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Review article

Reclassification, genotypes and virulence of *Paenibacillus larvae*, the etiological agent of American foulbrood in honeybees – a review*

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Abstract – American foulbrood, a worldwide bacterial disease of honeybee brood caused by the gram-positive bacterium *Paenibacillus larvae*, is one of the most serious bee diseases. This review will focus on recent achievements in the study of *Paenibacillus larvae* brought about by molecular methods introduced into the field over the last fifteen years. One topic will be the classification of the etiological agent, which has changed several times since the first description in 1906 and was most recently modified again. Different genetic and biochemical methods for subtyping *Paenibacillus larvae*, the analysis of differences in virulence and the implications of these differences will also be covered.

American foulbrood / *Paenibacillus larvae* / taxonomy / genotyping / virulence

Abbreviations: AFB (American Foulbrood), *P. l. larvae* (*Paenibacillus larvae* subsp. *larvae)*, *P. l. pulvifaciens* (*Paenibacillus larvae* subsp. pulvifaciens), *P. larvae* (*Paenibacillus larvae*), CFU (colony forming units), LC_{50} (lethal concentration killing 50% of the larvae).

1. INTRODUCTION

The oldest report on foulbrood disease of honeybees presumably dates back to Aristotle (384–322 b.c.) who described in book IX of his History of Animals a diseased condition which "is indicated in a lassitude on the part of the bees and in malodorousness of the hive". In 1769, the Saxon naturalist Schirach described a honeybee disease characterized by a foul smell and coined the name foulbrood (Schirach, 1769). In 1885, the cause of foulbrood disease was ascribed to *Bacillus alvei* (*B. alvei*) (Cheshire and Cheyne, 1885). In 1906, it became evident that there were actually two different bacterial brood diseases to which the name foulbrood was being applied (White, 1906): on the one hand a disease now known as European foulbrood (EFB) caused by *Melissococcus plutonius* with *B. alvei* as a frequent secondary invader (Bailey, 1957; Bailey, 1983); on the other hand what was then called American foulbrood (AFB) with *Bacillus larvae* isolated as etiological agent (White, 1906).

AFB is a serious bacterial disease of honeybee brood, not only able to kill infected individuals but also potentially lethal to infected colonies. Spreading of the disease within an apiary and between apiaries or even countries is facilitated by beekeeping practice like exchanging material between colonies, managing numerous hives in a confined area and the global trading of bees and honey. Meanwhile, AFB has spread worldwide. In many countries, AFB is a notifiable disease and most authorities consider burning of diseased colonies and contaminated hive material the only workable control measure. Thus, AFB causes

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considerable economic loss to beekeepers all over the world. In some countries, antibiotics (e.g. oxytetracycline) are used to suppress the clinical symptoms and, hence, the outbreak of AFB. The drawbacks of this treatment are that (i) antibiotics are not effective against spores and, therefore, only mask the disease, and that (ii) oxytetracycline-resistant *P. larvae*-strains have developed making the quest for new effective antibiotics already necessary (Miyagi et al., 2000; Mussen, 2000; Kochansky et al., 2001; Elzen et al., 2002; Wu et al., 2005).

Since the isolation and identification of the etiological agent of AFB in 1906 (White, 1906), the disease has become one of the best-studied honeybee diseases (for a recent review see: Hansen and Brødsgaard, 1999). However, many aspects of AFB remain elusive. In this review we will cover the great advances which have been made over the past fifteen years by applying molecular methods to the study of AFB. We will especially focus on the new taxonomic classification, on genotypes and differences in virulence.

2. RECLASSIFICATION OF THE ETIOLOGICAL AGENT

The bacterium causing AFB was first identified and described by White (1906). He classified the bacterium that was consistently found in diseased and dead larvae as *Bacillus larvae* based on the rod-shaped morphology of the vegetative form and the ability to sporulate under adverse conditions.

