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Characterization of plasmid pAW63, a second self-transmissible plasmid in *Bacillus thuringiensis* subsp. *kurstaki* HD73

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***Bacillus thuringiensis* subspecies *kurstaki* HD73, toxic for lepidopteran larvae, contains two large self-transmissible plasmids of approximately 75 kb, pHT73 and pAW63. The conjugative plasmid pHT73 has been studied extensively and has been shown to harbour the toxin gene *cry1Ac*, the transposon Tn*4430* and several insertion sequences. In this study it was demonstrated that the minor plasmid pAW63 is also self-transmissible and about 10–30 times more efficient in mobilizing plasmid pBC16. To facilitate direct selection for pAW63 transfer, the plasmid was tagged with the tetracycline resistance transposon Tn*5401* and in intraspecies matings it was found that after 2 h, all recipients had acquired a copy of the plasmid. Mating experiments demonstrated that pAW63 could be transferred to *Bacillus thuringiensis* subsp. *israelensis*, *Bacillus cereus*, *Bacillus licheniformis*, *Bacillus subtilis* and *Bacillus sphaericus*, and that the conjugative functions were expressed in these hosts. Hybridization studies showed that the replicons of pAW63 and pHT73 were distinct from one another. Sequences homologous to transposon Tn*4430* and several insertion sequences were, however, shown to reside on both plasmids.**

Keywords: *Bacillus thuringiensis*, HD73, self-transmissible plasmid, conjugation, mobilization

INTRODUCTION

Bacterial conjugation is a mechanism of genetic exchange that requires cell-to-cell contact and which is not susceptible to DNase present in the mating medium. Conjugation systems are encoded by large plasmids or by conjugative transposons (Clewell, 1993; Scott, 1993), which besides being capable of transferring themselves are able to co-transfer smaller mobilizable plasmids. Conjugation is a well-known process in Gram-negative bacteria with the F plasmid from *Escherichia coli* being particularly well studied. The cell-to-cell contact in Gram-negative bacteria is established by the sex pilus, which retracts and brings donor and recipient cells into close physical contact (Firth *et al*., 1996).

In Gram-positive bacteria, DNA transfer by conjugation has been found in an increasing number of species. In these bacteria cell-to-cell contact is not mediated by pili, but other, still scarcely characterized surface structures are involved. Recently, several Gram-positive systems capable of sustaining DNA transfer in liquid media have been discovered and characterized in some detail (for review, see Clewell, 1993). The best studied is the pheromone-induced conjugation system of *Enterococcus faecalis* (Dunny *et al*., 1978), which along with the conjugation systems of *Lactococcus lactis* (van der Lelie *et al*., 1991), *Lactobacillus plantarum* (Reniero *et al*., 1992) and the mosquito-toxic bacterium *Bacillus thuringiensis* (*B.t.*) subsp. *israelensis* (Andrup *et al*., 1993; Jensen *et al*., 1995), mediates plasmid transfer via the formation of large aggregates in liquid medium.

In 1982, González & Carlton reported the conjugative transfer of *B.t.* plasmids in mixed culture which led to the discovery that the insecticidal crystal protein genes were located on large conjugative or mobilizable plasmids in several subspecies (González *et al*., 1982). One of the subspecies analysed was *B.t. kurstaki* strain HD73. *B.t. kurstaki* flagella serotype 3ab comprises both strain HD1 (crystal serotype K-1) and HD73 (crystal serotype K-73) (Krywienczyk *et al*., 1978). Strain HD73 contains six plasmids ranging from 7·5 to 77 kb (González *et al*., 1981). The genetic basis of the insecticidal

Abbreviations: *B.t., Bacillus thuringiensis*; Cm, chloramphenicol; Em, erythromycin; Nal, nalidixic acid; Sm, streptomycin; Tc, tetracycline.

