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Review

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Taxonomie et phylogénie de *Cutibacterium* (ex-*Propionibacterium*) *acnes* et pathologies inflammatoires cutanées

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Abstract

Since its discovery, *Propionibacterium acnes* has undergone various name changes, and has been known since 2016 as *Cutibacterium acnes*. Herein we set out the history and rationale of these taxonomic changes together with a description of a new genus, *Cutibacterium*, which includes five species within the cutaneous ecosystem. Modern microbiological techniques allow finer distinction between species and subspecies while also enabling the identification of separate subtypes within the population of *Cutibacterium* acnes. Phylogeny and molecular typing techniques thus provide a better understanding of the subtypes involved in certain inflammatory skin diseases, including acne, folliculitis and progressive macular hypomelanosis.

Keywords: *Cutibacterium acnes*; phylotypes; MLST; SLST; folliculitis; acne; progressive macular hypomelanosis

Résumé

Depuis sa découverte, *Propionibacterium acnes* a subi différents changements de noms. Désormais appelé *Cutibacterium acnes* depuis 2016, nous présentons l'histoire et les bases de ces changements taxonomiques avec la description, au sein d'un nouveau genre *Cutibacterium*, de cinq espèces de l'écosystème cutané. En effet, les techniques microbiologiques modernes ont permis de mieux distinguer les espèces et sous-espèces mais elles ont aussi permis de séparer des sous-types au sein de la population de *Cutibacterium acnes*. La phylogénie et les techniques de typage moléculaire permettent ainsi une meilleure compréhension des sous-types impliqués dans certaines pathologies inflammatoires cutanées, notamment l'acné, les folliculites ou l'hypomélanose maculeuse progressive.

Mots clés : *Cutibacterium acnes*; phylotypes; MLST; SLST; folliculite; acné; hypomélanose maculeuse progressive

With the advent of new high-throughput sequencing techniques, the study of microbiota has revolutionised investigation of the cutaneous ecosystem. One of the predominant bacteria found within the pilosebaceous follicles and their orifices is *Propionibacterium acnes*. This cutaneous commensal forms part of the cutaneous microbiota [1]. It primarily colonises the sebaceous glands and hair follicles of the human skin, and the density of colonisation varies according to topography. Indeed, the number of pilosebaceous follicles on the face, shoulder, back and trunk varies, particularly in relation to gender [2]. Finally, this organism is also found in the mouth, nostrils, urogenital tract and large intestine [3].

Historically, *P. acnes* was first isolated over a hundred years ago from a patient presenting a chronic skin disease named "acne vulgaris" [4]. Following this discovery, *P. acnes* was the subject of several taxonomic errors. It was successively classified as a member of the *Bacillus* species and then of the *Corynebacterium* species [5,6]. However, in 1946, Douglas and Gunter showed this bacterial species to be closer to members of the *Propionibacterium* genus, since, in keeping with the other species in this genus, it ferments propionic acid from lactose under anaerobic conditions [7] to give an acidic cutaneous pH (favourable to this barrier flora) that hampers the implantation of pathogenic bacteria such as *Staphylococcus aureus* and *Streptococcus pyogenes* [8,9].

The genus *Propionibacterium*, described by Orla-Jensen in 1909, belongs to the phylum of *Actinobacteria* and to the class of *Propionibacteriales* [3,9,10]. The most well-known species within this genus are *Propionibacterium freudenreichii*, with its precious contribution to Swiss cheese [11], and *Acidipropionibacterium acidipropionici*, with its beneficial effect on bovine metabolism [12], but above all, *P. acnes*, due to its role in acne and its regrettable reputation for contaminating human samples [13,14]. Species within this genus have traditionally been separated into the so-called "classic *Propionibacteria*" on the one hand and "cutaneous *Propionibacteria*" on the other [15]. The informal expression "classic propionibacteria" is attributed to species isolated from dairy produce, by contrast with those found on the skin and formerly termed *Corynebacteria* [15].

