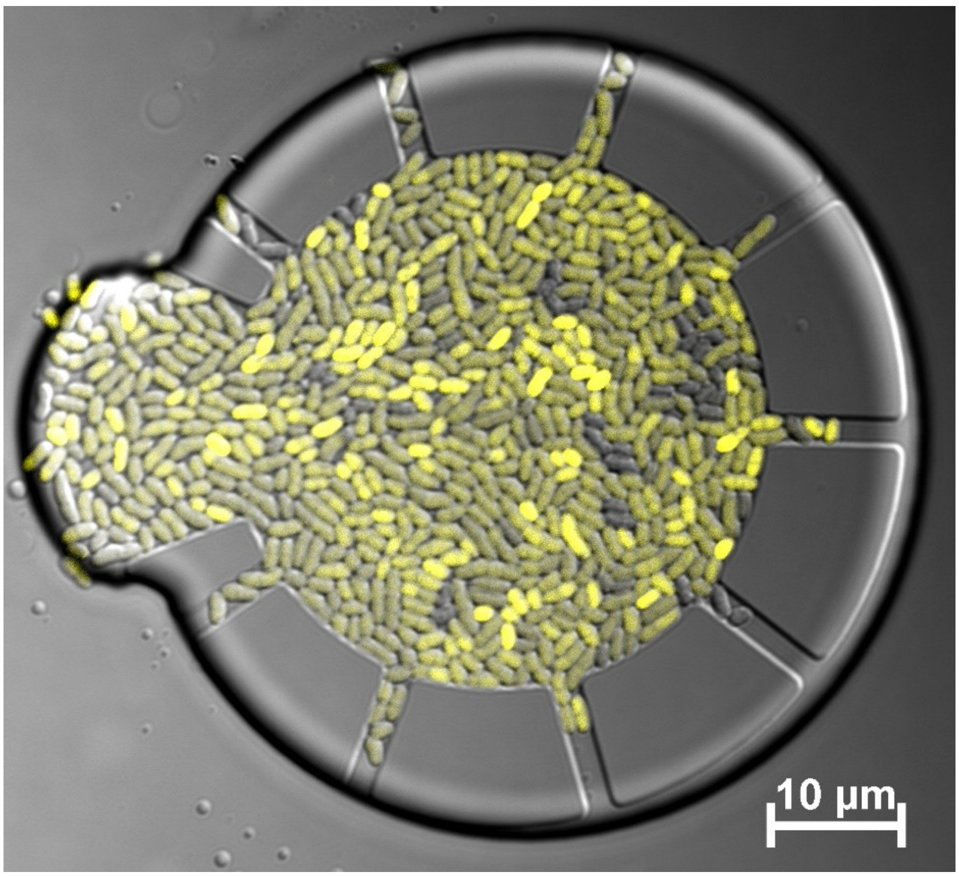
Food supplements: amino acids and vitamins

Mummy: we heard of a nasty disease of sailors in the olden days called scurvy: what is it?