Table 1. Strains and plasmids

Strain or plasmid	Relevant characteristics	Source or reference*
B.t. kurstaki		
KT_o		de Barjac (1981)
HD73	4D4	BGSC
AW05	HD73 cured of pAW63	This study
AW06	HD73 cured of pHT73	This study
AW16	AW05, Sm^R	This study
AW17	AW06, Sm^R	This study
AW21	HD73 cured of both pAW63 and pHT73, Sm^R	This study
AW43	HD73 cured of both pAW63 and pHT73, Nal^R	This study
AW46	AW06 electroporated with pEG922, Cm^R, Tc^R	This study
AW48	AW43 mated with AW46, pAW63::Tn*5401*, Nal^R, Tc^R	This study
B.t. israelensis		
GBJ001	Plasmid-cured derivative, Sm^R	Jensen *et al.* (1995)
GBJ002	Plasmid-cured derivative, Nal^R	Jensen *et al.* (1996)
AND940	GBJ002 containing pXO16, Nal^R	Jensen *et al.* (1996)
Other *Bacillus* species		
B. cereus	AH183	A. B. Kolstø
B. licheniformis	5A2	BGSC
B. subtilis	SB202	L. Boe
B. sphaericus	13A5	BGSC
Plasmids		
pBC16	Natural *B. cereus* plasmid, Tc^R	Bernhard *et al.* (1978)
pBC16Δ*Nde*I	pBC16 with a 498 bp *Nde*I deletion in the *mob* gene	Andrup *et al.* (1996)
pEG588-8	Vector containing *ori-44* from *B.t.* HD263	Baum *et al.* (1990)
pEG922	Tn*5401* delivery vector	Baum (1994)
pHT232	Containing IS*232*A, corresponds to pHTZ232	Menou *et al.* (1990)
pHT44	Vector containing Tn*4430*	Lereclus *et al.* (1986)
pHTA2	Vector containing IS*231*C and *cry1Ab*	Sanchis *et al.* (1988)

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toxin has been located to a 77 kb plasmid in strain HD73 and it was suggested that there may be a second plasmid of similar size (González *et al.*, 1981; Kronstad & Whiteley, 1984). Another *B.t. kurstaki* strain, KT_o, originating from Centre OILB (Institut Pasteur), has been shown to have a plasmid profile very similar to strain HD73 and the crystal toxin gene has been located to a 83 kb plasmid (Lereclus *et al.*, 1985). We suggest that the two toxin-encoding plasmids are identical and should be named pHT73 (G. T. Vilas Boas, unpublished). It has been assumed that strain KT_o was identical to HD73 (Lereclus *et al.*, 1985). However, as presented here, a plasmid analysis shows that strain KT_o only contains one large plasmid, the crystal toxin plasmid pHT73, whereas strain HD73 harbours a second plasmid, pAW63, somewhat smaller than pHT73. Plasmid pHT73 has been shown to contain a transposon, Tn*4430* (Lereclus *et al.*, 1986), and several insertion sequences (Menou *et al.*, 1990; Mahillon *et al.*, 1994) in addition to the toxin gene.

The objective of our study was to identify and analyse the conjugational activity of *B.t. kurstaki* strain HD73. Evidence is presented that each of the two large plasmids of about 77 kb found in this strain, pHT73 and pAW63, is capable of bringing about its own transfer as well as that of mobilizable plasmids. Transconjugants of *B.t. israelensis*, *Bacillus cereus*, *Bacillus licheniformis*, *Bacillus subtilis* and *Bacillus sphaericus* that inherited plasmid pAW63 were, in turn, effective donors. It appears that plasmid pAW63, under the circumstances used here, was the more efficient of the two plasmids, both with regard to conjugative transfer and as a mobilizing agent.

METHODS

Strains, plasmids and media. Bacterial strains and plasmids used in this study are listed in Table 1. Plasmids pEG922 and pEG588-8 were kindly provided by James A. Baum, Ecogen Inc., PA, USA. All cultures were grown in LB medium (Sambrook *et al.*, 1989) containing antibiotics (Sigma), when appropriate, at the following concentrations (μg ml^{-1}): streptomycin (Sm), 100; nalidixic acid (Nal), 15; tetracycline (Tc), 4; chloramphenicol (Cm), 6. Restriction enzymes were used as recommended by the supplier (Gibco-BRL).

Plasmid transfer. Broth mating was conducted as described by Andrup *et al*. (1996). Equal amounts of exponentially growing cells (250 µl per OD_{600} unit) were combined in 7 ml prewarmed LB medium and incubated at 30 °C with moderate shaking (180 r.p.m.). After 3 h or as indicated, appropriate dilutions were plated on selective media and controls of donors and recipients, grown separately, were tested in parallel.

To exclude plasmid transfer by transformation, 50 µg DNaseI ml^{-1} was added to mating mixtures. As donors *B.t. kurstaki* strains AW05 and AW06 containing pBC16 were used and matings to strain AW43 were performed. Transduction was excluded by adding supernatant from the donor cells filtered through a 0·2 µm filter to a potential recipient.