The "classic *Propionibacteria*" group includes species rarely involved in skin diseases [15]. Conversely, the cutaneous group includes the species *P. acnes*, *P. avidum* and *P. granulosum*. Recently, two new cutaneous species belonging to this group have been described in the literature. They are close to but different from *P. acnes* (Figure 1). The first, *P. namnetense*, was initially described in Nantes, in France, in a case of bone sepsis

originating in the cutaneous extremity of an external fixator [16,17]. The second, named *P. humerusii*, was isolated from a humerus by Butler et al., although it has never been taxonomically validated in accordance with the proper rules [18].

The arrival of new tools and technologies has resulted in a new taxonomic approach to this bacterial family, thanks in particular to genomic analysis (16S RNAr and Core Genome) [15]. Thus, all the former types of cutaneous *Propionibacterium* are now subsumed under a new bacterial genus named *Cutibacterium*. Henceforth, the names in standard use are *Cutibacterium acnes, Cutibacterium avidum, Cutibacterium granulosum, Cutibacterium namnetense* and *Cutibacterium humerusii*.

Within the population of *C. acnes* and its subtypes, which were initially determined by partial sequencing of the genes *recA* and *tly*, genomic techniques have enabled the identification of six phylogenetic groups: IA_1, IA_2, IB, IC, II and III [19]. In the last two years, it has been proposed that these phylotypes of *C. acnes* should be divided into three subspecies: *C. acnes* subsp. *acnes* for phylotype I, *C. acnes* subsp. *defendens* for phylotype II, and *C. acnes* subsp. *elongatum* for phylotype III, in particular on the basis of morphological differences [20,21]. These differences are observable at electronic microscopy as well as after Gram staining, thus further emphasising the pleiomorphism of this bacterium, with both short and long branched forms (Figure 2). This new taxonomy is based on phylogenetic differences and on percentage DNA-DNA hybridisation of less than 80% between subtypes. Further, in contrast with isolates of *C. acnes* type II and III, isolates of *C. acnes* type I, such as *C. avidum,* are described as β-haemolytic, (Figure 3) [22,23].

At the same time as this taxonomic shift, various molecular typing techniques have been developed connecting a subgroup of *C. acnes* to a given disease. In addition to phylotype analysis (IA1, IA2, IB, IC, II and III), different clones have been distinguished by means of the new typing of “household genes” either by partial sequencing (Multi-Locus Sequence Typing or MLST) allowing the determination of Sequence Type (ST) or Clonal Complex (CC) or by a single discriminatory sequence (Single-Locus Sequence Typing or SLST). A correlation exists between phylotypes and clonal complexes: IA1/CC18, IA2/CC28, IB/CC36, II/CCC53 and III/CC43 [24]. Because of the multiple methodologies available, a summary article recently proposed harmonisation and a simple approach to determine the phylogeny of an isolate of *C. acnes* based on the number of isolates to be analysed and on the desired degree of phylogenetic precision [24]. These techniques are used in other diseases involving *C. acnes*, such as infected equipment, prostatic adenoma and sarcoidosis.

