Emendation of Propionibacterium acnes subsp. acnes (Deiko et al. 2015) and proposal of Propionibacterium acnes type II as Propionibacterium acnes subsp. defendens subsp. nov

McDowell, A., Barnard, E., Liu, J., Li, H., & Patrick, S. (2016). Emendation of Propionibacterium acnes subsp. acnes (Deiko et al. 2015) and proposal of Propionibacterium acnes type II as Propionibacterium acnes subsp. defendens subsp. nov. *International Journal of Systematic and Evolutionary Microbiology*, *66*(12), 5358-5365. Advance online publication. https://doi.org/10.1099/ijsem.0.001521

Link to publication record in Ulster University Research Portal

Published in:
International Journal of Systematic and Evolutionary Microbiology

Publication Status:
Published online: 01/12/2016

DOI:
10.1099/ijsem.0.001521

Document Version
Author Accepted version

General rights
The copyright and moral rights to the output are retained by the output author(s), unless otherwise stated by the document licence.

Unless otherwise stated, users are permitted to download a copy of the output for personal study or non-commercial research and are permitted to freely distribute the URL of the output. They are not permitted to alter, reproduce, distribute or make any commercial use of the output without obtaining the permission of the author(s).

If the document is licenced under Creative Commons, the rights of users of the documents can be found at https://creativecommons.org/share-your-work/cclicenses/.

Take down policy
The Research Portal is Ulster University's institutional repository that provides access to Ulster's research outputs. Every effort has been made to ensure that content in the Research Portal does not infringe any person's rights, or applicable UK laws. If you discover content in the Research Portal that you believe breaches copyright or violates any law, please contact pure-support@ulster.ac.uk

Download date: 24/04/2025

1 **Title:**

2 Emendation of *Propionibacterium acnes* subsp. *acnes* (Deiko et al. 2015) and proposal of
3 *Propionibacterium acnes* type II as *Propionibacterium acnes* subsp. *defendens* subsp. nov

4

5 **Running title:**

6 Taxonomic reclassification of *P. acnes* types I and II

7

8 **Authors:**

9 Andrew McDowell[1], Emma Barnard[2], Jared Liu[2], Huiying Li[2,3], Sheila Patrick[4]

10

11 **Affiliations:**

12 [1]Northern Ireland Centre for Stratified Medicine, Biomedical Sciences Research Institute, C-
13 TRIC Building, Altnagelvin Area Hospital, University of Ulster, Londonderry, UK.

14 [2]Department of Molecular and Medical Pharmacology, Crump Institute for Molecular
15 Imaging, David Geffen School of Medicine, UCLA, California, USA.

16 [3]UCLA-DOE Institute for Genomics and Proteomics, Los Angeles, California, USA.

17 [4]Centre for Infection & Immunity, School of Medicine, Dentistry & Biomedical Sciences,
18 Queen's University, Belfast, UK.

19 **Correspondence**:

20 Dr Andrew McDowell; Email: a.mcdowell@ulster.ac.uk; Telephone +44 (0) 2871 675662
21 (extn 75662)

22 **Keywords:** *Propionibacterium acnes* subsp. *defendens* subsp. nov., type I, type II, Multilocus
23 Sequence Analysis, Genomics, Phenotype

24 **Subject category:** New taxa - Actinobacteria

25 **Abstract**

26 Recently, strains of *Propionibacterium acnes* from the type III genetic division have been
27 proposed as *Propionibacterium acnes* subsp. *elongatum* subsp. nov., with strains from the
28 type I and II divisions collectively classified as *Propionibacterium acnes* subsp. *acnes* subsp.
29 nov. Under such a taxonomic re-appraisal, we believe that types I and II should also have
30 their own separate rank of subspecies. In support of this, we describe a polyphasic taxonomic
31 study based on the analysis of publically available multilocus and whole genome sequence
32 datasets, alongside a systematic review of previously published phylogenetic, genomic,
33 phenotypic and clinical data. Strains of types I and II form highly distinct clades based on
34 multilocus sequence analysis (MLSA) and whole genome phylogenetic reconstructions. *In*
35 *silico* or digital DNA-DNA similarity values also fall within the 70-80% boundary recommended
36 for bacterial subspecies. Furthermore, we see important differences in genome content,
37 including the presence of an active CRISPR/Cas system in type II strains, but not type I, and
38 evidence for increasing linkage equilibrium within the separate divisions. Key biochemical
39 differences include positive tests for β-haemolytic, neuraminidase and sorbitol fermentation
40 activities with type I strains, but not type II. We now emend the description of
41 *Propionibacterium acnes* subsp. *acnes* (Deiko et al. 2015) and propose type II as
42 *Propionibacterium acnes* subsp. *defendens* subsp. nov. The type strain of *Propionibacterium*
43 *acnes* subsp. *defendens* subsp. nov. is ATCC 11828 (=JCM 6473=CCUG 6369).

44 *Propionibacterium acnes* is a Gram-positive anaerobic bacterium and a member of the
45 'cutaneous' group of human propionibacteria along with *Propionibacterium granulosum*,
46 *Propionibacterium avidum* and *Propionibacterium humerusii.* Although found predominately
47 on the skin, it can also be isolated from the oral cavity and the genitourinary and
48 gastrointestinal tracts (Patrick and McDowell, 2011). While the bacterium is most noted for
49 its association with the inflammatory skin condition acne vulgaris (Lomholt and Kilian, 2010,
50 McDowell et al., 2012, Fitz-Gibbon et al., 2013), there is now a growing recognition that the
51 spectrum of opportunistic infections and clinical conditions to which it may be associated has
52 been underestimated (Tunney et al., 1999, Cohen et al., 2005, Cavalcanti et al., 2011, Eishi,
53 2013; Barnard et al., 2016).