DNA isolation. Extraction of large plasmids from *B.t.* was conducted by the method of Jensen *et al*. (1995). Smaller plasmids were extracted by a modification of the alkaline-lysis method described by Andrup *et al*. (1993). DNA was analysed by horizontal gel electrophoresis (6–10 V cm^{-1}) in 0·5 or 0·8 % agarose (SeaKem GTG) with 1 × TBE buffer (Sambrook *et al*., 1989) for 2–3 h. After electrophoresis, the gel was stained in 1 µg ethidium bromide ml^{-1} for 5–10 min and destained in water. DNA fragments in agarose gels were purified using the QIAquick Gel Extraction Kit Protocol (Qiagen).

Electroporation. Electroporation was performed essentially as described by Bone & Ellar (1989). Exponentially growing culture (400 ml; $OD_{600} \approx 0{\cdot}2$) was chilled on ice and harvested in a precooled centrifuge. The cells were washed three times with cold water and resuspended in 500 µl cold 10 % (v/v) glycerol. The resuspended cells were mixed with 1 µg plasmid DNA and electroporated with a single pulse (1200 V, 100 Ω, 21 µF) using GeneZapper (International Biotechnologies). The cells were resuspended in 1 ml prewarmed LB medium and incubated at 30 °C with moderate shaking for 3–16 h before plating on selective medium.

Transposon insertion in pAW63. Transposon insertion was performed as described by Jensen *et al*. (1996). Strain AW06 containing plasmid pAW63 was electroporated with pEG922 isolated from a *B.t. israelensis* strain (Jensen *et al*., 1996). Electrotransformants were selected on LB plates containing Cm and Tc. An exponentially growing culture of one of the electrotransformants, AW46, was diluted (1:100) into LB broth containing Tc at 42 °C and incubated overnight at 42 °C. A mating was conducted (at 30 °C) with this culture as donor and a plasmid-cured, Nal^R strain (AW43) as recipient. Cm^S, Tc^R and Nal^R transconjugants were isolated and their ability to transfer the Tc^R phenotype to a plasmid-cured Sm^R derivative of *B.t. israelensis* (GBJ001) was assessed. One of the transconjugants containing pAW63::Tn*5401* was designated AW48 and subsequently used in matings to determine the transfer frequency of pAW63.

Southern blotting and hybridization. DNA was transferred from the agarose gel to Hybond-N+ (0·45 µm, Amersham) according to the method of Southern (1975). Plasmid DNA was labelled with 50 µCi (1·85 MBq) [α-^{32}P]dCTP [Amersham; 300 Ci mM^{-1} (11·1 TBq mM^{-1})] by nick-translation using the Stratagene Prime-It II Random Primer Labelling Kit. Hybridization with the labelled probe was carried out in Quickhyb solution (Hybaid) at 65 °C overnight. After hybridization, filters were washed twice under each of the following conditions: 2 × SSPE (Sambrook *et al*., 1989) for 15 min and 0·1 × SSPE for 15 min at room temperature.

RESULTS

Characterization of the large plasmids in strain HD73

We isolated plasmid-cured derivatives of strain HD73 to determine the conjugative ability of each the two large plasmids pHT73 and pAW63. Strain HD73 was cured of pAW63 (resulting in strain AW05, containing only pHT73) and pHT73 (resulting in strain AW06, containing only pAW63) by growth at 42 °C (Fig. 1, Table 1). To determine whether the two plasmids originated from a similar replicon, they were probed using the replication region *ori-44* cloned in pEG588-8 (Baum *et al*., 1990). The *ori-44* replicon is identical to that of pHT73 except for one nucleotide base change ($T^{301} \rightarrow C$) (Baum & Gilbert, 1991; Gamel & Piot, 1992). Hybridization with plasmid pEG588-8, containing *ori-44*, gave a signal only with AW05 containing pHT73 and no hybridization to AW06 was observed (Fig. 1). This indicates that the two plasmids are basically different with regard to their replication functions. In Fig. 1 the plasmid profiles (large plasmids) of the donor strains containing the mobilizable plasmid pBC16 are also shown.

