# M2/L2: Production of Enzymes in bioreactors

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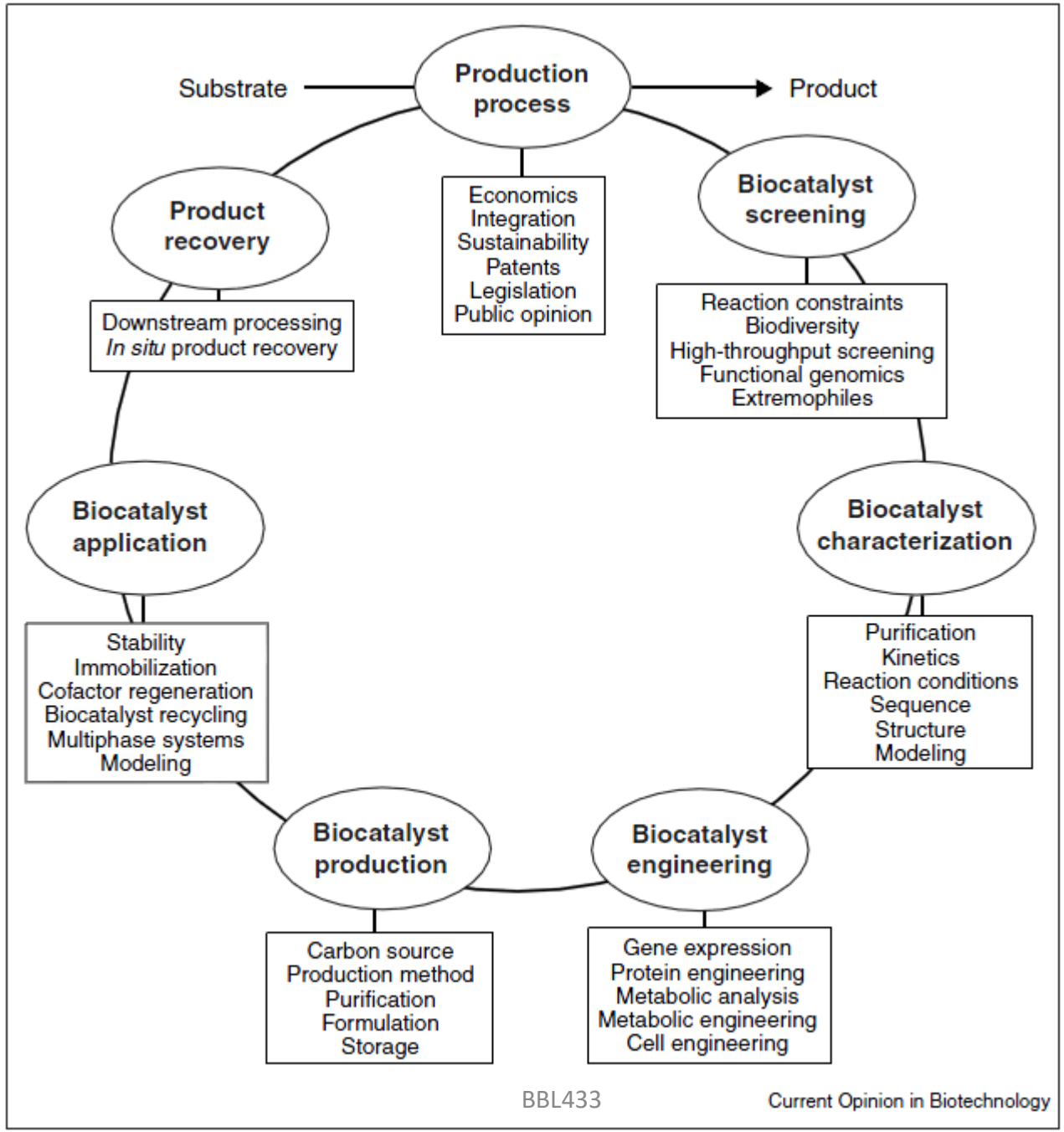
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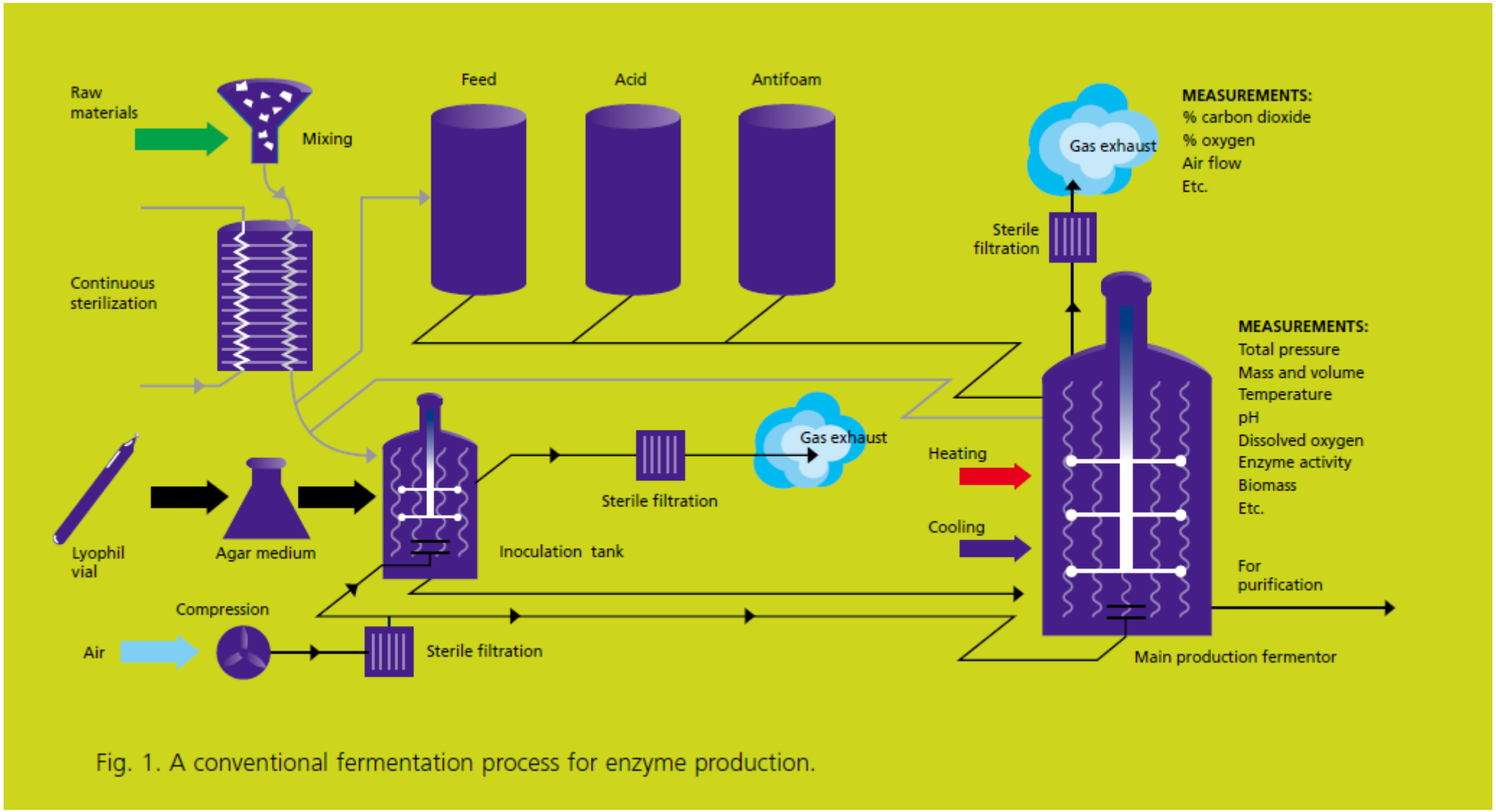