54 In the last 10 years, significant advances in our understanding of this bacterium at the
55 population genetic level have been made using single, multilocus and whole genome
56 sequence analyses (McDowell et al., 2005, McDowell et al., 2008, Lomholt and Kilian, 2010,
57 McDowell et al., 2012, Fitz-Gibbon et al., 2013, Tomida et al., 2013, Scholz et al., 2014). Such
58 work has demonstrated the phylogenetically distinct nature of the originally described *P.*
59 *acnes* serotypes, designated types I and II, and identified a new type, designated type III,
60 which displays an ability to form long filamentous cell structures not seen with types I and II
61 (McDowell et al., 2005, McDowell et al., 2008). These studies have also identified further
62 phylogenetic subdivisions within the type I clade (IA_1, IA_2, IB, IC) which differ in genome
63 content, inflammatory potential, association with disease, production of putative virulence
64 determinants, resistance to antibiotics used in the treatment of acne, as well as biochemical
65 and aggregative properties (Valanne et al., 2005, McDowell et al., 2013, Tomida et al., 2013,
66 Johnson et al., 2016, Scholz et al., 2016).

67 Very recently, Dekio *et al*. (2015) proposed that *P. acnes* type III be reclassified as
68 *Propionibacterium acnes* subsp. *elongatum* subsp. nov. based on phylogenetic, genomic and
69 phenotypic differences, with strains of type I and II classified as *Propionibacterium acnes*
70 subsp. *acnes* subsp. nov. (Dekio et al., 2015). In bacterial taxonomy, there are currently no
71 clear guidelines for the establishment of subspecies, and the proposal of such essentially
72 remains at the discretion of the researcher. Nevertheless, the proposal of a new bacterial
73 subspecies is normally based on consistent phylogenetic differences and phenotypic
74 variations between groups of strains within a species (Brenner et al., 2000). If the major
75 phylogroups of *P. acnes* are now to be reclassified within a subspecies framework, then strains
76 of types I and II also deserve separate taxonomic ranks of subspecies. In this paper, we
77 describe a polyphasic taxonomic study based on the analysis of publically available multilocus
78 sequence and whole genome datasets, alongside a review of published phylogenetic,
79 genomic, phenotypic and clinical data, that support the division and reclassification of *P.*
80 *acnes* type I and type II as distinct subspecies. We now emend the description of
81 *Propionibacterium acnes* subsp. *acnes* to include type I only since it contains the type strain,
82 and propose type II as *Propionibacterium acnes* subsp. *defendens* subsp. nov. Type III strains
83 remain as *Propionibacterium acnes* subsp. *elongatum* (hereafter described as type III) as
84 previously proposed (Dekio et al., 2015).

85 In 2005, we demonstrated that the *P. acnes* serotypes known as types I and II represented
86 highly distinct phylogenetic groups based on sequence analysis of the *recA* housekeeping
87 gene, as well as the putative haemolysin/ FtsJ-like methyltransferase gene *tly* (McDowell et
88 al., 2005). Application of *recA* typing was also central in the identification of strains
89 representing the type III phylogenetic division (McDowell et al., 2008). Since then, two key

90 MLSA methods based on eight ($MLSA_8$) and nine protein-encoding genes ($MLSA_9$) have been
91 described for this bacterium, both based on completely different sets of genetic loci (Lomholt
92 and Kilian, 2010, McDowell et al., 2012). With both independent MLSA schemes we find that
93 types I, II and III form highly distinct clades consistent with the original *recA* and *tly* analysis,
94 and supported by high bootstrap values (Fig. 1). This phylogenetic clustering is also highly
95 congruent with that obtained upon whole genome analysis of 124,731 SNPs in shared or 'core'
96 regions of 85 *P. acnes* genomes spanning all the major phylogenetic divisions (Fig. S1); the
97 average p-distance between each of the types based on core region analysis is 0.444 for types
98 I and II, 0.487 for types I and III, and 0.470 for types II and III (Table 1). Twenty six percent of
99 core region SNPs are unique to type I, with 22% unique to type II and 24% unique to type III
100 (Table 1). The genetic distance between types I and II is therefore similar to the distance
101 between type I and type III, and type II and type III. In addition, even though the 16S rRNA
102 gene of *P. acnes* demonstrates a high degree of intra-species sequence identity, the
103 observation of distinct and non-overlapping ribotypes for type I (RT1; RT3; RT4; RT5; RT8;
104 RT16; RT532), type II (RT2; RT6), and type III (RT9) provides further evidence for their different
105 phylogenies (Fitz-Gibbon et al., 2013, Barnard et al., 2016) (Fig S1).

106 Alongside phylogenetic analyses, previous whole genome typing patterns based on methods
107 such as Random Amplification of Polymorphic DNA (RAPD) and noncoding repeat sequences,
108 as well as the analysis of non-core regions, also support types I and II as highly distinct
109 divisions at the genome level (Perry et al., 2003, Tomida et al., 2013, Hauck et al., 2015).
110 While digital or *in silico* DNA-DNA hybridization values (GGDC 2.0 algorithm) between types I,
111 II and III are above the 70% cut-off value currently used for bacterial species demarcation,
112 thus confirming their membership of the same species, the whole genome relatedness values

113 are consistent with the proposal that types I and II are also placed in distinct taxonomic ranks
114 in line with that recently proposed for type III (Dekio et al., 2015). Strains representing the
115 different phylogroups within type I (IA_1, IA_2, IB, IC) share high *in silico* DNA-DNA hybridization
116 values of 91-100%, but this drops to 74.1-78.5% when analysed against the type II strains
117 ATCC11828 and JCM18920, and 72.0-72.8% with the type III strain JCM18909 (Dekio et al.,
118 2015). Strains of type II and III share relatedness values of 72.9-73.2% (Dekio et al., 2015).
119 These hybridization values between the major divisions are within the 70-80% similarity
120 boundary recently recommended for bacterial subspecies (Meier-Kolthoff et al., 2014).

