes, linear plasmids have been reported to exist in a number of *Streptomyces* spp., several Rhodococci and Mycobacteria, *Planobispora rosea*, the plant pathogen *Clavibacter michiganensis*, *Arthrobacter nitroguajacolicus*, *Brevibacterium* sp., and *Terrabacter* sp. (Dib et al. 2010b; Kalkus et al. 1993; Meinhardt et al. 1997; Overhage et al. 2005; Polo et al. 1998; Rose and Fetzner 2006; Stecker et al. 2003; for a monograph on microbial linear plasmids, see Klassen and Meinhardt 2007). Few of these elements have been characterized in some detail; current research particularly focuses on the replication machinery (Klassen and Meinhardt 2007; Kolkenbrock et al. 2010; Wagenknecht and Meinhardt 2011a, b; Tsai et al. 2012). Only quite recently, we have looked for plasmids in members of the genus *Micrococcus* isolated from high-altitude lakes (4,200–4,600 m) in the northwest of Argentina (extreme and

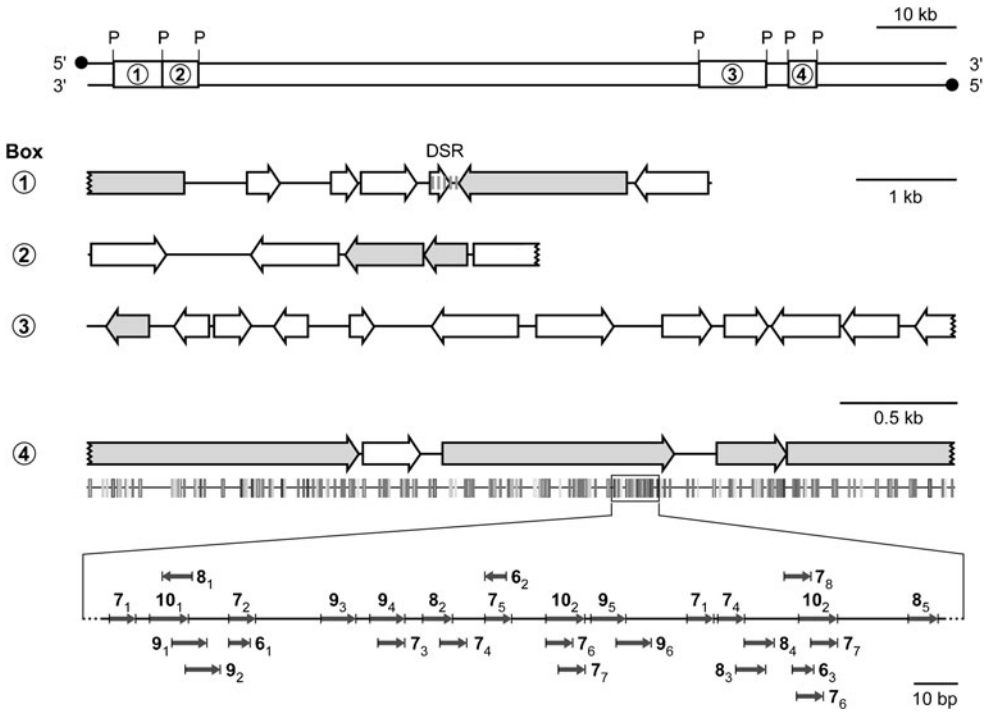

Fig. 2 Scheme of the micrococcal linear plasmid pLMA1. *Pst*I recognition sites (P) are indexed as such. The 5′-attached terminal protein is depicted as a *black dot*. The *consecutively numbered boxes* (1–4) represent the hitherto sequenced parts. In the *enlarged sections* below, predicted ORFs are shown as *arrows*. *Lacking arrowheads* and *zigzag lines* refer to truncated ORFs. *Gray arrows* indicate ORFs instrumental in transposition; for comprehensive information, we refer to Wagenknecht et al. (2010). The *vertical grey lines* in box 1 indicate five direct sequence repeats (DSR), considered to represent iterons as part of the replication origin. Short, repetitive sequences, a structural peculiarity of pLMA1, are exemplarily shown for box 4 indicated as *vertical lines* in different gray scales. Below, in an *enlarged section*, such sequences are depicted in more detail. Orientation and length of the sequence repeats are displayed by *gray arrows*. Repeat lengths are given by *bold numbers*. Identical repeats are earmarked by the *same subscript*

pristine environments, characterized by high UV radiation, oligothrophy, high arsenic concentration, and salinity). Unexpectedly, the isolated strains displayed multiple resistances to antibiotics (particularly macrolides) and, not equally surprising, to very high levels of UV radiation and arsenite (Dib et al. 2008, 2009a; Ordonez et al. 2009). Pulse field gel electrophoresis (PFGE) analyses identified five plasmids (Table 3), four of them differing in size: pLMA1 (110 kb), pLMH5 (110 kb), pLMV7 (90 kb), pLMA7 (80 kb), and pJD12 (75 kb) (Dib et al. 2009a, b, and own unpublished results). Since the migration of the plasmids remained constant relative to the marker under different PFGE conditions (switch time), the plasmids were considered to be linear rather than circular, as, depending on the switch time, the relative migration of circular plasmids changes in PFGE (Kalkus et al. 1990; Kinashi and Shimaji-Murayama 1991).