Fig. 1. A conventional fermentation process for enzyme production.

# Safety and GRAS status of microbial strain

- Genetic stability and safety of producing is microbial strain needs to be considered.
- Enzymes used in the food industry should have the GRAS (generally recognized as safe) status conferred by FDA.
- It is better to clone the enzyme structural gene into a GRAS host.
- Subsequently, structural and segregational instability of the cloning vector should be evaluated.

# Stages of enzyme production

The production process can be divided into four stages:

– Enzyme synthesis: it represents the propagation stage of the producing cells.

– Enzyme recovery: it represents the extraction of the enzyme from the producing cell system and involves solid–liquid separations, cell extraction and/or concentration.

– Enzyme purification: it represents a series of operations after enzyme recovery aiming to remove unwanted contaminants (mainly accompanying proteins).

– Enzyme product formulation: it consists in different operations aimed to give the enzyme product its final presentation; it includes final polishing operations, stabilization and standardization.

# Two methods of fermentation

- Submerged fermentation (SmF): The production of enzymes by microorganisms in a liquid nutrient media.

- Solid state fermentation (SSF): The cultivation of microorganisms for enzyme production is done on a solid or semi-solid substrate.

# Difference between SmF and SSF

| Parameters | SmF | SSF |
|---|---|---|
| Volumetric Productivity | Low | High |
| Product concentration | Low | High |
| Effluent generated | High | Low |
| Fermentation equipment | Complex | Simple |
| pH regulation | Easy | Difficult |
| Energy requirement | Low | High |

In most cases, SmF is the technology of choice as it is highly developed and automated for the production of most industrial enzymes