121 Detailed comparative analysis of type I and II whole genome sequences also reveals some
122 salient differences between the divisions. These include specific genomic inversions and
123 insertions present in type II strains, but not type I, which encode genes related to
124 carbohydrate processing and modification, ABC transporters, nickel import, bacitracin
125 resistance and hypothetical proteins (Fig. S2) (McDowell et al., 2013, Scholz et al., 2016). One
126 of the most striking differences relates to the presence in type II strains of clustered regularly
127 interspaced short palindromic repeats (CRISPR)/Cas locus (Brüggemann et al., 2012b, Fitz-
128 Gibbon et al., 2013). In contrast, type I and type III strains contain CRISPR/Cas gene remnants
129 within their genome, indicating deletion of the locus during the evolutionary history of these
130 phylogroups; the deletions are more extensive in type I strains compared to type III. The
131 deletion of the CRISPR/Cas system in type I and type III strains makes these divisions more
132 susceptible to horizontal gene transfer (HGT) and the acquisition of fitness or virulence traits.
133 The observation of such CRISPR/Cas gene remnants has led to the suggestion that the type I
134 and III divisions may constitute younger subpopulations than type II strains which are
135 descended from a more ancient lineage (Brüggemann et al., 2012a, Brüggemann et al.,

136 2012b). Since age = *ds*/(clock rate x 2), where *ds* is the mean number of synonymous
137 substitutions per site and clock rate is the synonymous molecular clock rate, calculation of
138 the *ds* values for strains currently representing the major type I, II and III divisions may give
139 deeper insights into their relative ages. Interestingly, using the Nei-Gojobori method (Jukes-
140 Cantor) (Nei and Gojobori, 1986) in MEGA v7.0 (Kumar et al., 2016), we observed that the *ds*
141 value for the entire type I division was slightly higher than type II based on an initial analysis
142 of concatenated $MLSA_8$ sequence data, while type III values were lower (Table 1). To
143 investigate this further, we examined the shared core-coding regions of 85 *P. acnes* genomes
144 currently available. Multiple sequence alignments were performed using MUSCLE (Edgar,
145 2004) and the Jukes-Cantor *ds* values calculated for each pair of sequences in the alignment
146 using the Nei-Gojobori method as implemented in the Bioperl package
147 Bio::Align::DNAStatistics (Stajich, 2002). As before, the resulting *ds* values obtained for type I
148 (0.008), type II (0.005) and type III (0.001) revealed higher synonymous nucleotide diversity
149 within the large type I clade compared to type II and type III, indicative of an older age. Further
150 studies are therefore required to provide clarity on the series of evolutionary events that have
151 given rise to the emergence and diversity of the current *P. acnes* clades now proposed as
152 subspecies, including the possible diversity-purging effects of periodic selection (Cohan,
153 2001).

154 *Propionibacterium acnes* has a clonal, epidemic population structure and is in linkage
155 disequilibrium, though rates of HGT within the population as a whole are statistically
156 significant (Lomholt and Kilian, 2010, McDowell et al., 2012, McDowell et al., 2013). Previous
157 studies have, however, found that rates of recombination appear to differ throughout the
158 population, and that the association of alleles is less significant when distinct phylogroup

159 populations are considered (McDowell et al., 2012, McDowell et al., 2013). In particular, we
160 see a drop in the index of association value (I_A) when strains from the type I and II divisions
161 are considered separately, indicating increasing linkage equilibrium within these distinct
162 clusters (McDowell et al., 2013); this can also be observed on a Neighbour-Net split graph
163 based on $MLST_8$ allelic profile data (Fig. 2). Detailed inspection of $MLSA_8$ datasets also
164 suggests conjugal transfer and replacement of unusually large chromosomal segments in the
165 genome dynamics of the type I clade, particularly between types IA_2 and IB (Lomholt and
166 Kilian, 2010, McDowell et al., 2012, McDowell et al., 2013). The idea that rates of genetic
167 interchange are more frequent within, but not between, the major divisions suggests
168 increasing sexual isolation which occurs with more genetically divergent organisms
169 (Majewski, 2001). Reduced rates of recombination may also indicate ecological differences
170 since members of the same habitat are more likely to undergo recombination events
171 (sympatric speciation); such population subdivisions can introduce linkage disequilibrium into
172 an analysis if isolates from different niches (Ecotypes) are included (Spratt and Maiden, 1999).
173 Comprehensive analysis of genome differences between the major types does indeed provide
174 potential evidence for distinct environmental challenges within the human host.

175 Studies by Johnson and Cummins (1972) first revealed types I and II as distinct phenotypes of
176 *P. acnes* based on serological agglutination tests and cell wall sugar analysis; type I strains
177 contain galactose in their cell wall, but this sugar is absent in type II strains which occasionally
178 also contain *meso*-Diaminopimelic acid (DAP) (Table 2). The development of more recent
179 monoclonal antibody typing methods for *P. acnes* have further highlighted differences
180 between the cell wall structures of type I and II, as well as type III, based on the expression of
181 unique antigenic determinants, including those in lipoteichoic acid and adhesin proteins

182 (Holland et al., 2010, McDowell et al., 2011, Bae et al., 2014). Differences in cell surface
183 hydrophobicity have also been described for types I and II, and upon growth in liquid media,
184 such as protease peptone yeast (PPY) or brain heart infusion (BHI) broth, type II strains form
185 a turbid solution with a slight fine sediment, while strains of type IA and IC can form a large
186 granular sediment or auto-aggregate with a clear solution (Cohen et al., 2005); type IB strains
187 behave as type II in respect to this characteristic. Types I and II can be differentiated from one
188 another and type III based on the analysis of bacterial whole cell proteins by matrix assisted
189 laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) fingerprinting,
190 highlighting further variation at the phenotype level (Nagy et al., 2013, Dekio et al., 2015).
191 Furthermore, differences in the susceptibility of types I and II to bacteriophage infection have
192 also been known for some time (Webster and Cummins, 1978, Liu et al., 2015). The main
193 phylogroups of *P. acnes* share a high degree of similarity with regard to their biochemical
194 phenotype, including traditional tests used to differentiate the bacterium from other
195 'cutaneous' propionibacteria (Table 2). Notable phylogroup differences, however, include β-
196 haemolytic and neuraminidase activity, as well as sorbitol fermentation, all of which are
197 essentially restricted to the type I division (McDowell et al., 2008, Lomholt and Kilian, 2010,
198 Niazi et al., 2010) (Table 2). The production of lipase also appears much lower amongst type
199 II strains versus those from the type I and III divisions (McDowell et al., 2008, Niazi et al., 2010)
200 (Table 2); we previously described how type II strains have deletions in the TATA box and open
201 reading frame of two candidate lipase genes which may explain this reduced activity (Tomida
202 et al., 2013).

