The Complete Genome Sequence of *Propionibacterium Acnes*, a Commensal of Human Skin

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trunk-ovisac junction, ovisac shape, and ramification of roots. Males of *O. frankpressi* are less than one-third the size of *O. rubiplumus* males. Male chaetae of *O. rubiplumus* have subrostral teeth and more capitium teeth.

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22. Phylogenetic analysis has its basis in 18S and 16S rDNA sequences. The amphinomid *Hipponoe* was chosen to root the tree. Additional polychaete sequences were obtained from GenBank. Ambiguously aligned sections of 18S rDNA were excluded from the analysis. PAUP*4.0b10 (D. Swofford, Florida State University) was used for maximum parsimony analysis with characters equally weighted and gaps treated as missing data. Bootstrap values were estimated with the use of 1000 heuristic searches. Bayesian analysis used MrBayes v3.0B4 (J. Huelsenbeck, University of Rochester, NY) with partitions for stems and loops using RNA secondary structure prediction via GeneBee (L. Brodsky, Moscow State University, Russia). Likelihood models were chosen with the use of ModelTest 3.06 (D. Posada, Universidad de Vigo, Spain). Further details are available at (*4*).

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Supporting Online Material

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Materials and Methods
Fig. S1
Tables S1 to S3

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The Complete Genome Sequence of *Propionibacterium Acnes*, a Commensal of Human Skin

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***Propionibacterium acnes* is a major inhabitant of adult human skin, where it resides within sebaceous follicles, usually as a harmless commensal although it has been implicated in acne vulgaris formation. The entire genome sequence of this Gram-positive bacterium encodes 2333 putative genes and revealed numerous gene products involved in degrading host molecules, including sialidases, neuraminidases, endoglycoceramidases, lipases, and pore-forming factors. Surface-associated and other immunogenic factors have been identified, which might be involved in triggering acne inflammation and other *P. acnes*–associated diseases.**

The details of the involvement of *Propionibacterium acnes* in acne—the most common skin disease, affecting up to 80% of all adolescents in the United States—are still obscure. Several mechanisms have been proposed to account for its role in the disease (*1*–*5*). First, damage to host tissues and cells might be accomplished by bacterial enzymes with degradative properties, such as lipases (*2*). Second, immunogenic factors of *P. acnes* such as surface determinants or heat shock proteins (HSPs) might trigger inflammation (*4*–*6*). Other diseases are also associated with *P. acnes*, including corneal ulcers; endocarditis; sarcoidosis; cholesterol gallstones; allergic alveolitis; pulmonary angitis; and synovitis, acne, pustulosis, hyperostosis, and osteitis (SAPHO) syndrome (*7*, *8*). Its genome sequence may provide a basis for finding alternative targets in therapy for acne and other *P. acnes*–associated diseases.

The genome of *P. acnes* strain KPA171202 (no. DSM 16379) consists of a single circular chromosome of 2,560,265 base pairs (*9*, *10*) (supporting online text and fig. S1). We predicted and annotated 2333 putative genes. The sequenced strain exhibited 100% identity on the 16S ribosomal RNA level to several clinical *P. acnes* isolates, as well as to the well-studied laboratory strain P-37. The main features of the genome sequence and comparative analyses are described in the supporting online material (supporting online text and fig. S2).