In 1950, another bacterial disease of honeybee brood was described which was characterized by larval remains forming powdery scales rather than the hard scales observed with AFB. Hence, the disease was called powdery scale disease. The bacterium isolated from those powdery scales resembled *Bacillus laterosporus* and even more so *Bacillus larvae*. On the basis of some characteristic differences it was nevertheless considered a different species and named *Bacillus pulvifaciens* (Katznelson, 1950). Powdery scale disease turned out to be an extremely rare condition. Only two reports describing the disease and the isolation of the etiological agent exist in the literature (Katznelson, 1950; Gilliam and Dunham, 1978). There are also conflicting reports on the pathogenicity or virulence of *Bacillus pulvifaciens*. Shortly after *Bacillus pulvifaciens* had been isolated as the causative agent of a honeybee larval disease, its pathogenicity was questioned (Katznelson and Jamieson, 1951) but then again confirmed by Hitchcock et al. (1979). Hence, *Bacillus pulvifaciens* was considered a honeybee pathogen rarely causing any visible damage to honeybees.

When molecular methods were introduced into bacterial taxonomy it became evident that the genus *Bacillus* is phylogenetically very heterogeneous. Comprehensive 16S rRNA gene sequence analyses revealed that it consisted of at least five phyletic lines (Ash et al., 1991). One of these lines, the rRNA group 3 bacilli, was shown to be phenotypically and phylogenetically sufficiently distinct to be assigned to a separate genus, *Paenibacillus* (Ash et al., 1993). Using a PCR probe test both, *Bacillus larvae* and *Bacillus pulvifaciens* were identified as members of the new genus *Paenibacillus* and, hence, renamed *Paenibacillus larvae* and *Paenibacillus pulvifaciens*, respectively (Ash et al., 1993).

In 1996, the taxonomic position of *Paenibacillus larvae* and *Paenibacillus pulvifaciens* was again reviewed using a polyphasic approach. Analysis of several type and reference strains of both species supported their reclassification into one species, *Paenibacillus larvae* (Heyndrickx et al., 1996). Especially the rDNA restriction patterns and DNA-DNA binding studies revealed high levels of similarity that did not support the former classification into two different species. However, at the infraspecific level phenotypic and genotypic differentiation into two subspecies, *Paenibacillus larvae larvae* (*P. l. larvae*) and *Paenibacillus larvae pulvifaciens* (*P. l. pulvifaciens*), seemed justified considering the different pathologies of the two former species (Heyndrickx et al., 1996). The emended descriptions of the two subspecies included some features differing between the subspecies. Besides differences in pathogenicity, *P. l. pulvifaciens* was described to differ from *P. l. larvae* e.g. by a striking orange-pigmented colony

Table I. Former subspecies of *P. larvae* and ERIC genotypes.

	Paenibacillus larvae			
Genotypes acc. to ERIC-PCR	ERIC I	ERIC II	ERIC III	ERIC IV
Colony morphology	greyish	orange-pigmented	orange-pigmented	greyish
Former subspecies designation	*P. l. larvae*	problematic	*P. l. pulvifaciens*	*P. l. pulvifaciens*
Diseased larvae show AFB-symptoms	yes	yes	yes	yes
Pest-like disease progression	yes	yes	questionable	questionable
Representatives referred to in this manuscript[a]	ATCC 9545^T	03-194 ger	LMG 16252	DSM 3615^T

Note: [a] Origin of strains: ATCC 9545^T (American Type Culture Collection), 03-194 ger (German field strain isolated from an AFB-outbreak in 2003), LMG 16252 (Belgian Type Culture Collection), DSM 3615^T (German Type Culture Collection).

morphology not seen with *P. l. larvae* and by production of acid from mannitol but not from salicin (Heyndrickx et al., 1996). However, although the ability to produce an orange pigment was solely ascribed to *P. l. pulvifaciens* (Heyndrickx et al., 1996), orange-pigmented colonies isolated from diseased brood with symptoms of AFB had been reported earlier (Drobnikova et al., 1994) and recently it was demonstrated that this morphology characterizes a certain genotype of *P. l. larvae* (Neuendorf et al., 2004). Hence, the validity of orange colony pigmentation as a distinctive feature was rebutted. Differentiation between the two subspecies on the basis of their differential acidification of salicin and mannitol (Heyndrickx et al., 1996) has also been questionable since the fermentation of mannitol and salicin has been reported to be a rather variable property of field strains of *P. l. larvae* (Carpana et al., 1995; Dobbelaere et al., 2001). The differentiation and classification of the two subspecies became even more dubious when the 16S rRNA gene sequences of DSM 3615^T, the type strain for *P. l. pulvifaciens*, and DSM 7030^T, the type strain of *P. l. larvae*, were proven to be identical (Kilwinski et al., 2004).