203 One defining difference between the type I and II phylogroups rests on their association with
204 acne vulgaris. On the basis of both culture and metagenomic analyses, widely disseminated

205 clonal lineages from the type I division have been described in association with acneic skin,
206 but not those from the type II or type III divisions which appear to be associated more with
207 blood, medical device and soft tissue infections (Lomholt and Kilian, 2010, McDowell et al.,
208 2011, McDowell et al., 2012, Fitz-Gibbon et al., 2013, Rollason et al., 2013). Recently, type III
209 strains have also been linked with the depigmenting skin condition progressive macular
210 hypomelanosis (Peterson et al., 2015, Barnard et al., 2016). Interrogation of the *P. acnes*
211 $MLST_8$ isolate database, which contains information on a large collection of geographically
212 widespread isolates and their clinical source, reveals a statistically significant enrichment
213 overall for strains from the type I clade in acneic versus healthy skin ($p<0.001$; Fishers exact
214 test, two tailed), while those from the type II clade appear to show no association overall
215 ($p=0.213$; Fishers exact test). More specifically, associations are found between acneic skin
216 and strains from the type IA_1 clonal complexes CC1 (RT1 and RT532) ($p<0.01$; Fishers exact
217 test), CC3 (RT1, RT4 and RT5) ($p=0.043$; Fishers exact test) and CC4 (RT8) ($p=0.021$; Fishers
218 exact test) (Fig 1 and S1). In a previous study, we found that a globally disseminated clonal
219 lineage with the MLST genotype ST6 (Warwick $MLST_7$ scheme analysis) or ST1 ($MLST_8$ analysis)
220 strikingly represented the majority of type IA_1 isolates we analysed from a cohort of patients
221 with acne (McDowell et al., 2011). In contrast, specific type II lineages (RT2 and RT6) belonging
222 to CC72 ($MLST_8$) appear associated with healthy skin based on metagenomic and culture-
223 based detection (McDowell et al., 2012, Fitz-Gibbon et al., 2013, Johnson et al., 2016). The
224 observation that type II strains, but not those from the type I clade, encode CRISPR/Cas
225 elements may be important in this context, thus preventing the acquisition of genetic loci that
226 may contribute to virulence and acne pathophysiology (Fitz-Gibbon et al., 2013). For example,
227 key type I lineages from CC3 ($MLST_8$; Fig. 1), believed to be associated with acne contain a
228 novel plasmid with a tight adhesion (Tad) locus and two unique genomic islands, known as

229 loci 1 and 2, that contain genes proposed to enhance virulence via increased bacterial
230 adhesion and host immune response (Fitz-Gibbon et al., 2013, Tomida et al., 2013, Kasimatis
231 et al., 2013).

232 To conclude, we now emend the description of *Propionibacterium acnes* subsp. *acnes* (Deiko
233 et al., 2015) as type I only, and propose type II strains as *Propionibacterium acnes* subsp.
234 *defendens* subsp. nov. based on a polyphasic taxonomy approach. The growing number of
235 genomes now becoming available for other propionibacteria will also provide an important
236 opportunity to re-examine the genus.

237 **Emendation of *Propionibacterium acnes* subsp. *acnes* (Deiko et al., 2015).**

238 Description as given by Deiko et al. (2015), emended as follows:

239 Four phylogenetically distinct *Propionibacterium acnes* subsp. *acnes* (type I) groups have been
240 described, known as type IA_1, IA_2, IB and IC; type IA_2, IB and IC represent phylogenetically tight
241 clusters compared to IA_1. Cells are Gram-positive, nonmotile, non-spore forming, and
242 anaerobic-to-aerotolerant. Colonies appear as lenticular, minute-to-4.0 mm, white, can
243 become tanned, pink or orange in 3 weeks. Growth is most rapid at 30-37°C. Surface colonies
244 on blood agar (horse or rabbit) are punctiform-to-0.5 mm, circular, entire-to-pulvinate,
245 translucent-to-opaque, white-to-gray, glistening. The cell shape after anaerobic culture in
246 broth medium ranges from small plump rods to ellipsoids which tend to occur in pairs joined
247 at a slight angle, and the size is approximately 0.4-to-0.5 by 0.8-to-0.9 µm. In defined medium
248 broth culture, type IA and IC strains form a turbid suspension, while in PPY or BHI broth they
249 form a settled granular sediment with a clear solution. In contrast, type IB strains form a slight
250 fine sediment and turbid solution containing suspended cells. In suitable media with good

