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Application of Microarrays in Process Analytical Technologies

A first step towards real time product release

Summary

Full implementation of **Process Analytical Technologies** (PAT) in a biopharmaceutical process makes real time product release feasible.[1]

On line process monitoring with **near infrared (nIR) spectroscopy** gives insight in product quality as it is formed.

Extensive process understanding is necessary to identify **critical quality attributes**. By monitoring these attributes with nIR, real time quality assurance and ultimately **real time product release** can be achieved.

Application of **microarrays** during process development helps to gain insight in biological processes involved in **product formation**, increasing **process understanding**.

The NVI is working on the **implementation** of this approach on the **cultivation process step** for the manufacturing of a whole cell vaccine against whooping cough disease, caused by the *Bordetella pertussis* bacterium.

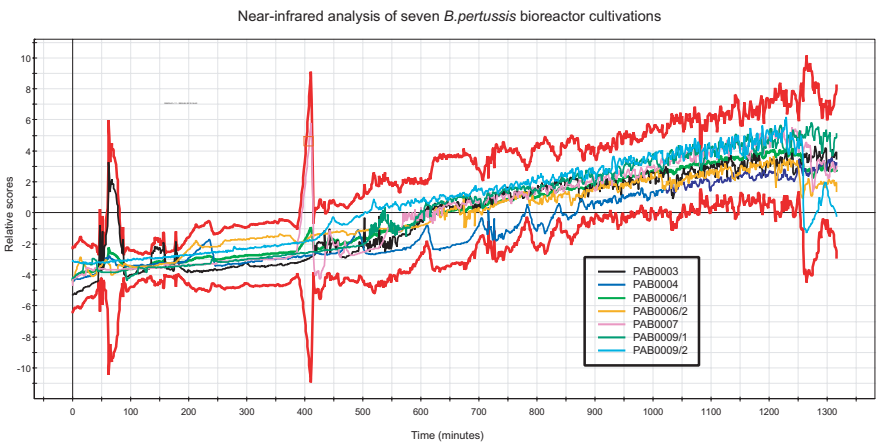


Figure 2. Near-infrared analysis of seven *B.pertussis* bioreactor cultivations with identical growth conditions. The *critical quality attributes* of each cultivation (*y*-axis) are plotted against time (*x*-axis), Statistical models (PCA, PLS analysis) were used to reduce the complex near-infrared spectrum to a single dot per time unit. The red lines indicates +/- 2 standard deviations.

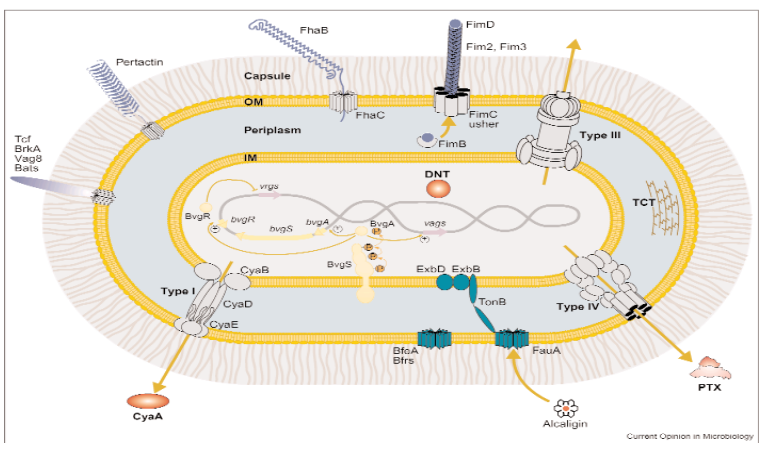
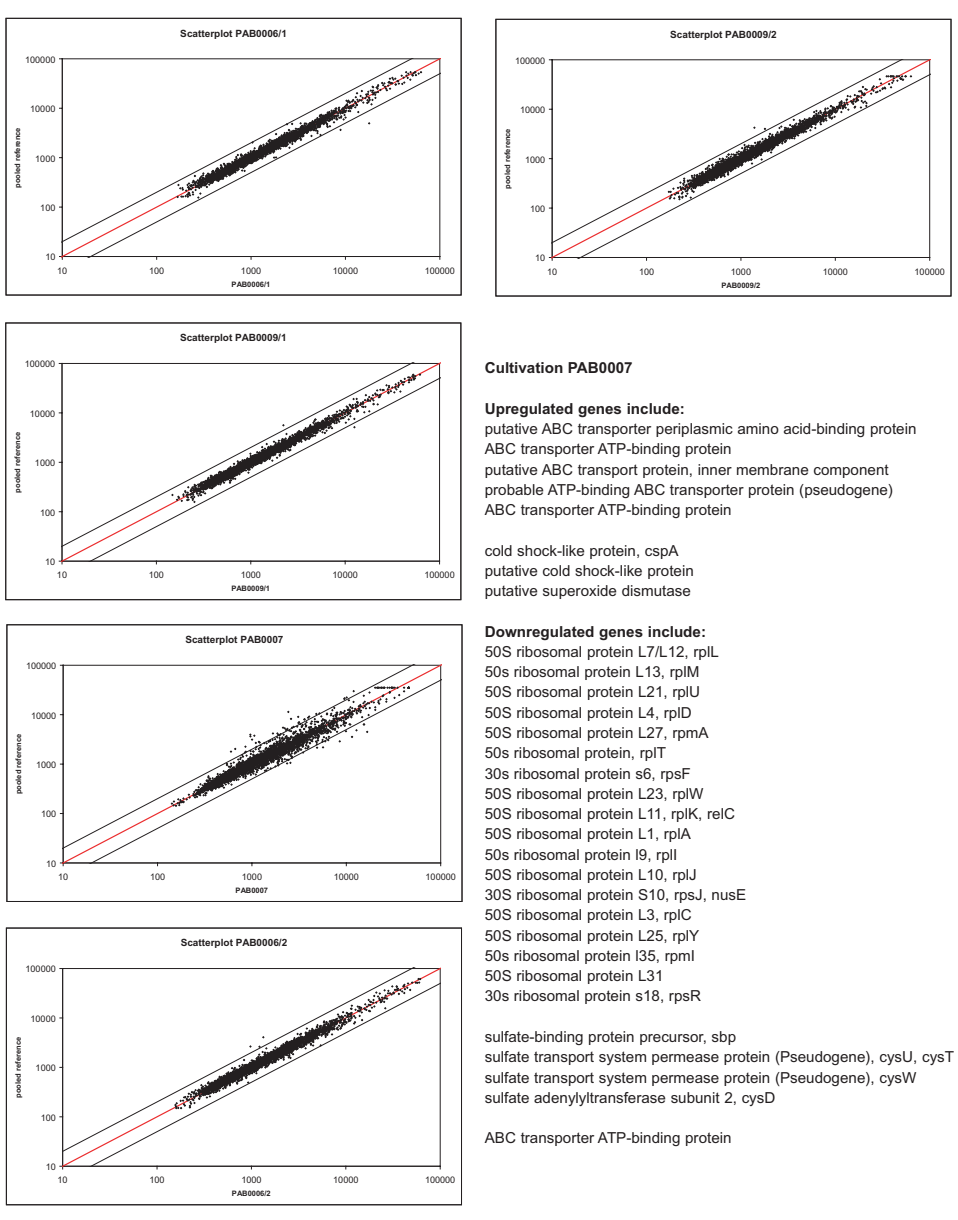