Consequently, a most recent revision of the classification within the species *Paenibacillus larvae* using a polyphasic approach, including not only type and reference strains from different culture collections but also field strains from Germany, Finland, and Sweden, resulted in the reclassification of the subspecies *P. l. larvae* and *P. l. pulvifaciens* as one species *P. larvae* without subspecies differentiation (Genersch et al., 2006). The collection of field strains used for this taxonomic study comprised not only non-pigmented but also orange-pigmented colony variants of the former subspecies *P. l. larvae*. All field and reference strains of the former subspecies *P. l. larvae* had been identified using a subspecies specific PCR protocol (Alippi et al., 2004) actually based on genotyping via rep-PCR performed with ERIC primers (Versalovic et al., 1994). Typing of all *P. larvae* strains with ERIC-PCR revealed that each former subspecies comprised two different genotypes resulting in a total of four *P. larvae* ERIC-genotypes (Tab. I). The pigmented strains of the former subspecies *P. l. larvae* formed one of the four genotypes (Genersch et al., 2006). Pulsed field-gel electrophoresis (PFGE) and SDS-PAGE profiling did not support the identity of the presumed *P. l. pulvifaciens* reference strains as a separate subspecies (Genersch et al., 2006). In contrast, the results strongly suggested that the presumed *P. l. pulvifaciens* reference strains should be reclassified as proposed in Kilwinski et al. (2004). The strongest argument for the two different subspecies had always been the different pathology of *P. l. pulvifaciens* (Katznelson, 1950; Gilliam and Dunham, 1978). Therefore, exposure bioassays were included in the revision of the taxonomy of *P. larvae* (Genersch et al., 2006). Honeybee larvae experimentally infected with either *P. l. larvae* or *P. l.*

pulvifaciens showed disease symptoms and died, clearly proving that both former subspecies were pathogenic to honeybee larvae. Dead larvae developed into a ropy mass with a glue-like consistency and dried down to a hard scale irrespective of the presumed subspecies used for infection. Therefore, the described differences in pathology could not be verified in exposure bioassays (Tab. I). However, clear differences in virulence could be observed for the analyzed *P. larvae* strains (Fig. 1). Those genotypes which belong to the former subspecies *P. l. pulvifaciens* killed infected larvae very quickly as already supposed earlier (Hitchock et al., 1979). It took the former subspecies *P. l. pulvifaciens* only around 2 days post infection to kill 50% and 7 days post infection to kill 100% of the infected larvae (Genersch et al., 2006). Therefore, nearly all infected larvae died during their larval stage before pupation and had been removed by the nurse bees under natural conditions. In contrast, ATCC 9545^T, the type strain of the former subspecies *P. l. larvae*, needed around 5 and 12 days post infection to kill 50% or 100% of the infected larvae, respectively (Genersch et al., 2005; Genersch et al., 2006). In this case, nearly half of the infected larvae died after pupation and had the chance to develop into a ropy mass and a hard scale under natural conditions. It is conceivable that these differences have an influence on the probability of a pest-like disease progression since the accumulation of spores and the spreading of the disease within a hive and between hives is dependent on the number of spores produced. The more larvae die after pupation the more spores are produced and the faster the disease will spread within the colony and between colonies. Following this line of thinking we would not expect representatives of the former subspecies *P. l. pulvifaciens* to cause classical AFB-outbreaks, although the finding of collapsed colonies with powdery scales (Katznelson, 1950; Gilliam and Dunham, 1978) may indicate that under certain conditions these *P. larvae* genotypes are also lethal to honeybee colonies (Tab. I). Therefore, for diagnostic purposes it may still be useful to differentiate between the different genotypes of *P. larvae*. Applying the PCR-protocol described by Alippi et al. (2004) obviously allows for the identification of the *P. larvae* genotypes ERIC I and ERIC II (Genersch et al., 2006) associated with classical AFB outbreaks. In combination with analysis of 16S rRNA (Govan et al., 1999) or metalloprotease-genes (Neuendorf et al., 2004), the PCR-based detection of *P. larvae* provides a highly sensitive and specific and, therefore, extremely useful tool for the correct laboratory diagnosis of AFB.