251 growth, the final pH is 4.5-5.0. Generally catalase positive, cultures need to be exposed to air
252 for 1 h before testing. All strains have an absolute requirement for pantothenate, while
253 thiamine, biotin and nicotinamide are stimulatory. Strains are co-haemolytic and variable for
254 β-haemolytic activity and produce a number of extracellular enzymes including ribonuclease,
255 neuraminidase, hyaluronidase, acid phosphatase, lecithinase and lipase. Strains of type IA
256 produce relatively low levels of the putative co-haemolytic Christie-Atkins-Munch-Peterson
257 (CAMP) factor 1, but type IB strains produce an abundance of this protein. The total quantity
258 of acid (especially the proportion of lactic acid) produced from fermentable carbohydrates is
259 highly variable. Cells ferment glucose, but not sucrose or maltose. Lactate is converted to
260 propionate by most strains but only if the initial oxidation-reduction potential of the medium
261 is sufficiently low, or if the initial growth rate is rapid. Sorbitol fermentation is a variable but
262 defining characteristic of type I strains. Gelatin is hydrolysed, and most strains produce indole
263 and reduce nitrate, but esculin is not hydrolysed. The major long chain fatty acid produced in
264 thioglycolate cultures is 13-methyltetradecanoic acid (32-62%) and iso-C15:0 FAME is the
265 predominant cellular fatty acid. Prominent mass ions obtained by MALDI-TOF mass
266 spectrometry are at 3,589 Da and 7,179 Da. Peptidoglycan contains alanine, glutamic acid,
267 glycine and LL-DAP. Cell wall sugars are glucose, mannose and galactose. Strains have been
268 isolated from the human skin, oral cavity and genitourinary tract. Type IA_1 and IC strains are
269 associated with acne vulgaris. The G+C content is ~ 60% based on whole genome sequencing
270 analysis.

271 **Description of *Propionibacterium acnes* subsp. *defendens* subsp. nov.**

272 *Propionibacterium acnes* subsp. *defendens* (de.fen'dens L. part. adj. *defendens,* defending,
273 guarding, protecting; referring to the fact that strains have an active CRISPR/Cas system which

274 guards or controls against foreign mobile genetic elements). Description based on McDowell
275 et al. (2008), Niazi et al. (2010), Patrick and McDowell (2011), and Dekio et al. (2015).

276 Cells are Gram-positive, nonmotile, non-spore forming, and anaerobic-to-aerotolerant. Their
277 cellular and colony morphology is similar to type I cells, but they may appear more coccoid
278 and are most similar to previous descriptions for 'Corynebacterium parvum' which is a
279 synonym for *P. acnes*. In defined medium broth culture, strains form a slight fine sediment
280 and turbid solution containing suspended cells. In addition to pantothenate, some strains
281 require haem and vitamin K to grow. Biochemical phenotype is similar to type I strains but
282 with some notable differences. Cells are negative for β-haemolysis, and neuraminidase and
283 lipase activity is infrequently found. Abundant levels of CAMP factor 1 are produced; similar
284 to that observed with strains of type IB. Sorbitol fermentation is negative. The predominant
285 cellular fatty acid is iso-C15:0 FAME and prominent mass ions obtained by MALDI-TOF mass
286 spectrometry are 3,628 Da and 7,258 Da. Peptidoglycan contains alanine, glutamic acid,
287 glycine, LL-DAP, and occasionally *meso*-DAP. Cell wall sugars are mannose and glucose, but
288 galactose is not present. Strains have been isolated from the human skin surface, oral cavity
289 and genitourinary tract. Strains are rarely associated with acne vulgaris and some may be
290 associated with skin health and others with opportunistic infection. The G+C content is ~ 60
291 % based on whole genome sequencing analysis.

292 The type strain of *Propionibacterium acnes* subspecies *defendens* subsp. nov. is ATCC11828
293 (=JCM 6473=CCUG 6369) isolated from a subcutaneous abscess (Genbank accession number
294 NC_017550).

Acknowledgments

EB, JL and HL are funded by the NIH grant R01GM099530 from NIGMS awarded to HL. JL is also supported by the Ruth L. Kirschstein National Research Service Award AI007323. This work was also supported by a grant of £11.5M awarded to Professor Tony Bjourson from European Union Regional Development Fund (ERDF) EU Sustainable Competitiveness Programme for N. Ireland; Northern Ireland Public Health Agency (HSC R&D) & Ulster University.

302 ## References

303 **Bae,Y., Ito, T., Iida, T., Uchida, K., Sekine, M., Nakajima, Y., Kumagai, J., Yokoyama, T., Kawachi, H.,**
304 **& other authors (2014).** Intracellular *Propionibacterium acnes* infection in glandular epithelium and
305 stromal macrophages of the prostate with or without cancer. *PLoS ONE* **9**: e90324.

306 **Barnard, E., Liu, J., Yankova, E., Cavalcanti, S. M., Magalhães, M., Li, H., Patrick, S. & McDowell, A.**
307 **(2016).** Strains of the *Propionibacterium acnes* type III lineage are associated with the skin condition
308 progressive macular hypomelanosis. *Sci Rep* **6**, 31968.

309 **Brenner, D., Stanley, J. & Krieg, N. (2000)**. Classification of prokaryotic organisms and the concept of
310 bacterial speciation. *In:* Boone, D. R., Castenholz, W. & Garrity, G. M. (eds.) *Bergey's manual of*
311 *systematic bacteriology.* 2 ed. New York, NY: Springer.

312 **Brüggemann, H., Lomholt, H. B. & Kilian, M. (2012a)**. The flexible gene pool of *Propionibacterium*
313 *acnes*. *Mob Genet Elements,* **2,** 145-148.

314 **Brüggemann, H., Lomholt, H. B., Tettelin, H. & Kilian, M. (2012b).** CRISPR/cas loci of type II
315 *Propionibacterium acnes* confer immunity against acquisition of mobile elements present in type I P.
316 acnes. *PLoS ONE,* **7,** e34171.

317 **Cavalcanti, S. M., De França, E. R., Lins, A. K., Magalhães, M., De Alencar, E. R. & Magalhães, V.**
318 **(2011).** Investigation of *Propionibacterium acnes* in progressive macular hypomelanosis using real-
319 time PCR and culture. *Int J Dermatol,* **50,** 1347-1352.

320 **Cohan, F. (2001).** Bacterial species and speciation. *Syst Biol,* **50**, 513–524.

321 **Cohen, R. J., Shannon, B. A., Mcneal, J. E., Shannon, T. & Garrett, K. L. (2005)**. *Propionibacterium*
322 *acnes* associated with inflammation in radical prostatectomy specimens: a possible link to cancer
323 evolution? *J Urol,* **173,** 1969-1974.