DNA homology between pHT73 and pAW63

As mentioned above there was no hybridization of the replicon of pHT73 to pAW63. To test whether pHT73 and pAW63 have sequences in common, hybridization with the crystal gene *cry1Ab* from *B.t. aizawai* 7.29 cloned into plasmid pHTA2 (Sanchis *et al*., 1988) was performed. *cry1Ab* shares 86 % identity to the crystal gene from pHT73, *cry1Ac* (Höfte & Whiteley, 1989). As

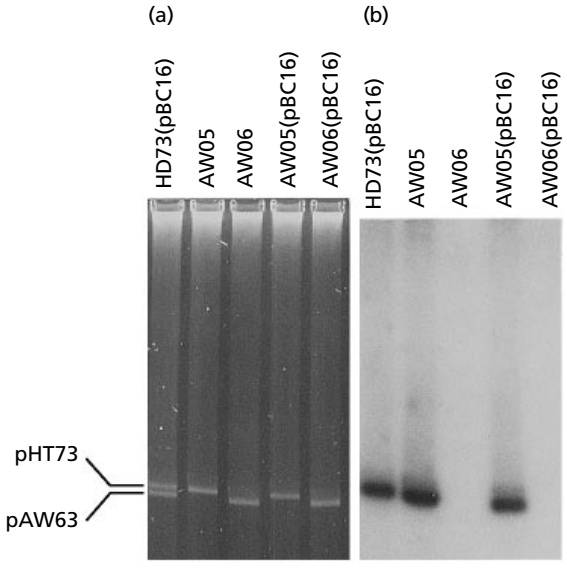

Fig. 1. (a) Agarose gel of the large plasmids from *B.t. kurstaki* strains (indicated above each lane) and (b) autoradiograph of Southern blot using ^{32}P-labelled pEG588-8 containing *ori-44* as probe.

probe we used a 1 kb internal *Hin*dIII fragment of *cry1Ab*. It was found that the *cry1Ab* probe only hybridized to fragments from pHT73 (Fig. 2b), so pAW63 contains no sequences with homology to the crystal gene. As seen in Fig. 2(b) the hybridization pattern of strains AW05, KT_0 and HD73 were identical, verifying that the largest plasmid, pHT73, is present in these strains. To determine whether pAW63 harboured structures similar to transposon Tn*4430* found on pHT73, a 4·2 kb *Kpn*I fragment from plasmid pHT44 (Lereclus *et al*., 1986), which carries a copy of Tn*4430*, was used as probe (Fig. 2c). It was found that Tn*4430* hybridized to fragments of both pHT73 and pAW63. Some of the fragments were shared by both plasmids but hybridization to additional bands on pHT73 revealed that the transposon structure is not identical on these plasmids. The restriction patterns of AW05, AW06 and KT_0 in Fig. 2(a) showed that KT_0 harbours plasmid pHT73, but no bands arising from plasmid pAW63 could be detected.

A plasmid preparation from strain AW06 was digested with *Hin*dIII and used as probe in hybridization studies with the following plasmids: pHT232 (containing an internal part of IS*232*A as a 1·7 kb *Cla*I fragment) restricted with *Cla*I; pHT44 (containing Tn*4430* as a 4·2 kb *Kpn*I fragment) restricted with *Kpn*I; pHTA2 (containing *cry1Ab* and a part of IS*231*C as a 0·5 kb *Kpn*I/*Pst*I fragment) restricted with *Kpn*I + *Pst*I (Fig. 3). It was found that the plasmid preparation from AW06 hybridized to fragments containing Tn*4430* and the insertion sequences IS*232*A and IS*231*C, while there was no hybridization to any fragment corresponding to *cry1Ab*. Hybridization to fragments of pHT73 (*Hin*dIII digest, Fig. 3) confirmed the partial homology between the two plasmids.

Kinetics of transfer of plasmid pAW63

By tagging pAW63 with the tetracycline resistance transposon Tn*5401*, resulting in the donor strain AW48 (see Methods), it was possible to follow the kinetics of transfer of pAW63::Tn*5401*. Broth matings to an HD73 strain resistant to streptomycin and cured of both large plasmids (strain AW21) were conducted. During mating appropriate dilutions of the mating mixture were plated on LB without antibiotics and after growth overnight, 100 colonies were picked on to selective media for the estimation of the number of donors, recipients and transconjugants. The results of such a mating experiment are shown in Fig. 4. It was found that after 2 h, practically all recipients had acquired a copy of plasmid pAW63::Tn*5401* and were resistant to tetracycline. That the mechanism of transfer was by the process of conjugation, rather than by transformation, was demonstrated by performing matings in the presence of DNase. DNase had no significant effect on the frequency of transfer of pBC16. To exclude phage-mediated plasmid transfer, supernatant from a donor strain was added to a potential recipient strain. No transfer of pBC16 was detected.