Figure 3. Schematic representation of the *B. pertussis* bacterial cell (the causative agent of whooping cough disease) and its most important virulence factors. All important virulence factors are regulated by the *bordetella virulence genes*-operon (*bvg*-operon) which is sensitive to environmental changes. Small disturbances during cultivation can switch off the *bvg*-operon, resulting in loss of protection inducing antigens on the cell surface. This severely compromises product quality.[1]



Cultivation PAB0007

Upregulated genes include:
putative ABC transporter periplasmic amino acid-binding protein
ABC transporter ATP-binding protein
putative ABC transport protein, inner membrane component
probable ATP-binding ABC transporter protein (pseudogene)
ABC transporter ATP-binding protein

cold shock-like protein, cspA
putative cold shock-like protein
putative superoxide dismutase

Downregulated genes include:
50S ribosomal protein L7/L12, rplL
50s ribosomal protein L13, rplM
50S ribosomal protein L21, rplU
50S ribosomal protein L4, rplD
50S ribosomal protein L27, rpmA
50s ribosomal protein, rplT
30s ribosomal protein s6, rpsF
50S ribosomal protein L23, rplW
50S ribosomal protein L11, rplK, relC
50S ribosomal protein L1, rplA
50s ribosomal protein l9, rplI
50S ribosomal protein L10, rplJ
30S ribosomal protein S10, rpsJ, nusE
50S ribosomal protein L3, rplC
50S ribosomal protein L25, rplY
50s ribosomal protein l35, rpmI
50S ribosomal protein L31
30s ribosomal protein s18, rpsR

sulfate-binding protein precursor, sbp
sulfate transport system permease protein (Pseudogene), cysU, cysT
sulfate transport system permease protein (Pseudogene), cysW
sulfate adenylyltransferase subunit 2, cysD

ABC transporter ATP-binding protein

Figure 1. Microarray analysis on samples from five *B. pertussus* bioreactor cultivations with identical growth conditions. Samples were taken at the end of the cultivations. These cultivations are also included in figure 2. mRNA-expression levels at the end of each cultivation were plotted per gene (*x*-axis) against the expression levels of the common reference, a mixture of mRNA from all five cultivations (*y*-axis). Differences in mRNA-expression result in a deviation from the red line (x=y). Cultivation PAB0007 shows the broadest scatter pattern. In the lower right panel, a selection of genes is presented that are either up or downregulated in this cultivation.

Conclusions

Near-infrared (nIR) monitoring shows clear **disturbances** at 60 minutes for PAB0003 and at 400 minutes for PAB0007 compared to other cultivations (figure 2). Both peaks coincide with **oxygen limitation** (dissolved oxygen <5% for 15 minutes). nIR monitoring provides online insight in how a process evolves compared to historical data

PAB0007 shows a **broader scatter pattern** in microarray analysis (figure 1), indicating a variation in gene expression. The genes involved indicate stress and a reduction in cellular activity. The effects of an oxygen limitation at the **beginning** of the cultivation have an effect on the mRNA expression levels at the **end** of the cultivation

Correlation between nIR monitoring data and microarray data during process development results in extensive **process understanding**. This is necessary to address problems associated with scale up and process optimisation.

Historical data can be combined and aligned to form a '**golden reference**'. This reference defines the **boundaries** within which product quality is assured. Online nIR data analysis allows **real time quality assurance** and ultimately **real time product release**.

Acknowledgements

We would like to thank Ingrid Maes and co-workers from Siemens Pharma Group (Zwijndrecht, Belgium) for their work on near-infrared data analysis.

Reference List

[2]Guidance for Industry: PAT - a Framework for Innovative Pharmaceutical Development, Manufacturing and Quality Assurance. http://www.fda.gov.

[1]Locht, Antoine and Jacob-Dubuisson, Bordetella pertussis, molecular pathogenesis under multiple aspects. Current Opinion in Microbiology 2001, 4:82–89.