# Process flow chart for SmF

Addition of sterilised nutrients

↓

Inoculations with selected microorganism

↓

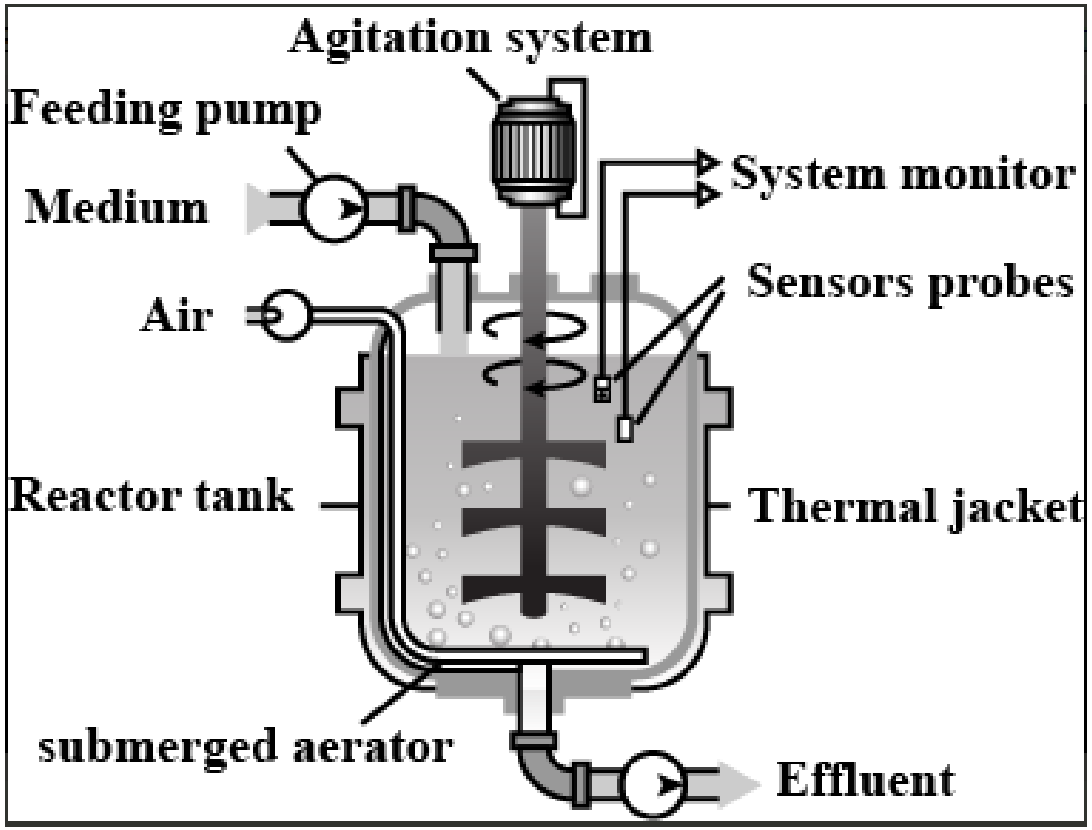
Fermentation in suitable environment

↓

Removal of biomass

↓

Extraction and purification of enzyme



# Bioreactor monitoring & process control

- SmF can be efficiently controlled in order to optimize productivity and product yield, and ensure reproducibility.
- The key physical and biochemical parameters involved are largely depend on:
  - The Bioreactor (Effect on mixing time and OTR)
  - Mode of operation (Effect on specific growth rate)
  - Microorganism being used (Effect on yield and productivity)
  - Type of production process (Inducible or constitutive)

# Type of enzyme production process

| Inducible Expression | Constitutive Expression |
|---|---|
| Regulated by promotor that allows transcription of gene only in response to specific stimuli. | Transcription of gene is unregulated and happens continuously in the cell |
| Chemical and physical factors such as alcohol, steroid, antibiotics, cold, heat etc can be the specific stimuli | Not affected by environmental and developmental factors |
| Enzyme production can be controlled | Enzyme production is active in all circumstances |

# Key factors influencing enzyme production

- Level of Inducer concentration
- Dissolved oxygen
- Temperature
- pH
- Carbon Source
- Nitrogen Source
- Off-gas composition

# Medium optimization

- Enzyme production is an energy intensive process and leads to metabolic burden on cellular environment.
- Transcription and translation rates must be balanced all the times.
- Enzyme production not only relies on good transcription but also the availability of charged tRNAs for the translation process to match the transcription rates.

# Medium optimization

- Since amino acids biosynthesis is the rate limiting as well as energy intensive step, medium optimization proves to have positive impact on enzyme production.
- In case of *De-novo* synthesis, genetic engineering of GOGAT pathway for increased ammonium ion assimilation along with non-repressing carbon sources has been shown to increase the amino acid biosynthesis rate.
- Thus the compromise of $\mu$ to attain maximum $Q_p$ can be adjusted with medium optimization.

REVIEW ARTICLE

# Toward minimal bacterial cells: evolution vs. design

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Received 27 June 2008; revised 29 September 2008; accepted 24 October 2008.
First published online December 2008.

DOI:10.1111/j.1574-6976.2008.00151.x

Editor: Michael Galperin

## Abstract

Recent technical and conceptual advances in the biological sciences opened the possibility of the construction of newly designed cells. In this paper we review the state of the art of cell engineering in the context of genome research, paying particular attention to what we can learn on naturally reduced genomes from either symbiotic or free living bacteria. Different minimal hypothetically viable cells can be defined on the basis of several computational and experimental approaches. Projects aiming at simplifying living cells converge with efforts to make synthetic genomes for minimal cells. The panorama of this particular view of synthetic biology lead us to consider the use of defined minimal cells to be applied in biomedical, bioremediation, or bioenergy application by taking advantage of existing naturally minimized cells.