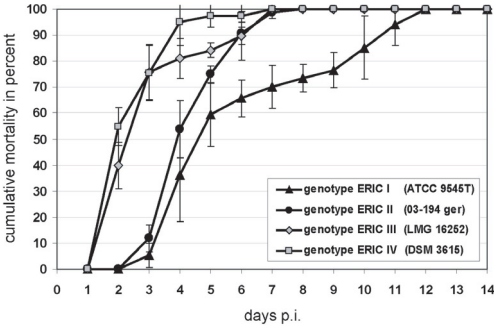

Figure 1. Time courses of infection for ERIC genotypes (modified from Genersch et al., 2006). Honeybee larvae were experimentally infected with one representative of each of the four ERIC genotypes of *P. larvae* using a spore concentration closest to the estimated LC_{50}. Mortality was monitored every day. Cumulative mortality was calculated per day post infection (p.i.) ± standard deviation (n = 3 for each strain) and expressed as percentage of infected hosts (i.e. all larvae that died from AFB in this experiment).

3. SUBTYPING OF *PAENIBACILLUS LARVAE*

Epidemiological studies investigate the time and spatial distribution of infectious diseases. In most cases, the organisms causing an outbreak are clonally related and share biochemical traits and genomic characteristics. Bacterial subtyping, therefore, is useful in determining the source of the infection, recognizing particularly virulent strains, and monitoring programs to combat AFB. The shortcomings of phenotypically based typing methods have led to the development of molecular typing methods.

Table II. Subtyping of *P. larvae* ERIC genotypes I and II with BOX A1R and MBO REP1 primers.

P. larvae genotypes according to rep-PCR using ERIC- or BOX A1R/MBO REP1 primers				
ERIC-genotypes[a]		ERIC I		ERIC II
BOX A1R/MBO REP1-genotypes[b]	*ab*	*Ab*	*aβ*	*AB*

Note: [a] Genersch et al., 2006; [b] Genersch & Otten, 2003.

First attempts to establish a molecular epidemiology of American foulbrood disease of honeybees were made a decade ago using DNA restriction endonuclease fragments patterns (REFP) (Djordjevic et al., 1994). Digestion of bacterial genomic DNA with *Cfo* I generated useful REFPs and allowed the differentiation of five clonal types of *B. larvae* (later *P. l. larvae*, now *P. larvae*) among geographically diverse isolates.

Repetitive element PCR fingerprinting, another method for molecular subtyping of bacterial isolates, also demonstrated considerable diversity within the former subspecies *P. l. larvae* (now *P. larvae*). Using BOX primers three closely related patterns were identified (Alippi and Aguilar, 1998a) and confirmed with additionally introduced primers REP (Alippi and Aguilar, 1998b). ERIC primers were reported to produce no band differences within the former subspecies *P. l. larvae* or between former subspecies, *P. l. larvae* and *P. l. pulvifaciens* (Alippi and Aguilar, 1998b). However, later reports did demonstrate differences between the two former subspecies, which even led to the development of a "subspecies"-specific PCR protocol (Alippi et al., 2004).

The analysis of a collection of German field isolates also using rep-PCR but combining primers BOX A1R and MBO REP1 (Genersch and Otten, 2003) revealed the existence of at least four genotypes (*AB*, *Ab*, *ab*, *αB*) causing AFB outbreaks in Germany. These genotypes showed remarkable constant geographic clustering over the four year observation period. In this study, rep-PCR performed with ERIC primers resulted in two banding patterns, in contrast to earlier reports (Alippi and Aguilar, 1998b). It is now known that the former subspecies *P. l. larvae* indeed splits up into two ERIC patterns (ERIC I, II) and the former subspecies *P. l. pulvifaciens* splits up in another two (ERIC III, IV) resulting in a total of four ERIC patterns within the species *P. larvae* (Genersch et al., 2006). ERIC pattern II is congruent with the BOX/MBO genotype *AB*, while the other BOX/MBO genotypes of the former subspecies *P. l. larvae* were shown to be subgroups of ERIC I (Tab. II). The type strains of the former subspecies *P. l. larvae* (e.g. ATCC 9545^T, DSM 7030^T) belong to ERIC group I and to the fifth BOX/MBO genotype *aβ* (Neuendorf et al., 2004).

