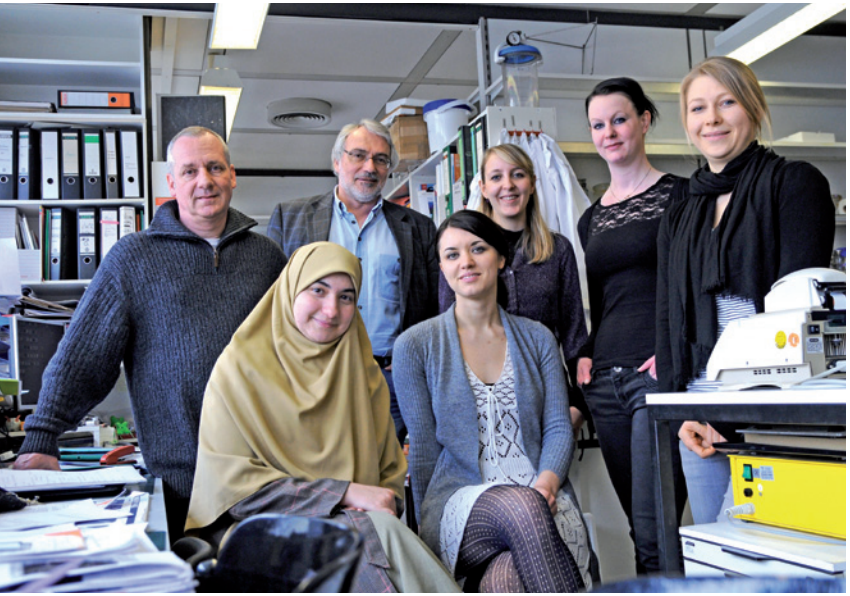

Institute of Microbiology and Biotechnology

Anaerobic Pathogens: Acne and Cancer Therapy

Head: Peter Dürre

Major projects involve spore formation, regulation of solvent formation in clostridia, development of gas fermentation by anaerobes as a novel biotechnological production platform, construction and application of recombinant clostridial endospores for cancer treatment, and identification of acne-causing enzymes in *Propionibacterium acnes* for selective inhibition and disease therapy. In molecular medicine, two projects are pursued. Clostridial endospores germinate only under hypoxic conditions found in mammals in the vicinity of tumors. Therefore, these endospores are ideally suited for targeting solid cancer structures. Apathogenic clostridia are provided with genes that encode tumor-attacking proteins, and the application of recombinant spores and their selective germination at the tumor allow multiplication there and specific therapy. *P. acnes* is a normal skin inhabitant and as an opportunistic pathogen it is also a major cause of acne vulgaris. This skin disease affects more than 85% of all teenagers. The complete genome of *P. acnes* has been sequenced and the annotation of the genome now opens the possibility of identifying factors responsible for pathogenesis and of looking for agents that specifically inhibit them. Another intention is to identify genes by encoding potential pathogenic factors transcribed in vivo. The aim is to classify acne-patients via expression analysis of genes by encoding potential pathogenic factors into the clinical classification of acne: acne vulgaris, comedonica and papulopustulosa.

Regulation of Carbon Metabolism in *Mycobacteria*

Head: Bernhard Eikmanns

Mycobacterium tuberculosis, the causative agent of tuberculosis, is able to persist within hosts for decades. It must adapt its carbon (C-) metabolism to tissue environments. It is assumed that the organism mainly subsists on fatty acids rather than on carbohydrates within the host. The aim of the project is to identify and analyze regulation of the genes by encoding the key enzymes of the central C-metabolism and to come to a better understanding of the coordination of the metabolism in *M. tuberculosis* and *M. bovis* BCG. We found the orf Rv0465c of *M. tuberculosis* in order to encode a protein with 56% identity to the transcriptional regulator RamB from *Corynebacterium glutamicum*, a well-studied organism used in the production of amino acids. We hypothesized that Rv0465c is an

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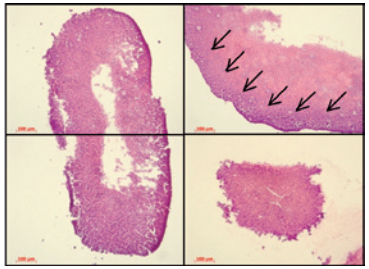
orthologue of *ramB* and involved in the regulation of the glyoxylate cycle genes, and possibly also in the regulation of other genes involved in fatty acid metabolism. We found that Rv0465c has a more specific regulatory function in *M. tuberculosis* than RamB in *C. glutamicum*. We now focus on other regulators involved in control of key enzymes in the C-metabolism of *M. bovis* BCG and *M. tuberculosis*.