Mobilization of pBC16 by pHT73 or pAW63

The mobilizable plasmid pBC16 from *B. cereus* was introduced by electroporation into strains HD73, AW05 and AW06. Matings were conducted to the following recipients: AW16 (Sm^R derivative of AW05), AW17 (Sm^R derivative of AW06) and AW43 (Nal^R and cured of both pHT73 and pAW63). Table 2 shows that both pHT73 and pAW63 were able to mobilize pBC16 to the recipients. The donor harbouring pAW63 (AW06) mobilized pBC16 at a frequency 10–30 times higher than the donor containing pHT73 (AW05) to recipients cured of pAW63 (AW16 and AW43). In contrast to this the two donors AW05 and AW06 mobilized pBC16 to the recipient harbouring pAW63 (AW17) with similar frequency. It was found that the frequency of mobilization drastically decreased when both donor and recipient harboured pAW63, indicating entry-exclusion. The HD73 donor, which contains both pAW63 and pHT73, had a reduced frequency of transfer to the three recipients compared to mobilization from strain AW06 alone.

Both strains AW05 and AW06 were able to mobilize pBC16 to strains of *B.t. israelensis* (Table 2) and there were no significant differences in the mobilization capacity of pHT73 and pAW63. Neither did the presence of the aggregation-encoding plasmid pXO16 (Jensen *et al*., 1995) in the recipient strain influence the frequency of transfer of pBC16. Analysis of the plasmid content of the transconjugants showed that besides pBC16 only transfer of pAW63 was detected. None of 28 *B.t. israelensis* transconjugants examined received pHT73, while about 50% of the transconjugants from a mating with strain AW06 acquired plasmid pAW63. Subsequently, the *B.t. israelensis* transconjugants harbouring pAW63 and pBC16 were able to mobilize pBC16 to *B.t. israelensis* recipients (data not shown).

Recently Andrup *et al*. (1996) found that the aggregation-mediated conjugation system of *B.t. israelensis* encoded by pXO16 is able to mobilize pBC16 deleted for the mobilization gene, *mob*, and for the origin of transfer *oriT*. To test whether a functional *mob* gene is necessary for mobilization by pHT73 and pAW63, the pBC16 derivative pBC16Δ*Nde*I, which contains a deletion spanning the first half of the coding region of the *mob* gene, was introduced into strains AW05 and AW06. Using these strains as donors, no transfer of pBC16-Δ*Nde*I to the recipient strain AW21 (HD73 cured of both large plasmids) was found.

Transfer of pAW63 to other species and subspecies

To investigate the host range of conjugative plasmid pAW63, matings to various *Bacillus* strains were conducted. Strain AW48 was used as donor of pAW63 after 3 h broth-mating transfer of pAW63::Tn*5401* to Sm^R recipient strains of *B.t. israelensis*, *B. cereus*, *B. licheniformis*, *B. subtilis* and *B. sphaericus* was demonstrated (data not shown). Transconjugants from these matings were also capable of transferring pAW63::Tn*5401* back