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The genome sequence offers insights into the traits that favor *P. acnes* as a ubiquitous commensal on human skin. Metabolic reconstruction reveals a capacity to cope with changing oxygen tensions, which confirms observations that strains of *P. acnes* can grow under microaerobic as well as anaerobic conditions. The genome sequence encodes all key components of oxidative phosphorylation that employs two terminal oxidases, a cytochrome aa_3 oxidase (PPA701/702) and a cytochrome d oxidase (PPA173-176), and a F_0F_1-type adenosine triphosphate synthase (PPA1238-1245). All genes of the Embden-Meyerhof pathway, the pentose phosphate pathway, and the tricarboxylic acid cycle are present. Under anaerobic conditions, strain KPA171202 can grow on several substrates such as glucose, ribose, fructose, mannitol, trehalose, mannose, *N*-acetylglucosamine, erythritol, and glycerol (*11*). In addition, several amino acid degrading pathways, similar to those of fermentative organisms, are present. Fermentative products are short-chain fatty acids, in particular propionic acid (*11*), whose production from pyruvate and methylmalonyl–coenzyme (CoA) is initialized by the methylmalonyl-CoA carboxyltransferase (PPA2005-2008). In addition to fermentative energy conservation, *P. acnes* possesses systems involved in anaerobic respiration such as nitrate reductase (PPA507-511), dimethyl sulfoxide reductase (PPA515-517), *sn*-glycerol-3-phosphate dehydrogenase (PPA2248-2250), and fumarate reductase (PPA950-952 and PPA1437-1439). Factors that might be involved in the life-style switch related to oxygen availability, as well as further aspects of the biology and biochemistry of *P. acnes*, are presented in the supporting online text.

Numerous genes have been found that can degrade and use host-derived substances (Table 1 and fig. S3). It has been proposed that free fatty acids, produced by *P. acnes* lipase activity on sebum, assist bacterial adherence and colonization of the sebaceous follicle (*2*, *12*). In

addition to the previously identified gene for the secreted triacylglycerol lipase (PPA2105), various other lipase/esterase genes can be found in the genome. Three of these possess a C-terminal Leu-Pro-X-Thr-Gly (LPXTG)–type cell-wall sorting signal. As previously known, a hyaluronate lyase (PPA380) degrades hyaluronan, an important constituent of the extracellular matrix of connective tissues, and presumably aids bacterial invasion (*13*, *14*). Genome sequencing revealed numerous additional enzymes putatively involved in host tissue degradation, such as two endoglycoceramidases (PPA644 and PPA2106) and four sialidases, two of them possessing a LPXTG motif (PPA1560 and PPA1569). Other degradative enzymes include a putative endo-β-*N*-acetylglucosaminidase (PPA990) and various extracellular peptidases. Five highly similar genes encoding homologs to CAMP (Christie, Atkins, Munch-Peterson) factors are also present in the genome of *P. acnes* (fig. S4). These factors are secreted proteins characteristic of some streptococcal species. CAMP factors have been shown to bind to immunoglobulins of the G and M classes and have long been known as pathogenic determinants. Recently, it was reported that CAMP factors can act as pore-forming toxins (*15*). These proteins could explain previously observed cytotoxic effects of *P. acnes* strains (*16*).

In several studies, the capability of *P. acnes* to interact with and stimulate the immune system has been investigated. Increased cellular, as well as humoral, immunity to *P. acnes* has been detected in patients with severe acne (*3*–*5*). The genome sequence encodes many factors with antigenic potential, for example, cell surface proteins that may also exhibit cell-adherent properties (Table 1 and fig. S3). Several of these (PPA1879-1881, PPA1955, PPA2127, and PPA2210) possess a C-terminal LPXTG-type cell-wall sorting signal that is required for attaching surface proteins to the cell-wall through the action of sortase (possibly encoded by candidate: PPA777), a mechanism employed by many Gram-positive bacteria (*17*). In all, 25 genes encoding proteins with a C-terminal LPXTG motif could be found in the genome. A few of these (PPA1880, PPA2127, and PPA2130) possess contiguous stretches of guanidine (G) or cytosine (C) residues, either in the putative promoter region or within the 5′ end. The sequences of several plasmids (of the shotgun library) that cover these regions were am-

Table 1. Selected factors of *P. acnes* that are putatively involved in degrading host molecules, conferring cell adhesion and/or mediating inflammation. ORF, open reading frame.