Recently, pulsed field-gel electrophoresis has also been used to genotype a range of *P. larvae* isolates originating from Australia and Argentina. Twelve distinct PFGE types were identified among a total of 44 isolates (Wu et al., 2005).

Biochemical characterization of *P. larvae* using traditional macro (Jelinski, 1985; Alippi and Aguilar, 1998a) or commercial micro methods (Carpana et al., 1995; Dobbelaere et al., 2001; Neuendorf et al., 2004) were also used in subtyping *P. larvae*. But it was not until genotyping was combined with a metabolic fingerprinting technique (BIOLOG system) that genotype-specific metabolic differences for *P. larvae* were demonstrated (Neuendorf et al., 2004). In this study, *P. larvae* genotype *AB* (ERIC II) exhibited the most striking metabolic pattern, since it was the only strain able to metabolize the carbohydrates D-fructose and D-psicose, and the only strain unable to use glycerol as carbon source. In addition, genotype *AB* (ERIC II) was the only one harboring plasmid DNA (Neuendorf et al., 2004). These results showed for the first time that different genotypes of *P. larvae* also differ in phenotypic characteristics.

4. VIRULENCE OF *PAENIBACILLUS LARVAE*

Varying definitions and uses of the terms pathogenicity and virulence exist in the

literature, especially in the discipline of invertebrate pathology (Thomas and Elkinton, 2004). A widely accepted definition indicates pathogenicity as a qualitative term describing the state of being pathogenic and virulence as a measure (i.e., the degree) of pathogenicity describing the disease producing power of a pathogen. For a given host and pathogen, pathogenicity is absolute whereas virulence is variable, e.g., due to strain or environmental effects (Steinhaus and Martignoni, 1970; Shapiro-Ilan et al., 2005). Differences in virulence of a pathogen are best analyzed in exposure bioassays because it requires all steps of pathogenesis: entering the host, establishing and reproducing, and causing disease. Three common measures for virulence can be derived from such bioassays: LD_{50} or LC_{50}, the respective dose or concentration it takes the pathogen to kill 50% of the hosts tested, and LT_{50}, the estimated time it takes to kill 50% of the infected individuals.

It has been long known that the course of AFB in infected colonies can be quite variable. Although *P. larvae* is able to kill affected colonies, some infected colonies not only survive, but never develop clinical disease symptoms visible to the apiculturist (Hansen and Brødsgaard, 1999). Hitherto, studies addressing these differences in the outcome of AFB only focused on aspects of host tolerance and hygienic behavior of honeybees (Tarr, 1938; Woodrow, 1942; Woodrow and Holst, 1942; Sturtevant and Revell, 1953; Brødsgaard et al., 1998; Hansen and Brødsgaard, 1999; Brødsgaard et al., 2000; Spivak and Reuter, 2001). Indeed, a study directly comparing a susceptible with a resistant bee line revealed that differences between bee strains might account for a factor of 2 in the spore dose needed for causing clinical symptoms in a colony (Hoage and Rothenbuhler, 1966). However, a most recent study using exposure bioassays for monitoring experimentally infected first instar bee larvae demonstrated that the impact of strain-specific differences in virulence of *P. larvae* on spore dose is much greater than the reported influence from bee tolerance to infection (Genersch et al., 2005). The LC_{50}s of different *P. larvae* strains, determined by exposure bioassays, varied with a factor of 10.