DNA Research **15**, 73–81, (2008) doi:10.1093/dnares/dsn002

# Enhanced Recombinant Protein Productivity by Genome Reduction in *Bacillus subtilis*

Takuya Morimoto[1,2,†], Ryosuke Kadoya[1,2,†], Keiji Endo[1,†], Masatoshi Tohata[1], Kazuhisa Sawada[1], Shengao Liu[1], Tadahiro Ozawa[1], Takeko Kodama[1,3], Hiroshi Kakeshita[1,4], Yasushi Kageyama[1], Kenji Manabe[1], Shigehiko Kanaya[2], Katsutoshi Ara[1], Katsuya Ozaki[1], and Naotake Ogasawara[2,*]

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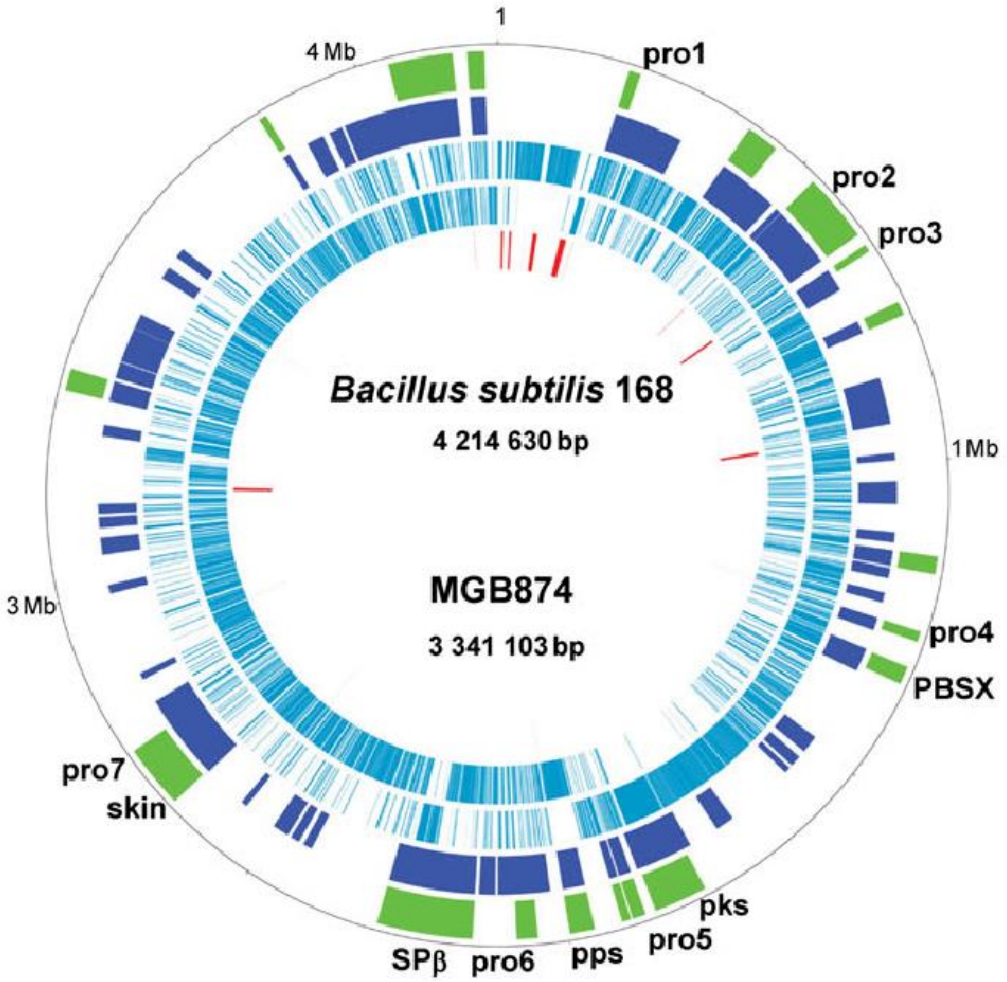
(Received 24 December 2007; accepted on 17 January 2008; published online 11 March 2008)

(A)