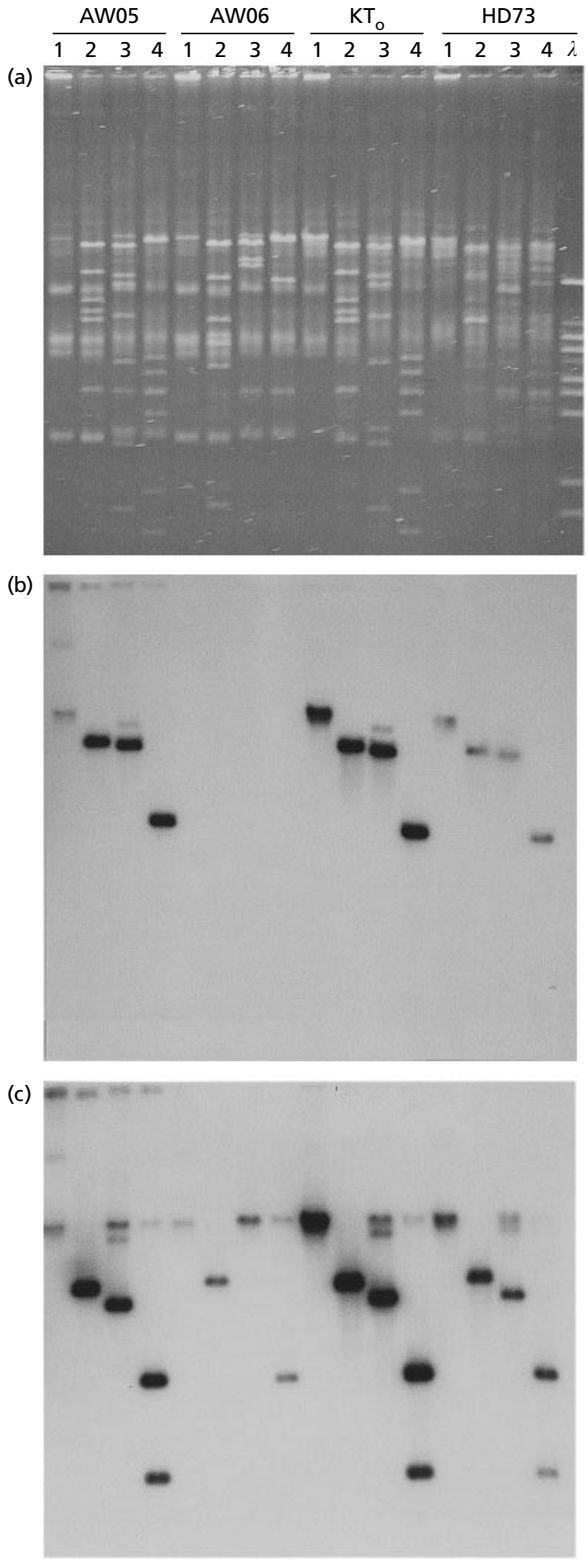

Fig. 2. (a) Agarose gel electrophoresis of plasmid DNA from strains AW05, AW06, KT$_o$ and HD73. Lanes: 1, undigested; 2, digested with *Bgl*II; 3, digested with *Bam*HI+*Bst*EII; 4, digested with *Bam*HI+*Pst*I; λ, λ DNA digested with *Bst*EII. (b) Autoradiograph of Southern blot using ^{32}P-labelled crystal toxin gene *cry1Ab* as probe. (c) Autoradiograph of Southern blot using ^{32}P-labelled transposon Tn*4430* as probe.