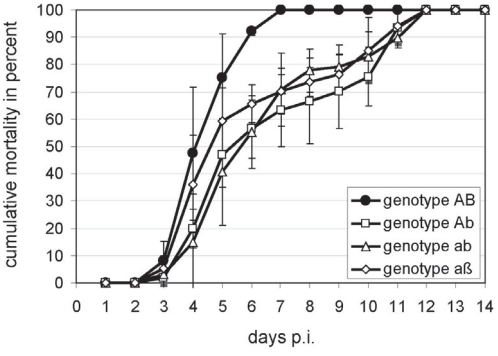

Figure 2. Time courses of infection for *P. larvae* BOX/MBO genotypes *AB*, *Ab*, *ab*, and *aβ* (modified from Genersch et al., 2005). Honeybee larvae were experimentally infected with several representatives of each of the four BOX A1R/MBO REP1 subtypes of genotypes ERIC I (*Ab*, *ab*, and *aβ*) and ERIC II (*AB*) of *P. larvae* (n = 6, 5, 5, 3 for *AB*, *Ab*, *ab*, and *aβ*, respectively) using spore concentrations around the estimated LC_{50}. Mortality was monitored every day. The mean cumulative mortality per day post infection (p.i.) ± standard deviation was calculated for each genotype.

Some highly virulent strains killed 50% of the larvae with less than 100 CFU mL^{-1} larval diet, whereas it took the least virulent strain tested around 800 CFU mL^{-1} larval diet to kill 50% of the exposed larvae. Taking into account an additional factor of 2 coming in from the differences in disease tolerance of the bees (Hoage and Rothenbuhler, 1966) spore concentrations needed to cause clinical symptoms may vary with a factor of at least 20. These results indicate that from quantifying the spore concentration present in brood comb honey it is difficult to predict whether or not clinical symptoms (ropy mass and foulbrood scales) will already be apparent in an infected colony.

In the same study another reason was presented to explain why spore concentration and visible clinical symptoms were not necessarily correlated. Analysis of the time course of disease progression (Fig. 2) revealed genotype-specific differences in the proportion of larvae dying after cell capping (i.e. dying later than approximately six days post infection). Since larvae which are moribund or dead before cell capping will most likely be removed

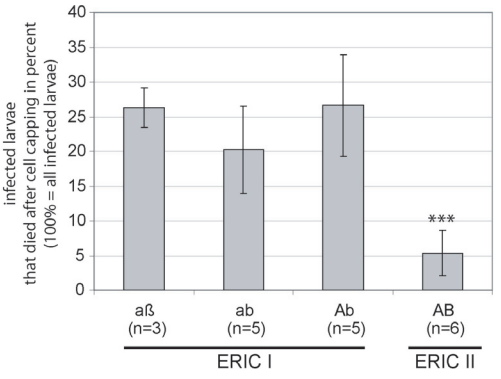

Figure 3. Correlation between time of larval death and genotype. Honeybee larvae were experimentally infected with several representatives of each of the four BOX A1R/MBO REP1 subtypes of genotypes ERIC I (*aβ*sg*b*, and *Ab*) and ERIC II (*AB*) of *P. larvae* using spore concentrations covering the range between 100 CFU mL^{-1} and 2000 CFU mL^{-1} for each strain. Mortality was monitored every day. The mean value ± standard deviation of AFB-dead larvae which died after cell capping was calculated for each genotype.