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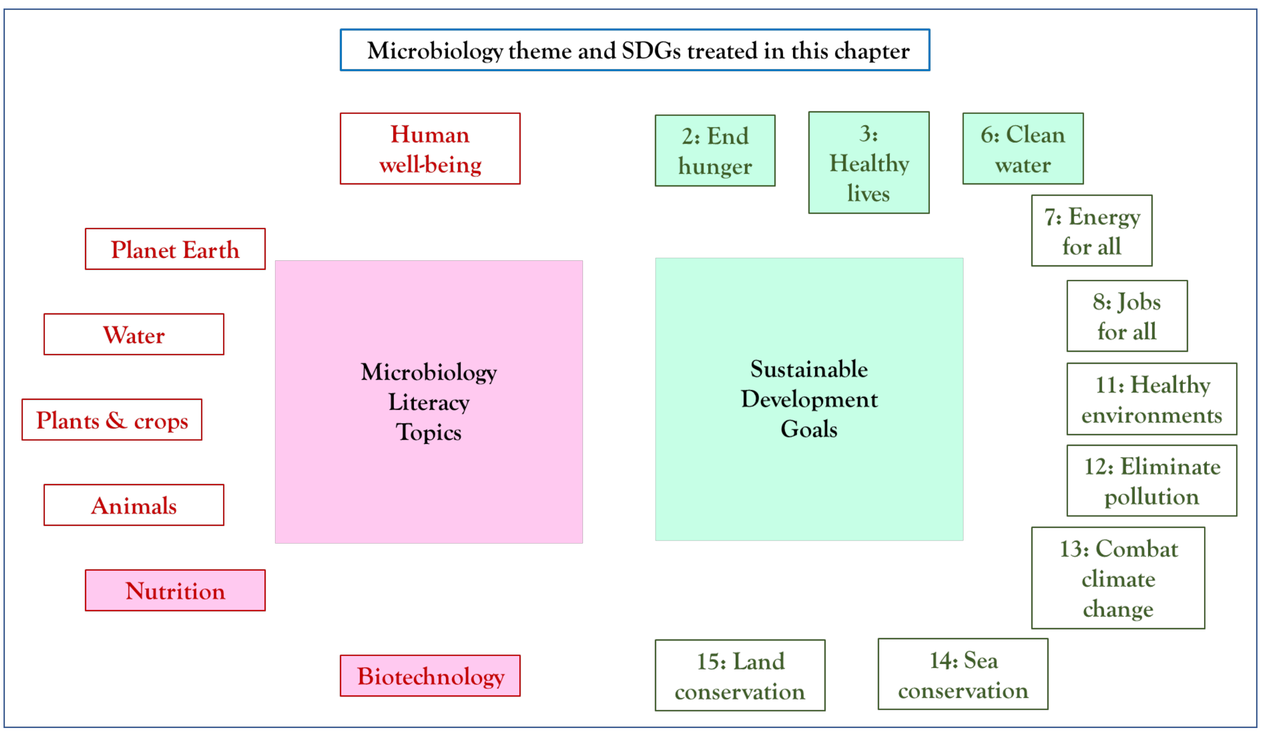
Storyline

Amino acids and vitamins are essential compounds of life. Amino acids are the constituents of proteins, which as enzymes or structural proteins, make up a large fraction of every cell on earth. Vitamins, although required in only small amounts, are mandatory for many metabolic reactions. Humans and animals have to obtain eight so-called essential amino acids, and most of the 13 vitamins in their food, because they cannot synthesize them by themselves. Various reasons lead to deficiencies in essential amino acids and vitamins, which have to be overcome by providing them as supplements. For this purpose, industrial production processes have been established. Almost all amino acids and some vitamins are produced with microorganisms. In some cases, such as the feed additive L-lysine, the production volume reaches several million tons per year. The supplementation of vegetable feed with biotechnologically produced amino acids has beneficial effects on feed consumption and in turn on the environment.

The Microbiology and Societal Context

The microbiology: production organisms, metabolic engineering, metabolism, essential amino acids, vitamins. *Sustainability issues*: health, food and feed, environmental pollution



Food supplements: the Microbiology

Amino acids

1. Proteins: essential players in every cell of every organism. Proteins make up a large part – about 20% – of the total human body mass. They fulfill numerous, mostly essential functions, in particular (i) as enzymes responsible for catalyzing hundreds of chemical reactions in our body, (ii) as structural proteins forming tissues, hair, nails, and much more, (iii) as contractile proteins (actin and myosin) allowing muscle function, (iv) as transport proteins, for example of oxygen (hemoglobin), (v) as protective proteins, for example antibodies or proteins responsible for blood coagulation, (vi) as hormones that control important processes in our body, or (vii) as storage proteins, for example of iron (ferritin). In short: without proteins, life is not possible.

2. Amino acids are the building blocks of proteins. Proteins can be considered as pearl necklaces where each pearl represents an amino acid. Amino acids are chemical compounds that contain an amino group ($-NH_2$) and a carboxylic acid group (-COOH). In proteins, the carboxylic acid group of one amino acid reacts with the amino group of the next amino acid to form a peptide bond. In this way, chains from a few up to several 1000 amino acids are formed. There are twenty different amino acids that make up proteins.