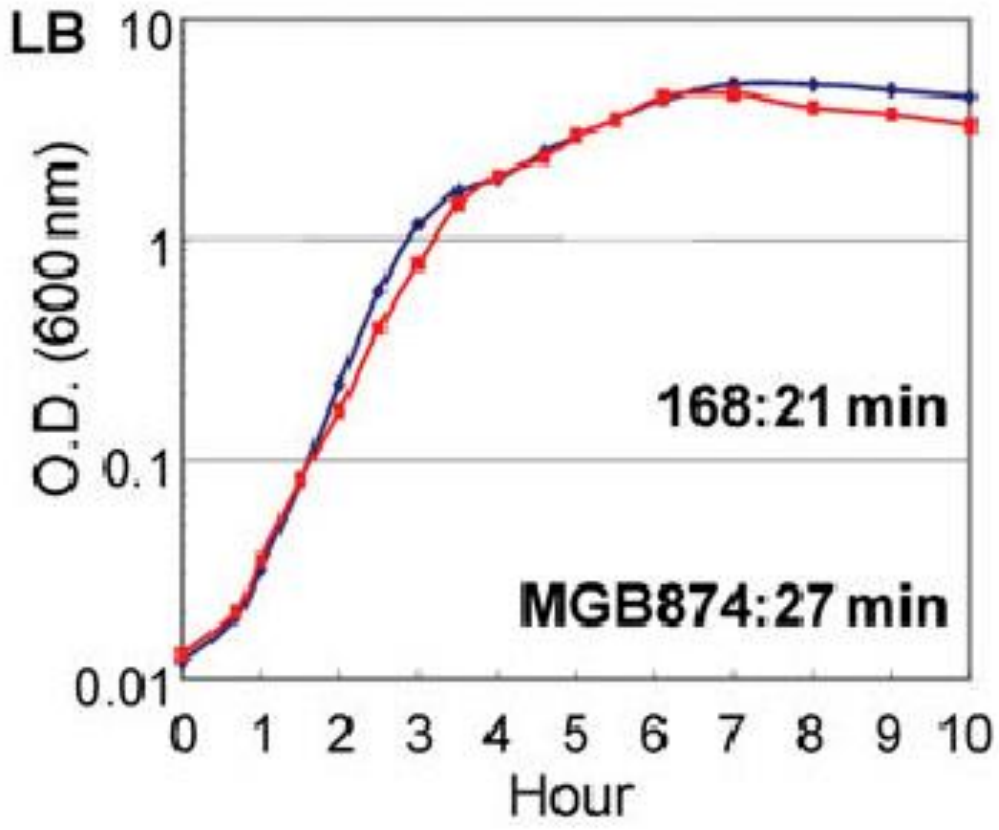


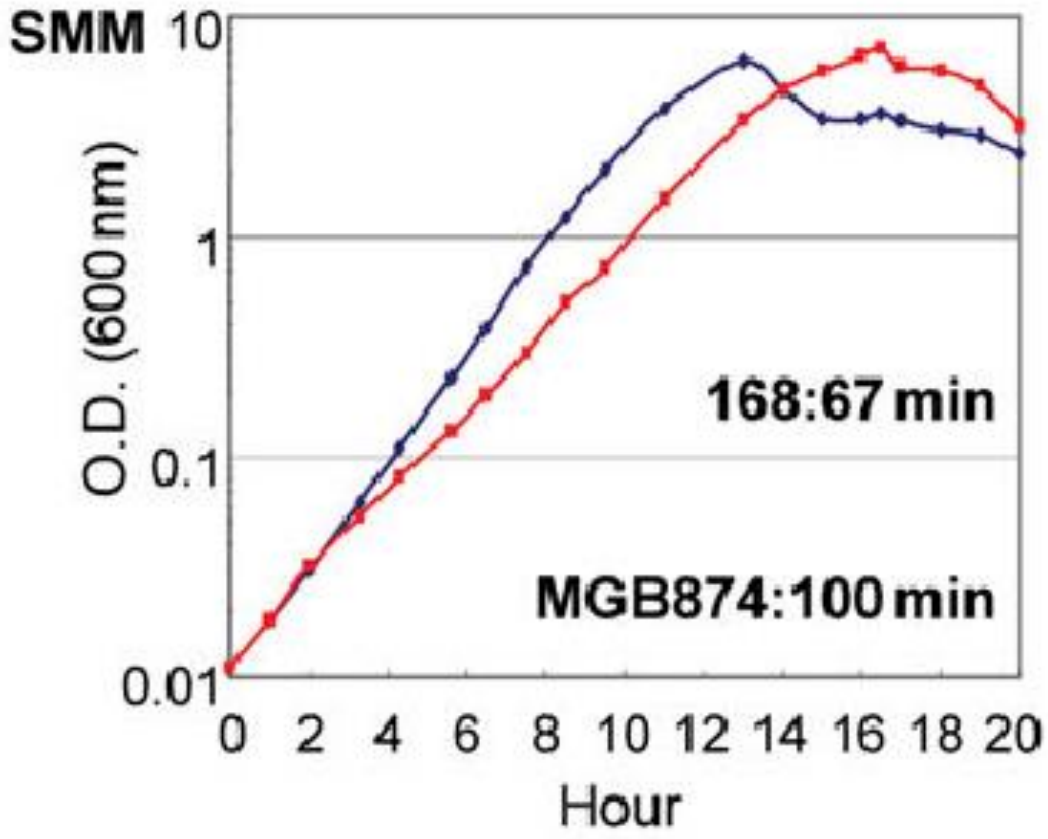

Outer concentric ring: genome coordinate (bases) of the B. subtilis 168 genome.