When the above five plasmids were purified from preparative pulse field gels and tested for their sensitivity to exonuclease III and λ exonuclease which hydrolyze dsDNA in 3′–5′ and 5′–3′ direction, respectively, all of them, as for pLMA1, pLMH5, and pLMV7 (Dib et al. 2010a, b), were degraded by exonuclease III but not by λ exonuclease. Such findings agree with other linear replicons from Actinobacteria and fungi known to have terminal proteins covalently attached to their 5′ DNA ends (Meinhardt et al. 1986). By checking the resistance profile of a cured plasmid-deficient derivative of *M. luteus* A1 (the host of pLMA1), we obtained evidence for the erythromycin resistance being plasmid encoded (Dib et al. 2010a).

Sequencing of the gel-purified pLMA1 by 454 Pyrosequencing (454 Life Sciences, http://www.454.com, Margulies et al. 2005) resulted in numerous exact reads but all efforts to assemble the data to generate large contigs failed. Eventually, it was a combination of the Sanger and the 454 technology applied to cloned pLMA1 fragments, covering altogether 23 kb of the total 110 kb, which allowed the identification of numerous sequence repeats varying in size, thus rendering an explanation for the failure to assemble the relatively short reads generated by 454 sequencing. Moreover, a high proportion of ORFs encoding proteins involved in transposition became evident. Besides the numerous irregularly spaced sequence repetitions, pLMA1 contains five regularly spaced repeats resembling iteron sequences, known from replication origins of linear plasmids (see Fig. 2 and Wagenknecht et al. 2010 for details). The determination of the entire sequences of pLMA1 and the other linear *Micrococcus* plasmids (pLMV7, pJD12, and pLMA7) by applying the Sanger method as well as the annotation of the ORFs is currently under way.

Conclusions

Micrococcus, the type genus of the Micrococcaceae, comprises a diverse group of soil bacteria with great industrial, environmental, and presumably also medical significance. The genes present on the plasmids can confer advantageous attributes to their respective hosts including antibiotic, and also heavy metal resistances, the ability to degrade xenobiotics or cholesterol, and osmotolerance.

Horizontal transfer and establishment of the *Micrococcus* plasmids in other Actinomycetes and also in rather distantly related bacteria, such as *E. coli* along with the routine appearance of transposable elements emphasize the apparent genetic plasticity. The well-characterized rather small pMEC2, which already proved its versatility in shuttle plasmid constructions, constitutes the most promising candidate for developing vectors for biotechnological purposes. However, there is still a high demand for proper genetic tools allowing efficient genetic work to take place. The recently discovered high G+C linear plasmids display a rather unique genetic composition as there are numerous sequence repetitions rendering it difficult to assemble the 454

Table 3 Linear plasmids in *Micrococcus*

Strain	Isolation source	Designation	Size (kb)[a]	GenBank accession number(s)	Associated phenotypes	HGT[d]	Reference(s)
M. luteus A1	Lake water	pLMA1	110	FN395369 FN692038-41[b]	Erythromycin resistance[c]	+	Dib et al. (2010a), Wagenknecht et al. (2010)
M. luteus H5	Lake water	pLMH5	110	–	n.d.	n.d.	Dib et al. (2010a)
M. luteus V7	Lake water	pLMV7	90	–	n.d.	n.d.	Dib et al. (2010a)
M. luteus A7	Lake water	pLMA7	80	–	n.d.	n.d.	Own unpublished results
Micrococcus sp. D12	Lake water	pJD12	75	–	n.d.	n.d.	Own unpublished results

n.d. not determined

[a] Approximately

[b] Partial sequences available

[c] Own unpublished results

[d] Horizontal gene transfer (own unpublished results)

sequencing data into contigs. Inferred from such findings and preliminary annotations, those elements seem to be very densely packed with numerous transposable elements (clearly exceeding the number in circular plasmids) which might, along with the capability for horizontal transfer, contribute to the necessary adaptiveness required to conquer very harsh environments, such as the high-altitude Argentinean lakes from which their hosts were isolated. The rather peculiar finding of numerous linear plasmid associated antibiotic resistances, again in association with the proven horizontal gene transfer, suspects the saprophytic *Micrococcus* strains and its extrachromosomal elements of constituting a reservoir of antibiotic resistance genes which can be transferred even to distantly related pathogenic bacteria. Ongoing research on both circular and linear extrachromosomal elements in *Micrococcus* will not only contribute to elucidate their role for the successful colonization of diverse harsh habitats but will also facilitate exploitation of this biotechnologically most interesting bacterial genus.

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