324 **Dekio, I., Culak, R., Misra, R., Gaulton, T., Fang, M., Sakamoto, M., Ohkuma, M., Oshima, K., Hattori,**
325 **M., & other authors (2015)**. Dissecting the taxonomic heterogeneity within *Propionibacterium acnes*:
326 proposal for *Propionibacterium acne*s subsp. acnes subsp. nov. and *Propionibacterium acnes* subsp.
327 elongatum subsp. nov. *Int J Syst Evol Microbiol,* **65,** 4776-4787.

328 **Edgar, R. C. (2004)**. MUSCLE: multiple sequence alignment with high accuracy and high throughput.
329 *Nucleic Acids Res* **32**, 1792–1797.

330 **Eishi, Y. (2013)**. Etiologic link between sarcoidosis and *Propionibacterium acnes*. *Respir Investig,* **51,**
331 56-68.

332 **Fitz-Gibbon, S., Tomida, S., Chiu, B. H., Nguyen, L., Du, C., Liu, M., Elashoff, D., Erfe, M. C., Loncaric,**
333 **A., & other authors (2013)**. *Propionibacterium acnes* strain populations in the human skin microbiome
334 associated with acne. *J Invest Dermatol,* **133,** 2152-2160.

335 **Hauck, Y., Soler, C., Gérôme, P., Vong, R., Macnab, C., Appere, G., Vergnaud, G. & Pourcel, C. (2015).**
336 A novel multiple locus variable number of tandem repeat (VNTR) analysis (MLVA) method for
337 *Propionibacterium acnes*. *Infect Genet Evol,* **33,** 233-241.

338 **Holland, C., Mak, T. N., Zimny-Arndt, U., Schmid, M., Meyer, T. F., Jungblut, P. R. & Brüggemann, H.**
339 **(2010)**. Proteomic identification of secreted proteins of *Propionibacterium acnes*. *BMC Microbiol,* **10,**
340 230.

341 **Huson, D. H. & Bryant, D. (2006).** Application of phylogenetic networks in evolutionary studies. *Mol*
342 *Biol Evol,* **23,** 254-267.

343 **Johnson, J. L. & Cummins, C. S. (1972)**. Cell wall composition and deoxyribonucleic acid similarities
344 among the anaerobic coryneforms, classical propionibacteria, and strains of Arachnia propionica. *J*
345 *Bacteriol,* **109,** 1047-1066.

346 **Johnson, T., Kang, D., Barnard, E. & Li, H. (2016)**. Strain-Level differences in porphyrin production and
347 regulation in *Propionibacterium acnes* elucidate disease associations. mSphere 1:e00023-15.

348 **Kasimatis, G., Fitz-Gibbon, S., Tomida, S., Wong, M. & Li, H. (2013)**. Analysis of complete genomes of
349 *Propionibacterium acnes* reveals a novel plasmid and increased pseudogenes in an acne associated
350 strain. *Biomed Res Int* **2013**, 918320

351 **Kumar, S., Stecher, G., & Tamura, K. (2016).** MEGA7: Molecular Evolutionary Analysis version 7.0 for
352 bigger datasets. Mol Biol Evol, 33, 1870-1874

353 **Lapage, S. P., Sneath, P. H. A., Lessel, E. F., Skerman, V. B. D., Seeliger, H. P. R. & Clark, W. A. (1992).**
354 *International Code of Nomenclature of Bacteria (1990 revision). Bacteriological Code,* Washington
355 D.C., ASM Press.

356 **Liu, J., Yan, R., Zhong, Q., Bangayan, N. J., Nguyen, L., Lui, T., Liu, M., Erfe, M.C., Craft, N., & other**
357 **authors (2015).** The diversity and host interactions of *Propionibacterium acnes* bacteriophages on
358 human skin. *ISME J*, **9**, 2078-2093.

359 **Lomholt, H. B. & Kilian, M. (2010).** Population genetic analysis of *Propionibacterium acnes* identifies
360 a subpopulation and epidemic clones associated with acne. *PLoS One,* **5,** e12277.

361 **Majewski, J. (2001).** Sexual isolation in bacteria. *FEMS Microbiol Lett,* **199,** 161-169.

362 **McDowell, A., Barnard, E., Nagy, I., Gao, A., Tomida, S., Li, H., Eady, A., Cove, J., Nord, C. E. & Patrick,**
363 **S. (2012).** An expanded multilocus sequence typing scheme for *Propionibacterium acnes*: investigation
364 of 'pathogenic', 'commensal' and antibiotic resistant strains. *PLoS One,* **7,** e41480.

365 **McDowell, A., Gao, A., Barnard, E., Fink, C., Murray, P. I., Dowson, C. G., Nagy, I., Lambert, P. A. &**
366 **Patrick, S. (2011).** A novel multilocus sequence typing scheme for the opportunistic pathogen
367 *Propionibacterium acnes* and characterization of type I cell surface-associated antigens. *Microbiology,*
368 **157,** 1990-2003.

369 **McDowell, A., Nagy, I., Magyari, M., Barnard, E. & Patrick, S. (2013).** The opportunistic pathogen
370 *Propionibacterium acnes*: insights into typing, human disease, clonal diversification and CAMP factor
371 evolution. *PLoS One,* **8,** e70897.

372 **McDowell, A., Perry, A. L., Lambert, P. A. & Patrick, S. (2008).** A new phylogenetic group of
373 *Propionibacterium acnes*. *J Med Microbiol,* **57,** 218-224.

374 **McDowell, A., Valanne, S., Ramage, G., Tunney, M. M., Glenn, J. V., McLorinan, G. C., Bhatia, A.,**
375 **Maisonneuve, J. F., Lodes, M., & other authors (2005)**. *Propionibacterium acnes* types I and II
376 represent phylogenetically distinct groups. *J Clin Microbiol,* **43,** 326-334.