In clinical dermatology, phylotype IA1, belonging to clonal complex 18 and to type SLST A1, is predominant in acne lesions, whether moderate or severe, on the face [25] and back [26]. Indeed, the development of acne does not appear to be correlated with an increase in the relative abundance of *C. acnes* in pilosebaceous follicles. Thus, when using either culture or a technique to analyse the microbiome (metagenomic analysis with amplification of the gene coding for 16S RNA), there is no qualitative difference between samples obtained from normal skin and those obtained from acne lesions (papules or nodules) [27,28]. In reality, it is more a question of imbalance of the bacterial flora within the same subgroups of *C. acnes*, with loss of diversity of the subgroups and overrepresentation of a single phylotype, particularly phylotype IA1 [26]. The actual host plays an important role in terms of the composition of individual sebum, microbiota and activation levels of innate immunity. Various teams have shown that the different phylotypes described to date modulate innate immunity in different ways [29,30]. Indeed, strains of *C. acnes* stimulate innate immunity through their interaction with Toll-Like Receptors (TLR), particularly TLR2 and TLR4 [31,32]. Moreover, depending on which phylotype it belongs to, the cutaneous innate immune response is modulated differently, with selective simulation of certain biomarkers [29,30,33]. Interactions within the microbiota have recently been found to play a noteworthy role in the physiopathology of this chronic inflammatory skin disease with a new key actor: *Staphylococcus epidermidis*. The latter agent acts on homeostasis of the cutaneous microbiota by interacting with the different phylogenetic groups of *C. acnes* [34–36]. Thus, microbial balance of the skin is partly related to the action of the bacteriocins of each species active against other species.

Within the domain of folliculitis, microbiological analysis of samples has demonstrated different types of *Cutibacterium* according to clinical presentation. Clones of *C. acnes* similar to those found in acne, and in particular those belonging to phylotype IA1 and to clonal complex CC18, have been reported in simple cases of folliculitis of the scalp, while *C. namnetense* tended to be associated rather with Quinquaud's folliculitis decalvans. Conversely, *S. aureus* is more commonly demonstrated in folliculitis keloidalis of the neck (acne keloidalis nuchae), and probably forms the source of the chronic suppuration associated with the pathogenic potency of this bacteria [37,38], which is also involved in atopic dermatitis [39].

Finally, two research teams have recently demonstrated clear predominance of *C. acnes* phylotype III belonging to clonal complex CC43 in patients presenting progressive macular hypomelanosis (PMH) [40,41]. A study published in 2016 reported for the first time

a detailed genetic analysis of populations of *C. acnes* isolated from samples of both lesioned and non-lesioned skin in patients presenting PMH. A strong statistical correlation was noted between strains belonging to phylotype III and the lesions observed. Comparative analysis of the genomes of the isolated strains demonstrated several genomic regions specific to or absent from strains of phylotype III in relation to other phylotypes, particularly the genes involved in metabolism, transportation and transcriptional regulation [41]. These findings were confirmed in 2017 by a Danish research team that reported predominance of this phylotype III in samples from the affected area, with almost 74% of the other analysed sequences belonging to this subtype. Interestingly, a therapeutic regimen effective against PMH (lymecycline and benzoyl peroxide) is associated with a change in the composition of the *C. acnes* population consisting of a considerable reduction in the proportion of *C. acnes* type III. Restoration of balance in the distribution of phylotypes of *C. acnes* results in clinical improvement [40]. These findings point to the possibility of new therapeutic approaches using pre- or probiotics in cutaneous diseases involving such imbalance in bacterial communities of *C. acnes*.

In conclusion, after being underestimated and almost invariably considered a contaminant of samples, *C. acnes* has been shown over the last decade to play a significant role, via its various subgroups, in several skin disorders. The development of new analytical methods has revealed the fabulous history of *C. acnes* not just as a contributor to stable cutaneous homeostasis, but also as a double-edged organism involved in various types of inflammatory dermatoses.