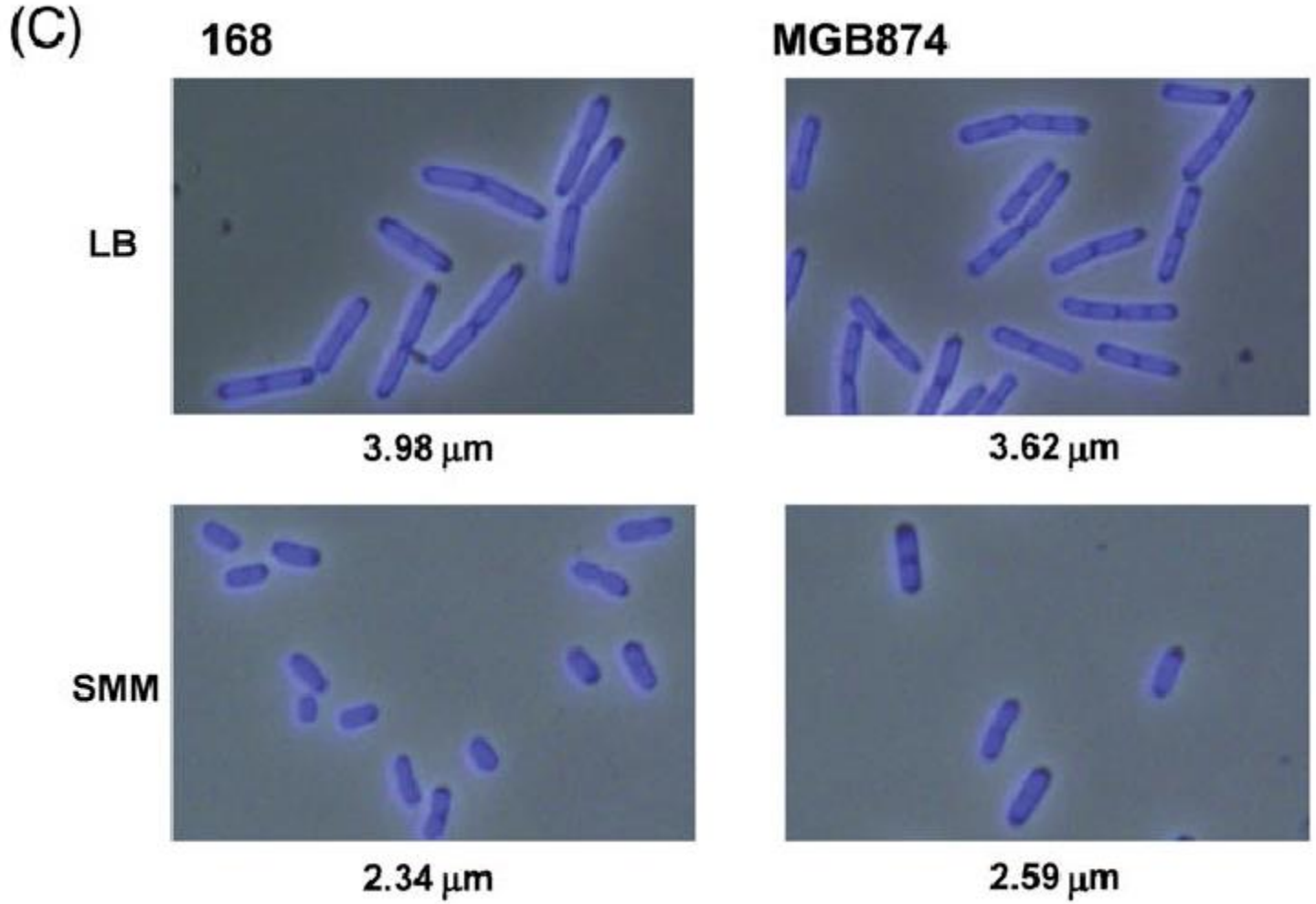
Ring 2 (green): positions of deleted sequences in MGB874, including prophages and prophage-like regions (SPb, PBSX, skin, pro1-7) and polyketide and plipastatin synthesis operons ( pks, pps).

