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Distribution of small native plasmids in *Streptococcus pyogenes* in India

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ABSTRACT

Complete characterization of a *Streptococcus pyogenes* population from a defined geographic region comprises information on the plasmids that circulate in these bacteria. Therefore, we determined the distribution of small plasmids (<5 kb) in a collection of 279 *S. pyogenes* isolates from India, where diversity of strains and incidence rates of *S. pyogenes* infections are high. The collection comprised 77 *emm*-types. For plasmid detection and discrimination, we developed PCRs for different plasmid replication initiation protein genes, the putative repressor gene *cop*G and bacteriocin genes *dys*A and *scn*M57. Plasmid distribution was limited to 13 *emm*-types. Co-detection analysis using aforementioned PCRs revealed four distinct plasmid sub-types, two of which were previously unknown. Representative plasmids pA852 and pA996 of the two uncharacterized plasmid sub-types were sequenced. These two plasmids could be assigned to the pMV158 and the pC194/pUB110 family of rolling-circle plasmids, respectively. The majority of small plasmids found in India belonged to the two newly characterized sub-types, with pA852- and pA996-like plasmids amounting to 42% and 22% of all detected plasmids, respectively. None of the detected plasmids coded for a known antibiotic resistance gene. Instead, all of the four plasmid sub-types carried known or potential bacteriocin genes. These genes may have influence on the evolutionary success of certain *S. pyogenes* genotypes. Notably, pA852-like plasmids were found in all isolates of the most prevalent *emm*-type 11.0. Together, *a priori* fitness of this genotype and increased fitness due to the acquired plasmids may have rendered type *emm*11.0 successful and caused the prevalence of pA852-like plasmids in India.

Introduction

The wide variety of infectious diseases that are caused by *Streptococcus pyogenes* ranges from uncomplicated superficial infections to severe invasive infections. Incidence rates of these infections and the mortality of invasive cases remain very high (Ralph and Carapetis, 2013). Moreover, *S. pyogenes* is a cause of severe immune sequelae (Chhatwal and Graham, 2008; Nitsche-Schmitz and Chhatwal, 2013).

High rates of lateral gene transfer occur in *S. pyogenes*. This is thought to be caused by the abundance of prophages and integrated conjugative elements in this species (Banks et al., 2002; Beres and Musser, 2007). The intraspecies horizontal exchange of these genetic elements is involved in the spread of virulence factors and antibiotic resistance, influencing the fitness of a strain. Interspecies horizontal gene transfer between *S. pyogenes* and related streptococcal species, such as *Streptococcus agalactiae* and both subspecies of *Streptococcus dysgalactiae* has also been reported (Beres and Musser, 2007; Davies et al., 2007a, 2007b, 2009; Franken et al., 2001; Rato et al., 2011; Stalhammar-Carlemalm et al., 1999). Plasmids are a further vector for the transmission of bacterial fitness factors such as antibiotic resistance genes and bacteriocins between streptococci. During the 1970s and 1980s several *S. pyogenes* plasmids were discovered that conferred erythromycin resistance to the bacteria (Table 1). To date, four natural plasmids of *S. pyogenes* have been completely sequenced and described (Table 1). Among them is the large, well characterized plasmid pSM19035 (GenBank: AY357120.1). This 28.9 kb plasmid encodes the *ermA* and *ermB*-genes that confer resistance to erythromycin to its host strain. The other three plasmids, pRW35 (Woodbury et al., 2008), pDN571 (Heng et al., 2004) and pDN281 (GenBank: AY995189.1) are less than 5 kb in size. Of these three small plasmids, pRW35 encodes the erythromycin resistance gene *ermT*. Plasmids pDN571 and pDN281 carry the bacteriocin genes *scnM57* or *dysA*, respectively. The *scnM57-gene* encodes streptococcin A-M57; a bacteriocin that has no bactericidal activity against *S. pyogenes* strains but against other Gram-positive bacteria (Heng et al., 2004). This suggests that *scnM57* has a role in streptococcal competition against other bacterial species. Dysgalacticin, which is encoded by *dysA*, kills *S. pyogenes* itself in a 'non-lytic' manner (Heng et al., 2006), suggesting that mode of action and function differ principally from streptococcin A-M57.

Plasmid-encoded fitness factors may contribute in shaping the population structure of bacteria in endemic regions. They may

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Table 1
Overview of known and newly identified *S. pyogenes* plasmids.

Prototype plasmid	Plasmid sub-type	Plasmid family	Factor	*rep* gene	Nucleotide sequence	Size		*emm* type	Reference
						kbp	Mol wt ($\times 10^6$)		
pA15	pA15	–	*erm*A, *erm*B	*rep*S	Partially	~19	–	19 different *emm* types	Liu et al. (2007)
	pA15-like (pA768)		*erm*A, *erm*B	*rep*S	No	~16	–	–	Liu et al. (2007)
pSM19035	pSM19035	–	*erm*A, *erm*B	*rep*S	Yes	28.98	18	–	GenBank: AY357120 (Ceglowski and Alonso, 1994)
	pSM22095		*erm*A, *erm*B	–	No	–	18	–	Malke (1974)
ERL1	ERL1	–	*erm*	–	No	–	19	–	Malke (1974)
pAC1 (pDC10535)	pAC1	–	*erm*	–	No	–	17	*emm*22	Clewell and Franke (1974)
pSM15346	pSM15346	–	*erm*	–	No	–	19	–	Malke (1974)
pSM10419	pSM10419	–	*erm*	–	No	–	15	–	Malke et al. (1981)
pRW35	pRW35	–	*erm*T	*rep2*	Yes	4.96	–	*emm*92 *emm*3 *emm*9 *emm*28	GenBank: EU192194 (Woodbury et al., 2008)
	pGA2000		*erm*T	*rep2*	Yes	4.96		–	GenBank: JF308631.1 (DiPersio et al., 2011)
pDN281	pDN281	pMV158	*dys*A	*rep*B	Yes	3.04	–	–	GenBank: AY995189
	pDN281-like		*dys*A	*rep*B	No		–	*emm*60 *emm*82 *emm*111 *st*11014	This study
pA852	pA852	pMV158	pA852_ORF3	repB	Yes	2.64	–	*emm*11	This study
	pA852-like		pA852_ORF3	repB	No		–	*emm*11 *emm*63 *emm*53 *emm*68	This study
pDN571	pDN571	pC194/pUB110	*scn*M57	*rep*A	Yes	3.35	–	*emm*57	GenBank: AY648561.1 (Heng et al., 2004)
	pDN571-like		*scn*M57	*rep*A	No		–	*emm*69 *emm*85	This study
pA996	pA996	pC194/pUB110	pA996_ORF5	*rep*A	Yes	3.62	–	*emm*44	This study
	pA996-like		pA996_ORF5	*rep*A	No		–	*emm*22 *emm*44 *emm*63 *emm*92	This study

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