3. Essential amino acids in our food. Eight of these amino acids (L-isoleucine, L-leucine, L-lysine, L-methionine, L-phenylalanine, L-threonine, L-tryptophan, L-valine) are called "essential", because we cannot synthesize them by ourselves, so they have to be obtained from external sources - usually through food intake. The daily requirement for the individual essential amino acids varies between 4 mg/kg bodyweight/day for L-tryptophan to 40 mg/kg bodyweight/day for L-leucine. For a person with a weight of 70 kg, this means an uptake of 0.28 g/day for tryptophan and 2.8 g/day for leucine. We acquire amino acids from proteins in our food. With a balanced diet, the daily requirement is generally satisfied. However, an amino acid deficiency may occur in the case of an unbalanced diet (e.g. eating only fast food), special diets, and certain diseases. For example, meat is rich in the amino acids we need, but plant food is often not, so vegetable-only or meat-poor diets may need to be supplemented. Soy beans are one plant product that is rich in proteins (about 34% of dry weight) with a high percentage of essential amino acids. Alternatively, supplementation with amino acids themselves can compensate for such deficiencies. For this purpose, amino acids have to be available in pure form. Pure amino acids are also required for patients in hospitals who are unable to eat. They are fed by infusion solutions (this is called total parenteral nutrition), which must contain all eight essential amino acids, besides other nutrients.

4. Adding amino acids to animal feed makes a big difference, as most feed crops are deficient in at least one amino acid. Not only humans require essential amino acids in their food, but also animals. This fact plays an important role in the breeding of pigs and poultry and in aquaculture (fish and shrimp). In conventional animal feed (corn, wheat, etc.), the amino acid composition does not meet the animals' demand. Depending on the type of feed and the animal, the essential amino acids lysine, methionine, threonine, and tryptophan are limiting, whereas other amino acids are in excess, but cannot be used and are excreted. To allow the animals to grow at their maximum rate, it is necessary to over-feed them to provide enough of the required amino acids. To prevent such an inefficient feed utilization, the feed can be supplemented with the right mixture of the limiting amino acids. In this way, the conventional feed can be effectively utilized, reducing the amount of required feed. At the same time, the area of arable land required for feed production and the amount of amino excretion that contributes to the formation of climate-wrecking gases, such as nitrous oxide or nitric oxide, is reduced.

5. Glutamate is responsible for the umami taste. Besides the eight essential amino acids, also the non-essential amino acid glutamate plays an important role in human nutrition. Glutamate is responsible for the umami taste. Besides sweet, sour, salty, and bitter, umami is one of the five basic tastes sensed by humans. Umami is a Japanese word meaning "pleasant savory taste" and has been described as brothy or meaty. For example, parmesan cheese, seaweed, miso, and mushrooms contain high levels of glutamate. Glutamate has been added to many processed foods, such as potato chips or soups, as a "flavour enhancer" (E621) for more than 100 years.

Vitamins

6. Vitamins are essential substances that, except for a few exceptions, the body cannot produce itself. Vitamins are essential nutrients for humans since they are required for many metabolic reactions. Humans need 13 vitamins, four of which are fat-soluble (vitamins A, D, E, and K), whereas the others are water-soluble (vitamins B_1, B_2, B_3, B_5, B_6, B_7, B_9, B_{12}, and C). Compared to essential amino acids, only small amounts of vitamins are needed. The daily requirement varies between 3 µg for vitamin B_{12} and 100 mg for vitamin C. Similar to the essential amino acids, a balanced diet covers the vitamin requirements. However, certain forms of nutrition (like veganism), malabsorption, pregnancy, or illness may lead to vitamin deficiencies. A famous example of a vitamin deficiency resulting from malnutrition is scurvy. Scurvy is a disease resulting from a chronic lack of vitamin C (ascorbic acid) that is associated with skin lesions, wound healing disorders, and gum inflammation. Until the 18^{th} century, scurvy was the most common cause of death among sailors spending months at sea without vitamin C-containing food. In industrialized countries enabling a balanced diet, nutrition-related vitamin deficiency should be rare, but nevertheless is observed for some vitamins like B_{12} and D. A deficiency in vitamin D is, however, not due to missing uptake, as we can synthesize it ourselves, but caused by insufficient exposure to sunlight, which is required for the synthesis.

In order to treat or prevent vitamin deficiency, some foods and beverages are supplemented with certain vitamins. Alternatively, vitamin pills offer an easy way to avoid vitamin deficiency. However, it should be noted that excessive intake of fat-soluble vitamins can have negative effects on health due to accumulation in the body. Pure vitamins are used for food supplementation and pills, but also for infusion solutions in the hospital and other purposes. For example, yellow vitamin B_2 (riboflavin, E101) is used as a food colouring agent and vitamin C (ascorbic acid; E300) is used as antioxidant, stabilizer, acidifying, or buffering agent. A large part of the vitamins produced today is used in animal breeding and aquaculture to avoid vitamin deficiencies and support the healthiness of animals and fish.