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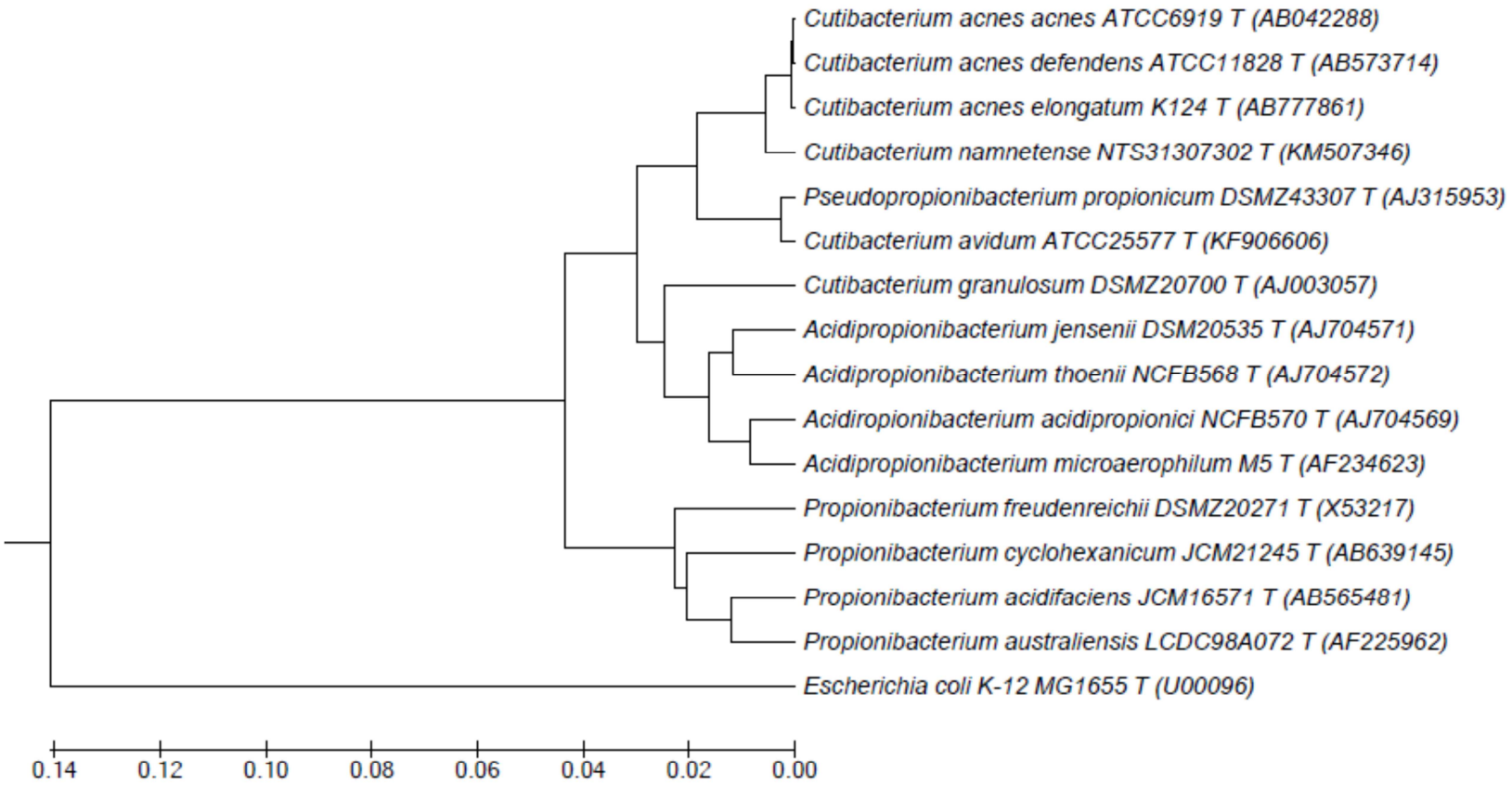
Figure 1: Phylogenetic tree following analysis of 16 nucleotide sequences of 1173 bp of the gene coding for 16S RNA in different types of *Propionibacteriaceae* using Kimura's two-parameter method and the MEGA6 software package.