377 **Meier-Kolthoff, J. P., Hahnke, R. L., Petersen, J., Scheuner, C., Michael, V., Fiebig, A., Rohde, C.,**
378 **Rohde, M., Fartmann, B., & other authors (2014)**. Complete genome sequence of DSM 30083(T), the
379 type strain (U5/41(T)) of *Escherichia coli,* and a proposal for delineating subspecies in microbial
380 taxonomy. *Stand Genomic Sci,* **9,** 2.

381 **Nagy, E., Urbán, E., Becker, S., Kostrzewa, M., Vörös, A., Hunyadkürti, J. & Nagy, I. (2013)**. MALDI-
382 TOF MS fingerprinting facilitates rapid discrimination of phylotypes I, II and III of *Propionibacterium*
383 *acnes*. *Anaerobe,* **20,** 20-26.

384 **Nei, M. & Gojobori, T. (1986).** Simple methods for estimating the numbers of synonymous and
385 nonsynonymous nucleotide substitutions. *Mol Biol Evol*, **3**, 418–426.

386 **Niazi, S. A., Clarke, D., Do, T., Gilbert, S. C., Mannocci, F. & Beighton, D. (2010).** *Propionibacterium*
387 *acnes* and *Staphylococcus epidermidis* isolated from refractory endodontic lesions are opportunistic
388 pathogens. *J Clin Microbiol,* **48,** 3859-3869.

389 **Patrick, S. & McDowell, A. (2011).** The Propionibacteriaceae. *In:* Goodfellow, M., Kämpfer, P., Busse,
390 H.-J., Trujillo, M. E., Suzuki, K.-I., Ludwig, W. & Whitman, B. W. B. (eds.) *Bergey's Manual of Systematic*
391 *Bacteriology.* 2 ed. New York, NY: Springer.

392 **Perry, A. L., Worthington, T., Hilton, A. C., Lambert, P. A., Stirling, A. J. & Elliott, T. S. (2003)**. Analysis
393 of clinical isolates of *Propionibacterium acnes* by optimised RAPD. *FEMS Microbiol Lett,* **228,** 51-55.

394 **Petersen, R., Lomholt, H. B., Scholz, C. F. & Brüggemann, H. (2015)** Draft genome sequences of two
395 *Propionibacterium acnes* strains isolated from progressive macular hypomelanosis lesions of human
396 skin. *Genome Announc.* **3**, 6.

397 **Rollason, J., McDowell, A., Albert, H. B., Barnard, E., Worthington, T., Hilton, A. C., Vernallis, A.,**
398 **Patrick, S., Elliott, T. & Lambert, P. (2013)** Genotypic and antimicrobial characterisation of
399 *Propionibacterium acnes* isolates from surgically excised lumbar disc herniations. *Biomed Res Int*,
400 **2013**, 530382.

401 **Scholz, C. F., Brüggemann, H., Lomholt, H. B., Tettelin, H. & Kilian, M. (2016).** Genome stability of
402 *Propionibacterium acnes*: a comprehensive study of indels and homopolymeric tracts. *Sci Rep,* **6,**
403 20662.

404 **Scholz, C. F., Jensen, A., Lomholt, H. B., Brüggemann, H. & Kilian, M. (2014).** A novel high-resolution
405 single locus sequence typing scheme for mixed populations of *Propionibacterium acnes in vivo*. *PLoS*
406 *One,* **9,** e104199.

407 **Spratt, B. G. & Maiden, M. C. (1999).** Bacterial population genetics, evolution and epidemiology.
408 *Philos Trans R Soc Lond B Biol Sci,* **354,** 701-710.

409 **Stajich, J.E. (2002).** The Bioperl Toolkit: Perl Modules for the Life Sciences. *Genome Res*, **12**, 1611–
410 1618.

411 **Tomida, S., Nguyen, L., Chiu, B. H., Liu, J., Sodergren, E., Weinstock, G. M. & Li, H. (2013)**. Pan-genome
412 and comparative genome analyses of *Propionibacterium acnes* reveal its genomic diversity in the
413 healthy and diseased human skin microbiome. *mbio*, **4,** e00003-13.

414 **Tunney, M. M., Patrick, S., Curran, M. D., Ramage, G., Hanna, D., Nixon, J. R., Gorman, S. P., Davis,**
415 **R. I. & Anderson, N. (1999).** Detection of prosthetic hip infection at revision arthroplasty by
416 immunofluorescence microscopy and PCR amplification of the bacterial 16S rRNA gene. *J Clin*
417 *Microbiol,* **37,** 3281-3290.

418 **Valanne, S., McDowell, A., Ramage, G., Tunney, M. M., Einarsson, G. G., O'Hagan, S., Wisdom, G. B.,**
419 **Fairley, D., Bhatia, A., & other authors (2005).** CAMP factor homologues in *Propionibacterium acnes*:
420 a new protein family differentially expressed by types I and II. *Microbiology,* **151,** 1369-1379.

421 **Webster, G. F. & Cummins, C. S. (1978).** Use of bacteriophage typing to distinguish *Propionibacterium*
422 *acne* types I and II. *J Clin Microbiol,* **7,** 84-90.

423

424

Table 1. Genetic characteristics of *P. acnes* phylogroups

Genetic Grouping	p-distance (core SNPs)			*ds**	% unique core region SNPs
	Type I	Type II	Type III		
Type I	-	0.444	0.487	0.006 ± 0.001	26
Type II	0.444	-	0.470	0.005 ± 0.001	22
Type III	0.487	0.470	-	0.002 ± 0.001	24
Type I, II, III	-	-	-	0.024 ± 0.003	-

*Based on the analysis of concatenated $MLSA_8$ sequence data using the Nei-Gojobori method (Jukes-Cantor) in MEGA v5.0.