ORF number(s)	Function	Comment	Homology
380	Hyaluronate lyase	Polysaccharide lyase family protein	94% identity to *P. acnes* clone 49/51
570, 1035, 1101, 1224, 1425, 1631, 1745, 1761, 1839, 1953, 1967, 2036, 2142, 2150, etc.	Putative lipases/esterases	ORFs 570, 1745, and 2150 have a C-terminal LPXTG motif	Diverse
644, 2106	Endoglycoceramidases	Hydrolyzes glycosphingolipids	*Rhodococcus* sp., *Cyanea nozakii*
684	Sialidase L	Trans-sialidase	*Macrobdella decora* (leech)
685	Sialidase A	Exo-α-sialidase	*Clostridium perfringens*, *C. septicum*
687, 1198, 1231, 1340, 2108	CAMP factors	Immunoglobulin-binding, pore-forming toxin	*Streptococcus* species
990	Endo-β-N-acetylglucosaminidase		*Streptomyces plicatus*
1396	Putative hemolysin		*M. ulcerans*, *M. leprae*
1560, 1569	Sialidases/neuraminidases	With LPXTG motif	*Micromonospora viridifaciens*
1796, 2105	Triacylglycerol lipases	ORF 2105 is 100% identical to *gehA*	*P. acnes* P-37
1819–1821	Putative chitobiase/ β-N-acetylhexosaminidase	Possible frameshift, with LPXTG motif	*Arthrobacter*
2139	Putative cutinase		*Botryotinia fuckeliana*
109	Myosin-crossreactive antigen		*Rhodopseudomonas palustris*
125–134, 145–150, 1692–1700, 1185, 1791, 2181	Glycosyltransferases, uridine diphosphate–*N*-acetylglucosamine 2-epimerase, polysaccharide biosynthesis proteins	Slime/capsular polysaccharide biosynthesis	*Staphylococcus aureus* and others
453, 1772, 1773	GroEL, Cpn10	The homolog of ORF 737 is a major immune reactive protein in mycobacteria	100% identity to GroEL and DnaK of *P. acnes* strain P-37
916, 2038	DnaJ2, DnaJ		
2039	GrpE		
2040, 1098	DnaK		
737	18-kD antigen		
721, 1962	Homologs of invasion-associated protein p60	NlpC/P60 family	*Listeria welshimeri*
571	Putative secreted surface protein	WD domain, senescence marker protein-30 domain	
765	Immunogenic protein, antigen 84	*divIVA* domain	*Corynebacterium efficiens*, *M. leprae*, *M. tuberculosis*
1983, 1984, 1906–1908, 1663–1666	Putative adhesions	Thrombospondin type 3 repeats, PKD and SEST domains	
1955	Surface-associated protein	RTX toxin domain, LPXTG motif	
1879, 1880, 1881, 2127	PTRPs	Putatively regulated by phase variation, LPXTG motifs	
1715, 2210, 2270	PTRPs	ORF 2210 has a LPXTG motif	
2130	Outer membrane protein A family protein	Putatively regulated by phase variation	

biguous with respect to the length of the poly(C)/(G) stretches (supporting online text and figs. S5 and S6). Such variable homopolymeric C or G stretches, generated in the course of replication because of slipped-strand mispairing, have been reported in other species to be involved in phase variation, an adaptation strategy to generate phenotypic variation (*18*) (supporting online text). Although showing no similarity to database entries, PPA1880 and PPA2127 both contain characteristic multiple repeats of the dipeptide proline-threonine [PT repetitive protein (PTRP)]. Such PT repeats have been detected in antigenic proteins of *Mycobacterium tuberculosis* (*19*). Additional PTRPs (PPA1715, PPA2210, and PPA2270) were found in *P. acnes* with characteristics of surface proteins, which may represent further host-interacting factors (Table 1 and supporting online text).