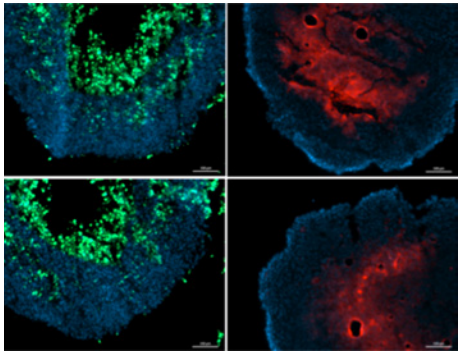
Tumor-Targeting with Bifidobacteria by Using Three-dimensional Tumor Models

Head: Christian Riedel

Bifidobacteria are Gram-positive, anaerobic bacteria of the normal human intestinal microbiota. They are able to colonize and replicate in hypoxic or necrotic regions of solid tumors following oral, intravenous or intratumoral application in animal models. Due to their non-pathogenic nature, genetically engineered bifidobacteria are promising candidates as life vectors for delivery and expression of therapeutic genes to inhibit tumor growth. We developed three-dimensional in vitro tumor models. Cryo-sections of three-dimensional tumors were stained and analyzed by microscopy for morphological and histological tumor characteristics. We could show that bifidobacteria survived in these three-dimensional in vitro tumors. In a first attempt to generate recombinant bifidobacteria for tumor therapy, cytosine deaminase was expressed in *B. bifidum* and *B. longum/infantis*. The recombinant strains metabolized the prodrug 5-fluorocytosine into the toxic substance 5-fluorouracil which inhibits DNA synthesis. Culture supernatants of the recombinant bifidobacterial strains showed an inhibitory effect on the growth of tumor cell lines.



H&E staining of cryo-sections of three-dimensional in vitro HT-29 tumors. The arrows indicate the outer layers of the tumors consisting of actively proliferating cells. This area is distinct from the inner core of the tumor consisting of necrotic tissue.



Apoptosis (TUNEL, green) and hypoxia staining (Pimonidazole, red) of cryo-sections of in vitro generated three-dimensional HT-29 tumors. Both stains label the inner core of the tumors indicating that hypoxia and apoptotic areas inside these tumors largely overlap and suggest that these areas especially may allow the survival of anaerobic bifidobacteria.

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Selected Publications:

- *Brüggemann H, Henne A, Hoster F, Liesegang H, Wiezer A, Strittmatter A, Hujer S, Dürre P, Gottschalk G (2004): The complete genome sequence of Propionibacterium acnes, a commensal of human skin. Science 305: 671-673.*
- *Theys J, Pennington O, Dubois L, Landuyt W, Anné J, Burke P, Anlezark G, Dürre P, Wouters BG, Minton NP, Lambin P (2006): Repeated systemic treatment cycles of Clostridium-directed enzyme prodrug therapy results in sustained anti-tumour effects in vivo. Br J Cancer 95: 1212-1219.*
- *Köpke M, Held C, Hujer S, Liesegang H, Wiezer A, Wollherr A, Ehrenreich A, Liebl W, Gottschalk G, Dürre P (2010): Clostridium ljungdahlii represents a microbial production platform based on syngas. Proc Natl Acad Sci USA 107: 13087-13092.*
- *Micklinghoff JC, Breitinger KJ, Schmidt M, Geffers R, Eikmanns BJ, Bange FC (2009): Role of transcriptional regulator RamB (Rv0456c) in the control of the glyoxylate cycle in Mycobacterium tuberculosis. J Bacteriol 191:7260-7269.*
- *Gleinser M, Grimm V, Zhurina D, Yuan J, Riedel CU (2012): Improved adhesive properties of recombinant bifidobacteria expressing the Bifidobacterium bifidum-specific lipoprotein BopA. Microb Cell Fact 11:80.*
- *Sun Z, Baur A, Zhurina D, Yuan J, Riedel CU (2012): Accessing the inaccessible: molecular tools for bifidobacteria. Appl Environ Microbiol 78:5035-5042.*