Metabolit Primer

Nur Hidayat

Pendahuluan

- Metabolit primer adalah metabolit yang dihasilkan dari proses metabolisme primer
- Primary metabolism involves an interrelated series of enzyme - mediated catabolic, amphibolic, and anabolic reactions which provide biosynthetic intermediates and energy, and convert biosynthetic precursors into essential macromolecules such as DNA, RNA, proteins, lipids, and polysaccharides
- Commercially, the most important primary metabolites were amino acids, vitamins, fl avor nucleotides, organic acids, and alcohols

Amino Acids

- Production of amino acids amounted to 2.3 million tons in 2002.
- The most important acids made at least partly by biological methods were glutamate (1.6 million tons per year), lysine - HCl (700 000 tons), threonine (70 000 tons), phenylalanine (13 000 tons, including that by chemical synthesis), aspartic acid (10 000 tons made enzymatically), and tryptophan (3000 tons including that made enzymatically

MSG

- Monosodium glutamate (MSG) is used as a potent fl avor enhancer.
- The glutamic acid fermentation was discovered in Japan in 1957 by Kinoshita, Udaka, and Shimono of the Kyowa Hakko Kogyo Company.
- MSG was manufactured using various species of the genera *Corynebacterium and Brevibacterium*

MSG

- Normally, glutamic acid overproduction would not be expected to occur because of feedback regulation.
- Glutamate feedback controls include repression of PEP carboxylase, citrate synthase, and NADP - glutamate dehydrogenase; the last – named enzyme is also inhibited by glutamate

MSG - Biotin

- Glutamate excretion was intentionally effected by various manipulations, such as limitation of biotin in *Corynebacterium glutamicum ; all glutamate overproducers* were natural biotin auxotrophs
- Biotin is a cofactor of acetyl - CoA carboxylase which is essential for biosynthesis of fatty acids

MSG

- The commonality in the various manipulations that were found to bring about high - level production of L - glutamic acid, that is, (i) limitation of biotin, (ii) addition of penicillin, or (iii) fatty acid surfactants (e.g., tween 60) to exponentially growing cells, was recognized and the permeability mechanism was strongly supported
- By genetic removal of homoserine dehydrogenase, a glutamate - producing wild - type *Corynebacterium was converted into a* lysine - overproducing mutant that could not grow unless methionine and threonine were added to the medium

MSG

- *E. coli strains were constructed with plasmids bearing amino acid biosynthetic* operons. Plasmid transformation was also accomplished in *Corynebacterium , Brevibacterium*, and *Serratia so that rDNA technology could be used to improve these* commercial amino acid - producing strains

Vitamins

- Vitamins are made at a rate of 70 000 tons per year by synthesis and fermentation.
- Of these, ribofl avin was produced by these two methods at an annual rate of 4000 tons. Most fungi produce enough ribofl avin (vitamin B 2) to satisfy their growth requirements, but a few were natural overproducers of this vitamin.
- This tendency of uncontrolled synthesis of riboflavin was found primarily in two species of fungi, *Eremothecium ashbyi and Ashbya gossypii .*

Vitamins

- *E. ashbyi was replaced in industry by the* more stable *A. gossypii which produced, after genetic manipulation, over 20 g/l* of the vitamin.
- An rDNA process was developed for ribofl avin in *Corynebacterium ammoniagenes* by cloning and overexpressing the organism ' s own ribofl avin biosynthesis genes and its own promoter sequences. The resulting culture produced 15 g/l riboflavin in 3 days.

Vitamins

- Genetic engineering of a *Bacillus subtilis strain already containing* purine analog - resistance mutations led to production of 15 g/l riboflavin
- Bacterial formation of vitamin B 12 by bacteria is a very old phenomenon
- In the late 1940s, a Merck scientist discovered that *Streptomyces griseus and Pseudomonas denitrifi cans could form vitamin B 12*
- Other studies showed that the vitamin could also be produced by *Propionibacterium shermanii as well as other* bacteria. *P. denitrifi cans and P. shermanii became the industrial producing organisms.*

Organic Acids

- Organic acids have been an important product of biotechnology
- The most important commercial organic acids are citric, acetic, and lactic acids.
- Fermentation processes are also available for production of succinic, gluconic, oxogluconic, pyruvic, itaconic, shikimic, malic, propionic, butyric, oxalic, kojic, fumaric, erythorbic, *trans - epoxysuccinic, tartaric, itatartaric, and long - chain α , ω - dicarboxylic acids.*

Citric Acid

- Citric acid production has historic significance since it was the first industrial fermentation to be developed. It had been exclusively produced via isolation from lemons.
- In 1916, citric acid production by black aspergilli was described by Charles Thom and J.N. Currie
- Mutants producing higher concentrations were obtained by Miles Laboratories, James *et al. and Hannan et al.*

Citric Acid

- About 1.5 million tons of citric acid are produced by *A. niger per year.*
- *The commercial* process employs *A. niger in media defi cient in iron and manganese.*
- Other factors contributing to high citric acid production were the inhibition of isocitrate dehydrogenase by citric acid, and the low pH optimum (1.7 – 2.0)
- Alternative processes were developed for the production of citric acid by *Candida yeasts, especially from hydrocarbons*

Acetic Acid

- The acetic acid bacteria are Gram - negative, obligately aerobic bacteria composed of species of *Acetobacter, Gluconoacetobacter, and Frateuria.*
- *Acetobacter suboxydans was used to produce vinegar as far back as 4000 BC*
- Industrial production of acetic acid was carried out solely by the conversion of sugar until the latter part of the 1800s when the distillation of wood became competitive with the fermentation process.
- Petroleum then became a major source of synthetic acetic acid

Acetic Acid

- Today, acetic acid fermentation is a two - step process in which the yeast *Saccharomyces cerevisiae converts glucose to ethanol and Acetobacter aceti produces* acetic acid from the ethanol.
- Cloning of aldehyde dehydrogenase from *Acetobacter polyoxogenes on a plasmid vector into A. aceti subsp. xylinum increased the rate of* acetic acid production by over 100% (1.8 g/l per h to 4 g/l per h) and titer by 40% (68 – 97 g/l)

Acetic Acid

- Another group of organisms considered for acetic acid production has been the anaerobic, thermophilic anaerobes of the genus *Clostridium .*
- *In 1940, Wieringa* isolated *Clostridium aceticum which was subsequently lost.*
- *However in 1942,* Fontaine *et al. isolated Clostridium thermoaceticum which converted sugar* quantitatively to acetic acid via the Embden – Meyerhof pathway to pyruvate which was then converted to acetate.

Lactic Acid

- lactic acid is used in the food industry as a preservative and as flavor enhancer and in the chemical and pharmaceutical industries
- In 1878, Joseph Lister isolated the first pure culture of any bacterium which he called *Bacterium lactis , later to be renamed Lactobacillus lactis ssp. lactis.*
- the fungus *Rhizopus is also a producer of* the acid.
- *Rhizopus oryzae synthesizes solely the L - (+) isomer of lactic acid, whereas* most lactobacilli produce mixed isomers of the acid.