Several HSPs have been identified as major targets of the immune response in bacterial pathogens. Homologs of GroEL and DnaK were found and characterized in *P. acnes* (*6*). The genome sequence contains several more HSPs, such as DnaJ (PPA916 and PPA2038), GrpE (PPA2039), and an 18-kD protein (PPA737), the homologs of which in mycobacteria are major immune reactive proteins (*20*). Further proteins show similarities to known bacterial immunogenic factors, such as PPA765, to antigen 84 of *M. tuberculosis* and *M. leprae*, which is a highly immunogenic protein involved in the symptoms of multibacillary leprosy (*21*). Porphyrins are produced by *P. acnes* in high amounts, and these are also thought to be involved in inflammation (*1*) (supporting online text). In the presence of increasing oxygen tension, the interaction of molecular oxygen with released porphyrins generates toxic, reduced oxygen species, which can damage keratinocytes and lead to cytokine release (fig. S7).

In summary, the genome sequence clearly reveals many proteins involved in the ability of *P. acnes* to colonize and reside in human skin sites as well as a pronounced potential to survive a spectrum of environments. This capacity helps to explain the ubiquity of *P. acnes* and also its potential hazards, for example, the public health problems associated with Blood Bank contaminations, or the contamination of the human genome sequence database with *P. acnes* sequence. The GenBank entry AAH14236.1, a proposed human protein, is in fact a *P. acnes* protein (PPA1069).

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Materials and Methods
SOM Text
Figs. S1 to S7
Table S1
References and Notes

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Synthetic Mammalian Prions

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Recombinant mouse prion protein (recMoPrP) produced in *Escherichia coli* was polymerized into amyloid fibrils that represent a subset of β sheet–rich structures. Fibrils consisting of recMoPrP(89–230) were inoculated intracerebrally into transgenic (Tg) mice expressing MoPrP(89–231). The mice developed neurologic dysfunction between 380 and 660 days after inoculation. Brain extracts showed protease-resistant PrP by Western blotting; these extracts transmitted disease to wild-type FVB mice and Tg mice overexpressing PrP, with incubation times of 150 and 90 days, respectively. Neuropathological findings suggest that a novel prion strain was created. Our results provide compelling evidence that prions are infectious proteins.

Prion diseases are responsible for some devastating neurological diseases, including Creutzfeldt-Jakob disease in humans, and present as infectious, genetic, and sporadic illnesses (*1*). The production of a new prion strain in Tg mice expressing an artificial, chimeric PrP transgene (*2*) encouraged us to renew our effort to produce synthetic wild-type prions. Earlier, we and others were unable to produce prion infectivity with the use of recombinant wild-type PrP refolded into β sheet–rich isoforms (*3*, *4*); hence, we turned to mutant PrPs.

Mice expressing high levels of MoPrP-(P101L), which harbors a mutation (Pro^{101} → Leu) analogous to the human prion protein mutation that causes Gerstmann-Sträussler-Scheinker syndrome, develop neurodegeneration spontaneously at an early age. Brain extracts prepared from these Tg mice transmit prion disease to Tg mice expressing low levels of MoPrP(P101L), designated Tg196 mice (*5*). About 30% of Tg196 mice develop spontaneous illness at ~550 days of age. We synthesized a 55–amino acid peptide composed of MoPrP residues 89 to 143 with the P101L mutation and folded it into a β-rich conformation. The aggregated peptide produced neurologic dysfunction in Tg196 mice within ~1 year after inoculation, whereas the non–β-rich form did not (*6*). The incubation time for these mutant prions did not change upon serial passage (*7*). Even though the disease-causing $MoPrP^{Sc}$(P101L) readily formed amyloid in the brains of Tg196 mice, resistance of the protein to limited proteolysis could be demonstrated only under "mild" digestion conditions.

Although PrP amyloid deposition in brain is pathognomonic of prion disease, it is a nonobligatory feature (*8*). Moreover, full-length PrP^{Sc} does not polymerize into amyloid, whereas N-terminally truncated PrP^{Sc} (designated PrP 27-30) assembles into amyloid fibrils (*9*). On the basis of the foregoing findings, we reasoned that

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