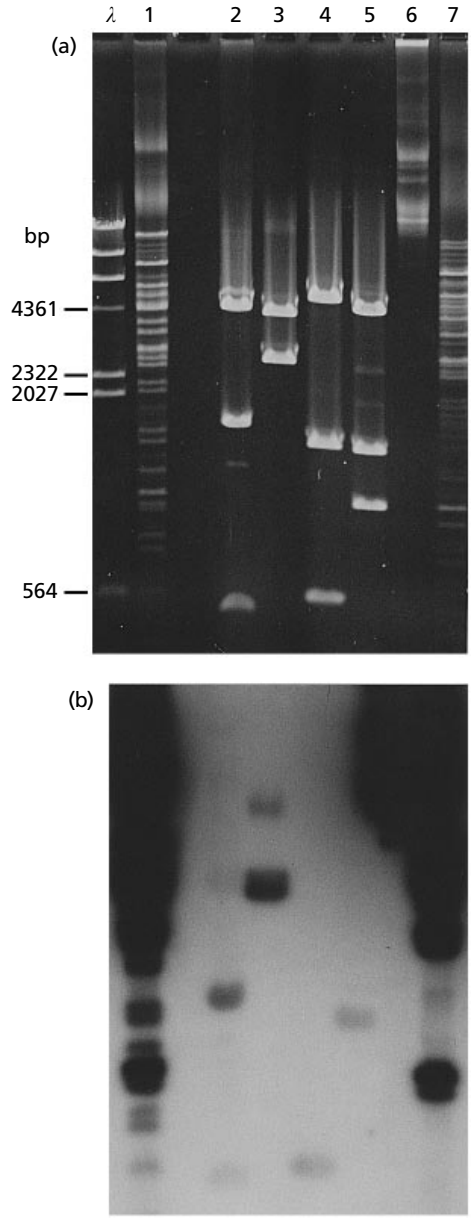

Fig. 3. (a) Agarose gel electrophoresis of: lane 1, pAW63 DNA digested with *Hin*dIII; lane 2, pHT232 digested with *Cla*I; lane 3, pHT44 digested with *Kpn*I; lane 4, pHTA2 digested with *Kpn*I and *Pst*I; lane 5, pHTA2 digested with *Hin*dIII; lane 6, pHT73; lane 7, pHT73 digested with *Hin*dIII. Lane λ, λ DNA digested with *Hin*dIII. (b) Autoradiograph of Southern blot using a ^{32}P-labelled plasmid preparation of pAW63 digested with *Hin*dIII as probe.

to a plasmid-cured HD73 (data not shown), indicating that pAW63 is also functional in these species.

DISCUSSION

This study demonstrates that *B.t. kurstaki* HD73 harbours two self-transmissible plasmids, pHT73 and pAW63. González *et al*. (1982) reported for the first time

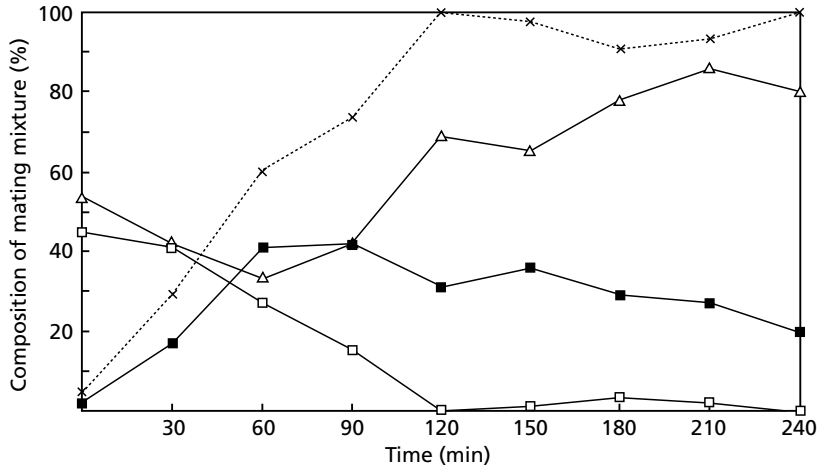

Fig. 4. Kinetics of transfer of pAW63::Tn*5401* from donor strain AW48 to recipient strain AW21. Colonies (100) were picked every 30 min on selective agar plates for determination of the numbers of donor cells (△), recipient cells (□) and transconjugants (■). The frequency of transfer was calculated as the no. of transconjugants per recipient (dotted line).

Table 2. Mobilization of pBC16 by plasmids pHT73 and pAW63

The data shown are from at least three experiments. Values shown are ± SD. The transfer frequency was 10^{-3} transconjugants per recipient. ND, Not determined.

Recipients	Donors		
	HD73 (pHT73, pAW63, pBC16)	**AW05 (pHT73, pBC16)**	**AW06 (pAW63, pBC16)**
HD73			
AW16(pHT73)	0·73 ± 0·07	0·29 ± 0·26	3·7 ± 1·0
AW17(pAW63)	0·084 ± 0·017	0·20 ± 0·16	0·26 ± 0·28
AW43	8·7 ± 1·3	0·47 ± 0·13	12·6 ± 2·6
B.t. israelensis			
AND940(pXO16)	ND	0·22 ± 0·19	1·2 ± 1·1
GBJ002	ND	0·31 ± 0·30	0·52 ± 0·32

the transfer of pHT73 from HD73 in a conjugation-like process in broth mating. They also reported the transfer of a smaller (66 kb) plasmid (González & Carlton, 1982), presumably pAW63, and they speculated whether the transfer of pHT73 was due to mobilization by the 66 kb plasmid. However, our work shows that both pHT73 and pAW63 are independently able to mobilize pBC16. Furthermore, pAW63 is self-transmissible to *B.t. israelensis*, *B. cereus*, *B. licheniformis*, *B. subtilis* and *B. sphaericus*. Under the conditions used, pAW63 was more efficient than pHT73 both regarding conjugative transfer and as a mobilizing agent. Strain KT_0 has been reported to be able to mobilize smaller non-conjugative plasmids (Lereclus *et al.*, 1985) and was formerly assumed to be identical to HD73. However, this work showed that KT_0 only harbours pHT73 and not pAW63, hence mobilization must be caused by pHT73.