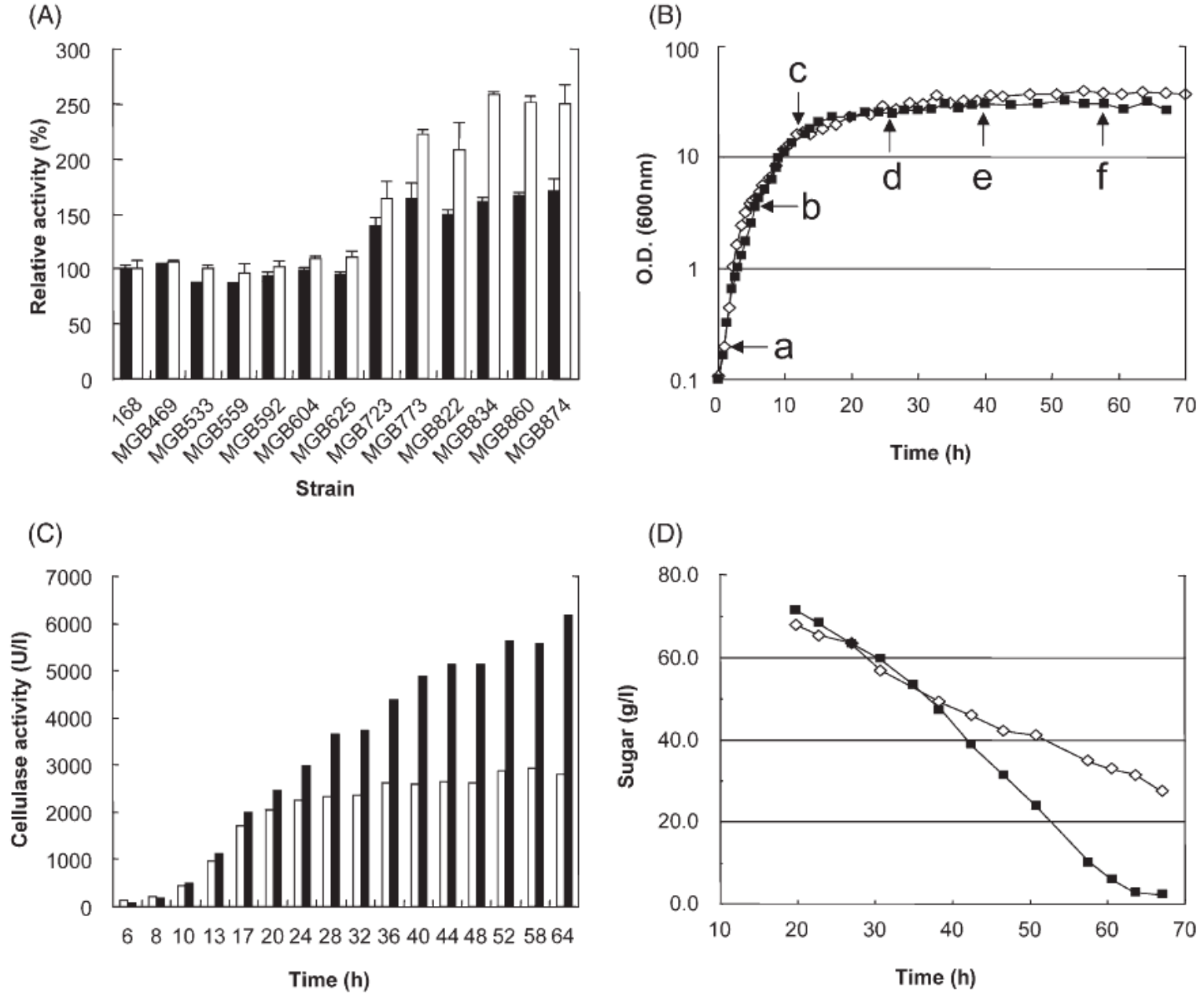
Ring 3 (dark blue): regions of single deletion. Rings 4 and 5 (light blue): protein coding regions in clockwise (Ring 4) and counterclockwise (Ring 5) orientations. Ring 6 (red): rRNA and tRNA
genes.

(B)


LB
O.D. (600 nm)
10
1
0.1
0.01
168:21 min
MGB874:27 min
0 1 2 3 4 5 6 7 8 9 10
Hour


SMM
O.D. (600 nm)
10
1
0.1
0.01
168:67 min
MGB874:100 min
0 2 4 6 8 10 12 14 16 18 20
Hour


(C)
168
MGB874
LB
3.98 μm
3.62 μm
SMM
2.34 μm
2.59 μm


(A)
Relative activity (%)
300
250
200
150
100
50
0
168
MGB469
MGB533
MGB559
MGB592
MGB604
MGB625
MGB723
MGB773
MGB822
MGB834
MGB860
MGB874
Strain
(B)
O.D. (600 nm)
100
10
1
0.1
a
b
c
d
e
f
0
10
20
30
40
50
60
70
Time (h)
(C)
Cellulase activity (U/l)
7000
6000
5000
4000
3000
2000
1000
0
6 8 10 13 17 20 24 28 32 36 40 44 48 52 58 64
Time (h)
(D)
Sugar (g/l)
80.0
60.0
40.0
20.0
0.0
10
20
30
40
50
60
70
Time (h)

Manabe *et al. Microbial Cell Factories* 2013, **12**:18
http://www.microbialcellfactories.com/content/12/1/18

MICROBIAL CELL FACTORIES

RESEARCH Open Access

# Improved production of secreted heterologous enzyme in *Bacillus subtilis* strain MGB874 via modification of glutamate metabolism and growth conditions

Kenji Manabe[1,2†], Yasushi Kageyama[1†], Takuya Morimoto[1], Eri Shimizu[3], Hiroki Takahashi[4], Shigehiko Kanaya[5], Katsutoshi Ara[6], Katsuya Ozaki[1*] and Naotake Ogasawara[2*]