by nurse bees, the typical clinical signs of AFB like ropy mass in capped cells and foulbrood scales only develop when larvae or pupae die after cell capping and are not cleaned out by nurse bees. Therefore, the more infected larvae die after cell capping, the more frequent clinical symptoms will be in an infected colony. On the other hand, the more larvae die before cell capping the less frequent clinical symptoms will be and the more difficult it will be to clinically diagnose AFB in such a case. Hence, it is remarkable that only 5.4 ± 3.2% of representatives of *P. larvae* BOX/MBO genotype *AB* (ERIC II) were found to survive until after cell capping, whereas 26.6 ± 7.3, 20.2 ± 6.3, and 26.3 ± 2.8% of larvae infected with BOX/MBO genotypes *Ab*, *ab*, and *aβ*, respectively, died after capping (Fig. 3) (Genersch et al., 2005). The differences between the three genotypes *Ab*, *ab*, and *aβ* (all belong to ERIC I) and the genotype *AB* (ERIC II) when infected larvae died were highly significant, with *P* values of 0.0006, 0.002, and 0.0004, respectively (one-way analysis of variance: $df = 3$, $F = 14.06$, $P = 0.0002$, followed by a post hoc test, Newman-Keuls test). These results indicate that irrespective of the spore concentration the likelihood to detect clinical symptoms is very low for infections caused by genotype *AB* (ERIC II) because most of the diseased larvae will be removed due to the hygienic behavior of the nurse bees. Nevertheless, the remaining larvae dying from the disease after cell capping are still sufficient to cause colony collapse and a pest-like disease progression as can be deduced from the fact, that this genotype is frequently isolated from AFB outbreaks in Germany, Sweden, and Finland (Genersch and Otten, 2003; Genersch et al., 2006; Peters et al., 2006). In contrast, infections caused by any other BOX/MBO genotype (ERIC I) can be diagnosed on the basis of clinical symptoms at an early stage and, hence, official measures can be taken directly. Because of these genotype-specific differences in virulence we recommend that genotyping of *P. larvae* isolated from colonies not showing clinical symptoms should be considered, especially for countries allowing artificial swarming as a possible control means (Fries et al., 2006), to enable the veterinarian to decide on a rational basis for the most appropriate measures.

5. PERSPECTIVES

Although the molecular pathogenesis of American foulbrood is still far from being understood the work of the past fifteen years added a lot to the understanding of this deleterious bacterial disease of honeybee brood. With the establishment of *P. larvae* genotypes and their phenotypic differences including variation in virulence, we are now in a situation where we can start to unravel certain aspects of the interaction between the pathogen *P. larvae* and its host, the honeybee larvae, at the molecular level.

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Résumé – Reclassification, génotypes et virulence de *Paenibacillus larvae*, agent de la loque américaine des abeilles. L'agent de la loque américaine (AFB) a été classé au cours des temps comme *Bacillus larvae*, *Paenibacillus larvae* et *Paenibacillus larvae larvae.* En même temps une bactérie qui tuait les larves et les desséchait en croûtes poudreuses était caractérisée comme *Bacillus pulvifaciens*, *Paenibacillus pulvifaciens* et *Paenibacillus larvae pulvifasciens.* L'étude moléculaire des souches types et des souches de références des deux sous-espèces et des souches de terrain a montré qu'il s'agissait d'une seule espèce, *Paenibacillus larvae.* L'analyse par PCR-répétitive à l'aide de quatre amorces ERIC a permis de différencier quatre génotypes. Les tests d'infection expérimentale n'ont montré aucune différence de pathologie entre les deux sous-espèces. Toutes les souches étudiées étaient pathogènes pour les larves et les décomposaient en une masse filante qui se desséchait en une croûte dure. En Allemagne seuls deux des quatre génotypes de *P. larvae* existent, comme le montre l'analyse par PCR-rep : il s'agit des génotypes ERIC I et ERIC II. On les différencie en combinant les amorces BOX A1R et MBO REP1. Cette méthode permet de scinder encore plus le groupe ERIC I en deux. En Allemagne on trouve principalement les génotypes BOX/MBO *Ab, ab* et *αβ*. Le groupe ERIC II est identique au génotype BOX/MBO *AB*. Les différences entre les deux groupes sont principalement phénotypiques. Le génotype *AB*/ERIC II présente une pigmentation orange sur gélose au sang de mouton et il est le seul à pouvoir métaboliser le fructose et le psicose. Les génotypes du groupe ERIC I se comportent exactement de façon opposée ; en outre le génotype *AB*/ERIC I est le seul à posséder un plasmide. Les différences de virulence de *P. larvae* ont été étudiées à l'aide d'infections expérimentales. La CL_{50}, i.e. la concentration en spores pour laquelle 50 % des larves exposées meurent d'AFB, est spécifique à la souche et varie d'un facteur de 10. On a trouvé des différences dans le déroulement de la loque américaine spécifiques à la souche. Il est remarquable qu'environ 5 % des larves infectées par le génotype *AB*/ERIC II étaient encore en vie après l'operculation, tandis qu'environ 75 % de celles infectées par les autres génotypes survivaient à l'operculation. Puisque les larves, qui sont moribondes ou mortes avant l'operculation, ont très vraisemblablement été éliminées par les ouvrières, pour un même nombre d'insectes infectés il faut s'attendre à des symptômes cliniques plus faibles avec le génotype AB/ERIC II qu'avec les autres génotypes.