Mobilization of non-conjugative plasmids mediated by a co-resident conjugative plasmid by the process of donation (Clark & Warren, 1979) requires some functions on the non-conjugative plasmid: a *trans*-acting *mob* gene (encoding a mobility protein) and a *cis*-acting origin of transfer (*oriT*) (for a review, see Lanka & Wilkins, 1995). This has been shown also to apply to mobilization of pBC16, on which a *mob* gene and a site (RS_A) suggested to function as origin of transfer (*oriT*) have been identified (Selinger *et al.*, 1990), by the conjugative plasmid pLS20 in *B. subtilis*. However, recently Andrup *et al.* (1996) found that the aggregation-mediated conjugation system in *B.t. israelensis* is capable of mobilizing derivatives of pBC16 deleted for both these loci. In this study, we have demonstrated that both pAW63- and pHT73-mediated mobilization require the presence of the *mob* gene on pBC16, hence the mechanism of conjugation is different from the aggregation-mediated conjugation system encoded on pXO16 in *B.t. israelensis*.

Tagging plasmid pAW63 with the tetracycline resistance transposon Tn*5401* enabled us to follow this plasmid in broth matings. We found very high transfer frequencies of pAW63 to a plasmid-cured derivative of HD73. After 2 h of mating more than 90% of the recipients were Tc^R. This is comparable with the transfer frequency found for pXO16, the conjugative plasmid from *B.t. israelensis* (Jensen *et al.*, 1996), but in contrast to this system neither pHT73- nor pAW63-mediated conjugation gene-

rated visible aggregates during broth mating. By microscopic examination the mating pairs were found to comprise 2–10 cells.

The transfer of pHT73 tagged with a gene encoding erythromycin resistance has been observed (G. T. Vilas Boas and others, unpublished). Matings were conducted both in broth culture, in soil and in infected larvae to a *B.t. thuringiensis* recipient. In broth mating pHT73-Em^R transferred at frequencies between $2{\cdot}4 \times 10^{-4}$ and $1{\cdot}6 \times 10^{-3}$ c.f.u. per recipient c.f.u. This is comparable to the frequency we found for pAW63::Tn*5401* transfer to recipients of *B.t. israelensis*.

To date, eight conjugative plasmids have been found in *B.t.* Two of these plasmids carry a gene for crystal production: pHT73 (from HD73 and KT_0) and pXO12 (from *thuringiensis*) (Battisti *et al.*, 1985). The other plasmids, i.e. pXO11 from *B.t. thuringiensis* (Battisti *et al.*, 1985), pXO13 from *B.t. morrisoni*, pXO14 from *B.t. toumanoffi* and pXO15 from *B.t. alesti* (Reddy *et al.*, 1987) have, apart from their conjugative ability, no known functions. Reddy *et al.* (1987) found DNA homology between pXO11, pXO12, pXO13 and pXO14 and showed that if donor and recipient contained the same conjugative plasmid, there was a reduced efficiency of pBC16 transfer, while transfer of pBC16 was enhanced if donor and recipient harboured different conjugative plasmids. Like Reddy *et al.* (1987) we observed entry-exclusion when both donor and recipient harboured pAW63; the transfer frequency, however, was reduced when donor and recipient each contained a different conjugative plasmid. This could indicate that pAW63 and pHT73 compete in the transfer process, perhaps by using the same system for mobilization and conjugation.

B.t. thuringiensis also harbours two conjugative plasmids: pXO12, containing the crystal toxin gene, and the cryptic plasmid pXO11 (Battisti *et al.*, 1985). Both of these plasmids are capable of promoting the transfer of pBC16 to *Bacillus anthracis* and *B. cereus*, and the transconjugants harbouring either pXO12 or pXO11 are also efficient donors of pBC16. When *B.t. thuringiensis* is the donor, plasmid pXO12 is more efficient in transferring pBC16 than pXO11. This is in contrast to the situation in strain HD73 where the crystal plasmid, pHT73, is less efficient than pAW63. The benefit for a cell to have two conjugative plasmids is unknown and it would be interesting to determine whether pAW63 harbours genes, other than *cry* genes, but expressing adaptive functions advantageous to *B.t.*

Work is in progress to map pAW63 and isolate relevant genes for conjugation and replication, hopefully thereby shedding some light on the structure and properties of pAW63 and conjugation in *B.t.* in general.

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