Figure 2: Direct examination of *Cutibacterium acnes* of phylotypes IA1 (a), II (b) and III (c)

Figure 3: Cultures of *Cutibacterium acnes*: (a) phylotype IA1 presenting beta-haemolysis; (b) non-haemolytic phylotype II from an acne sample; (c) non-haemolytic phylotype III from a sample of progressive macular hypomelanosis


Cutibacterium acnes acnes ATCC6919 T (AB042288)
Cutibacterium acnes defendens ATCC11828 T (AB573714)
Cutibacterium acnes elongatum K124 T (AB777861)
Cutibacterium namnetense NTS31307302 T (KM507346)
Pseudopropionibacterium propionicum DSMZ43307 T (AJ315953)
Cutibacterium avidum ATCC25577 T (KF906606)
Cutibacterium granulosum DSMZ20700 T (AJ003057)
Acidipropionibacterium jensenii DSM20535 T (AJ704571)
Acidipropionibacterium thoenii NCFB568 T (AJ704572)
Acidiropionibacterium acidipropionici NCFB570 T (AJ704569)
Acidipropionibacterium microaerophilum M5 T (AF234623)
Propionibacterium freudenreichii DSMZ20271 T (X53217)
Propionibacterium cyclohexanicum JCM21245 T (AB639145)
Propionibacterium acidifaciens JCM16571 T (AB565481)
Propionibacterium australiensis LCDC98A072 T (AF225962)
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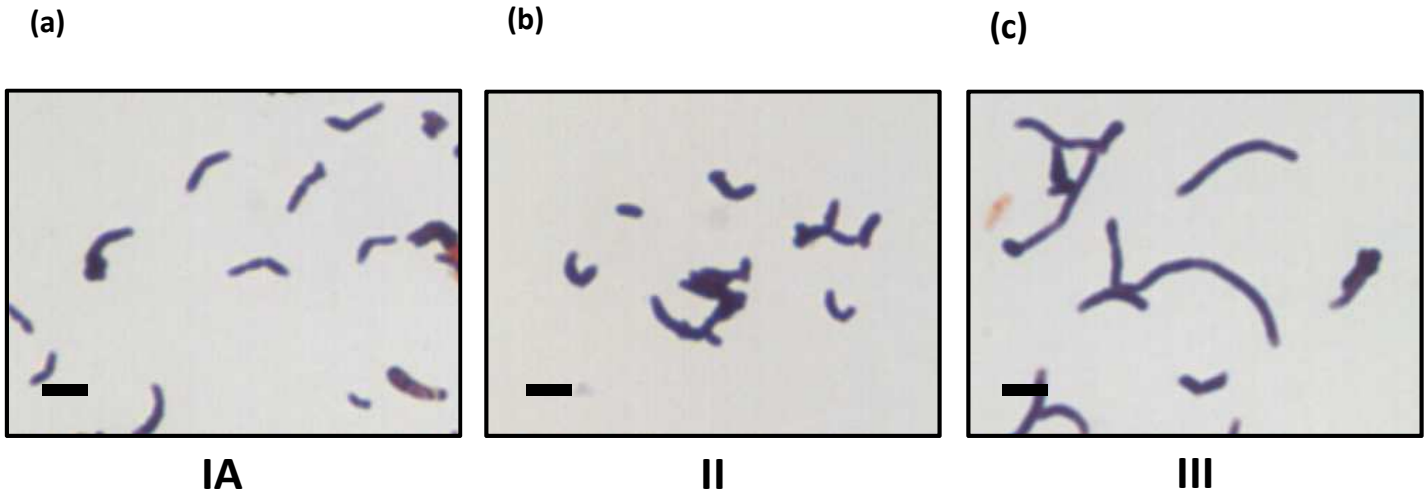
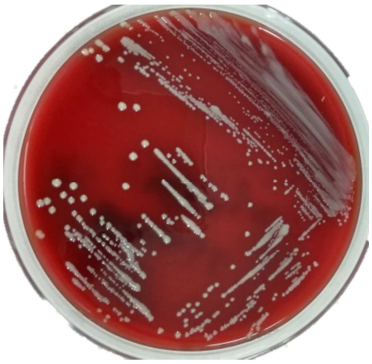

(a)
(b)
(c)
IA
II
III

(a)

IA

(b)



II

(c)

III