Table 2. **Key phenotypic similarities and differences between type I, II and III strains**

Characteristic*	Type I	Type II	Type III
Indole production	+	d+	+
Catalase activity	+	+	+
Nitrate reduction	+	+	d+
Gelatin liquefaction	+	+	-
Aesculin Hydrolysis	-	-	-
β-haemolysis (5d at 37°C)	d+	-	-
Neuraminidase	d+	-	-
Lipase	d+	d-	d+
L-pyrrolydonyl arylamidase	d+	d-	-
Pyruvate	d+	+	-
Fermentation of:			
Sorbitol	d+	-	-
Maltose	-	-	-
Sucrose	-	-	-
Glycerol	d+	d+	+
Ribose	d-	d+	-
Cell wall components			
Dermatan sulphate-binding adhesins	d+	-	-
A_2pm isomer	LL-	LL- (*meso*)	ND
Amino acids	Ala, Gly, Glu	Ala, Gly, Glu	ND
Sugars	Galactose, Glucose, Mannose	Glucose Mannose	

+90% isolates are positive; -90% isolates are negative; d+40-89% isolates positive; d-11-39% isolates are positive.

*Key phenotypic characteristics were compiled from the data of one or more of the following publications: McDowell et al. (2005), McDowell et al. (2008), Lomholt and Kilian (2010), Niazi et al. (2010), McDowell et al. (2011), Patrick and McDowell (2011), Dekio et al. (2015).

Figure Legends.

Fig. 1. Minimum evolution phylogenetic tree (MEGA v7.0) (Kumar et al., 2016) of concatenated gene sequences (4253 bp) from all STs currently represented in the $MLST_8$ database (http://pubmlst.org/pacnes/), and covering all major genetic divisions**.** Sequence input order was randomized, and bootstrapping resampling statistics were performed using 500 data sets. Bootstrap values (≥70%) are shown on the arms of the tree. Horizontal bar represents genetic distance. CC= clonal complex.

Fig. 2. Neighbour-net split graph (SplitsTree v4.14.4) of allelic profiles from all STs currently represented in the $MLST_8$ database (http://pubmlst.org/pacnes/), and covering all major genetic divisions (Huson and Bryant, 2006). A distance matrix was generated from the allelic profile data and saved in NEXUS format for input to SplitsTree. Parallelogram formations indicative of recombination/ reticulation events are evident within the major type I and II divisions.

Figure 1

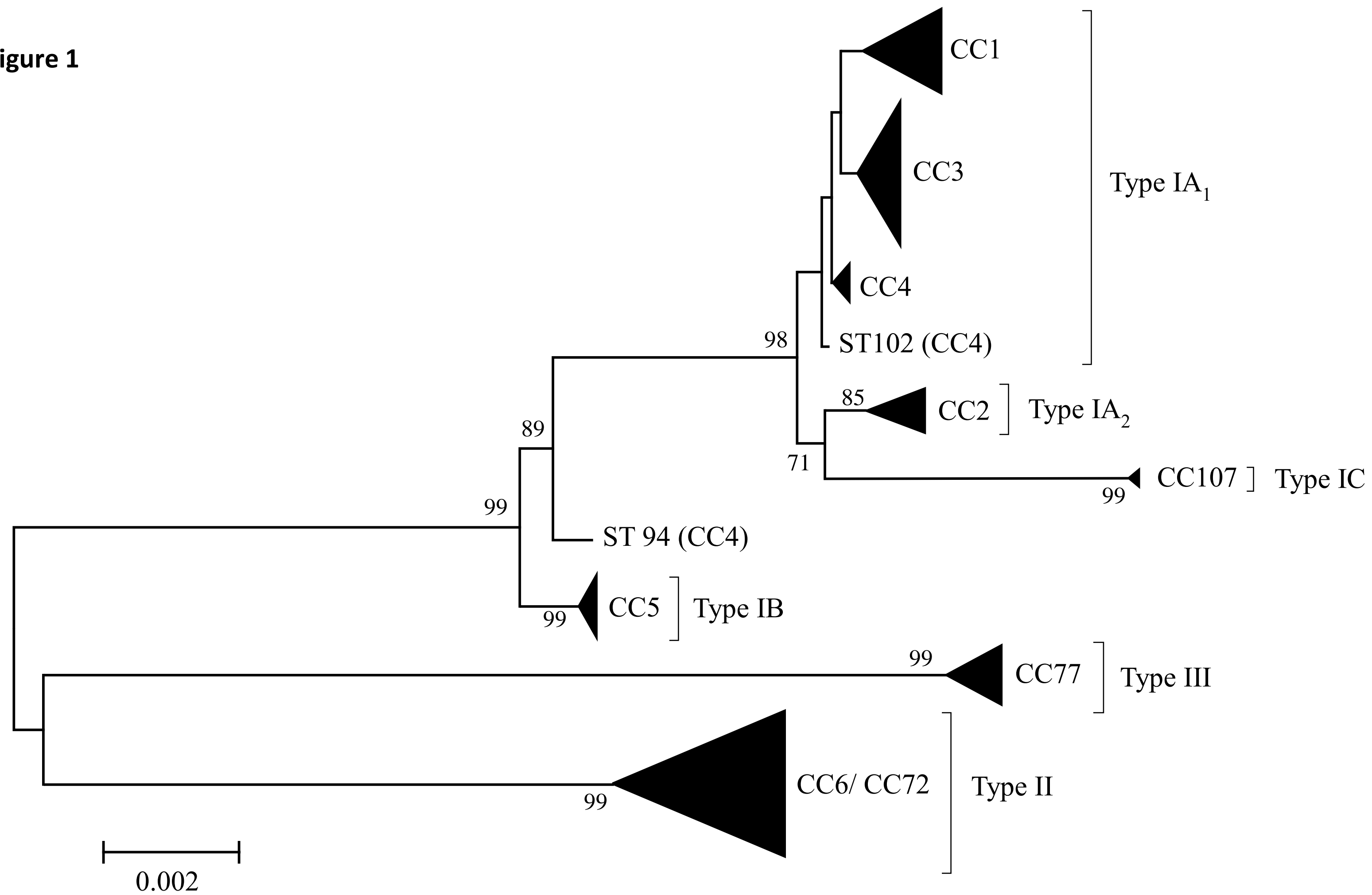

Figure 2