***Paenibacillus larvae* / loque américaine / taxonomie / virulence / caractérisation du génotype**

Zusammenfassung – *Paenibacillus larvae* und die Amerikanische Faulbrut der Bienen – Reklassifizierung, Genotypen und Virulenz. Der Erreger der Amerikanischen Faulbrut (AFB) wurde im Verlauf der Zeit bisher als *Bacillus larvae*, *Paenibacillus larvae* und *Paenibacillus larvae larvae* klassifiziert. Parallel dazu wurde ein Bakterium, welches Larven tötet und zu Puderschorfen eintrocknen lässt, als *Bacillus pulvifaciens*, *Paenibacillus pulvifaciens* und *Paenibacillus larvae pulvifaciens* bezeichnet. Eine neuerliche molekulare Untersuchung etlicher Typ- und Referenzstämme von beiden Subspezies als auch von Feldisolaten ergab nun, dass es nur eine Spezies, *Paenibacillus larvae*, gibt, die mittels rep-PCR unter Verwendung von ERIC-Primern in vier Genotypen differenziert werden kann. Experimentelle Infektionsversuche zeigten keine unterschiedliche Pathologie für die beiden Subspezies. Alle untersuchten Stämme waren pathogen für die Larven und zersetzten die Larven zu fadenziehender Masse, die wiederum zu einem harten Schorf eintrocknete. In Deutschland kommen nur zwei der vier *P. larvae*-Genotypen, wie sie mit ERIC-PCR dargestellt werden können, vor: ERIC I und ERIC II. Diese Genotypen lassen sich noch weiter differenzieren, wenn statt der ERIC-Primer BOX A1R- und MBO REP1-Primer verwendet werden und diese Ergebnisse kombiniert werden. Mit dieser Methode lässt sich die ERIC I-Gruppe weiter aufspalten, wobei in Deutschland hauptsächlich die BOX/MBO-Genotypen *Ab*, *ab* und *aβ* vorkommen. ERIC II ist mit dem BOX/MBO-Genotyp *AB* identisch. Phänotypische Unterschiede bestehen hauptsächlich zwischen den beiden ERIC-Gruppen. So zeigen Kolonien von *AB*/ERIC II eine orange Pigmentierung auf Schafblutagar. *AB*/ERIC II kann Glycerin nicht verstoffwechseln, wohl aber mit Fructose und Psicose als einziger Kohlenstoffquelle wachsen. Vertreter der Gruppe ERIC I verhalten sich genau umgekehrt. Bisher wurden außerdem nur bei *AB*/ERIC II Plasmide nachgewiesen. Untersuchungen zu Virulenzunterschieden bei *P. larvae* wurden mittels experimenteller Infektionsassays durchgeführt. Es zeigte sich, dass die LC_{50}, d.h. die Sporenkonzentration, bei der 50 % der exponierten Larven im Verlauf des Versuchs an AFB starben, stammspezifisch ist und mindestens innerhalb einer Zehnerpotenz schwankt. Bei den zeitlichen Verläufen der AFB wurden Genotyp-spezifische Unterschiede gefunden. Wichtig war hier vor allem, dass Larven, die mit Vertretern von *AB*/ERIC II infiziert worden waren, nur zu ca. 5 % nach der Verdeckelung gestorben wären, während die Vertreter der anderen Genotypen die Larven langsamer töteten und dadurch ca. 25 % der infizierten Larven erst im verdeckelten Stadium gestorben wären. Da Larven,

die vor der Verdeckelung moribund oder tot sind, mit großer Wahrscheinlichkeit von den Ammenbienen ausgeräumt werden, ist bei einer Infektion mit *AB*/ERIC II bei gleicher Anzahl infizierter Tiere mit einer weniger ausgeprägten klinischen Symptomatik zu rechnen als bei Infektionen mit den anderen Genotypen.

***Paenibacillus larvae* / Taxonomie / Virulenz / Genotyping / Amerikanische Faulbrut**

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