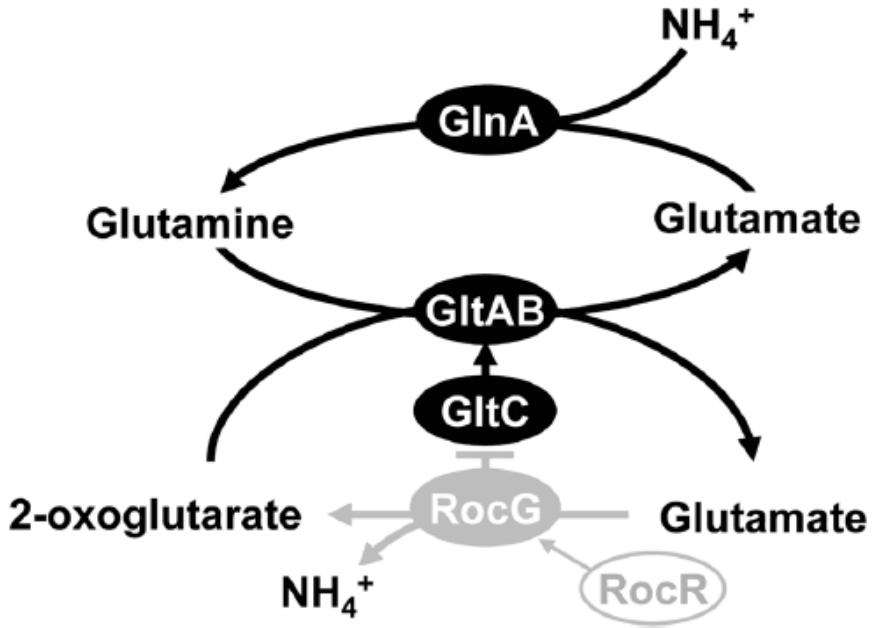



**Figure 1 Major reactions and regulation involved in glutamate metabolism in *B. subtilis*.** Proteins are shown as ovals. RocG, glutamate dehydrogenase; GltAB, glutamate synthase (GOGAT); GlnA, glutamine synthetase (GS). In *B. subtilis*, glutamate can be degraded by RocG. *B. subtilis* has a glutamine synthetase-glutamate synthase (GS-GOGAT) pathway for assimilation of ammonia. The RocR and GltC transcription factors positively regulate *rocG* and *gltAB*, respectively, and GltC can be inhibited via interaction with RocG. Open and closed gray ovals indicate proteins corresponding to genes that have been deleted or inactivated, respectively, in strain MGB874. Deletion of *rocR* in strain MGB874 decreases expression of *rocG*, which leads to an increase in expression of *gltAB* due to activation of GltC via disinhibition by RocG.

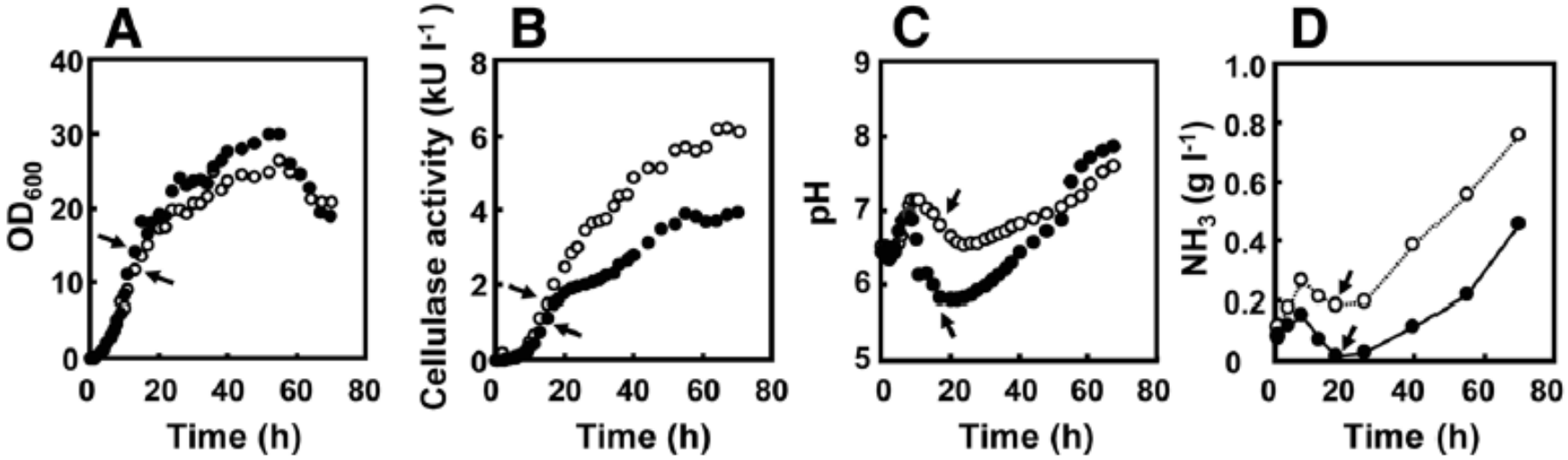



**Figure 2 Growth characteristics of strains MGB874 and 874ΔrocG producing the alkaline cellulase Egl-237.** Strains MGB874 (open circles) or MGB874ΔrocG (closed circles) were transformed with pHYS237 for production of alkaline cellulase Egl-237. The strains were cultured in 2xL medium containing 7.5% (w/v) maltose monohydrate by batch fermentation with a 30-L jar fermentor. Cell yield (**A**), extracellular cellulase activity (**B**), external pH of the growth media (**C**) and ammonia concentration in the growth media (**D**) were measured at the indicated times. Arrows indicate the point at which transcriptome analyses were conducted.

# Thank you