7. Commercial production of amino acids and vitamins. Several methods for the production of amino acids are available: (i) extraction out of proteins, (ii) chemical synthesis, (iii) enzymatic catalysis, or (iv) fermentation. Today, most of the commercial production of amino acids relies on fermentation. In fermentation, suitable microorganisms such as *Corynebacterium glutamicum* or *Escherichia coli* convert sugars and ammonia into the desired amino acid. The synthesis process takes place via a multitude of intermediate steps within the cells. One example is the production of glutamate from glucose. In this process, about 2 kg of glucose result in 1 kg of glutamic acid.

The market for biotechnologically produced amino acids is huge. The world market for amino acids is quantitatively dominated by glutamate (>3 million tonnes per year; flavour enhancer) followed by lysine (>2.8 million t/y; feed additive), and methionine (>2 million t/y; feed additive). Whereas methionine is synthesized mainly chemically, glutamate and lysine are produced in fermentative processes using selected bacteria as production hosts.

8. Bioreactors of 750 m^3 or more are used for large scale amino acid production. The fermentative production of glutamate and lysine is performed in bioreactors with a volume up to 750 m^3. This corresponds to a water content of about 5,000 bathtubs. In such a bioreactor, the number of bacteria at the end of the fermentation is around 2×10^{19}, which is a quite huge number (20,000,000,000,000,000,000). Assuming that the amount of lysine at the end of the fermentation is 200 g/l, such a fermentation yields 150×10^6 g or 150 tons. Assuming that each fermentation requires one week, the yearly production of such a bioreactor would be 7,500 tons. For the production of 2 million tons of lysine per year, 260 such bioreactors are needed.

9. Selected vitamins are produced almost entirely by fermentation. In contrast to amino acids, chemical processes dominate the industrial production of vitamins. Only vitamins B_2 and B_{12} are synthesized entirely by fermentation. Whereas in the case of vitamin B_2 the biotechnological process replaced a chemical process due to its cost efficiency, for vitamin B_{12} an alternative chemical production process never existed. The quantitatively most important vitamin C is produced in a mixed process starting by chemical reduction of glucose to D-sorbitol, which is subsequently transformed with the help of two different bacteria to 2-keto-gulonic acid. This compound is then chemically rearranged to vitamin C, which is also called ascorbic acid. The global vitamin C production in 2016 was estimated at about 110 000 tons annually.

10. Microbial production strains for amino acids and vitamins. Microbes have been used by mankind for production of wine, beer and bread for thousands of years, but it remained unknown that they were crucial for these processes before their existence was discovered in the 17th century by Antonie van Leeuwenhoek. In recent decades, the chemical industry has begun to produce various chemicals by biological means from renewable carbon sources rather than in the traditional way by chemical means from fossil carbon sources, predominantly oil. In some cases, microorganisms isolated from nature (so-called wild-type strains) can be directly used for production processes, for example in cases like ethanol, butanol, or lactic acid, which are formed by certain microbes as an end product of their metabolism. Also in the case of vitamin C production, wild-type strains of the acetic acid bacteria *Gluconobacter oxydans* and *Ketugulonicigenium vulgare* can be used for the biotransformation of D-sorbitol into 2-keto-L-gulonic acid.

In other cases, however, wild-type strains are not suitable for industrial production of target metabolites such as amino acids, as their biosynthetic pathways are tightly regulated in order to produce only as much as is required for their own growth. To allow the overproduction of an amino acid, such as L-lysine, the metabolism of the microbes has to be altered to abolish regulatory mechanisms and enable accumulation and excretion of the amino acid into the medium. Since the

1950s, this was done for example by random mutagenesis with certain chemicals or UV light and laborious screening of the resulting mutant library for cells that showed improved production of the amino acid. Since the 1980s, with the establishment of gene technology, metabolic engineering is used for creation of producer strains. Metabolic engineering is a rational approach to optimize the metabolism and transport capacities of the producer cells for the conversion of the substrate, which often is sugar, to the desired product, e.g. an amino acid. Important features to be considered in metabolic engineering approaches are shown in Fig. 1. An overview on selected biotechnologically synthesized amino acids and vitamins, the microorganisms used for production, and companies involved in that business is given in Table 1.

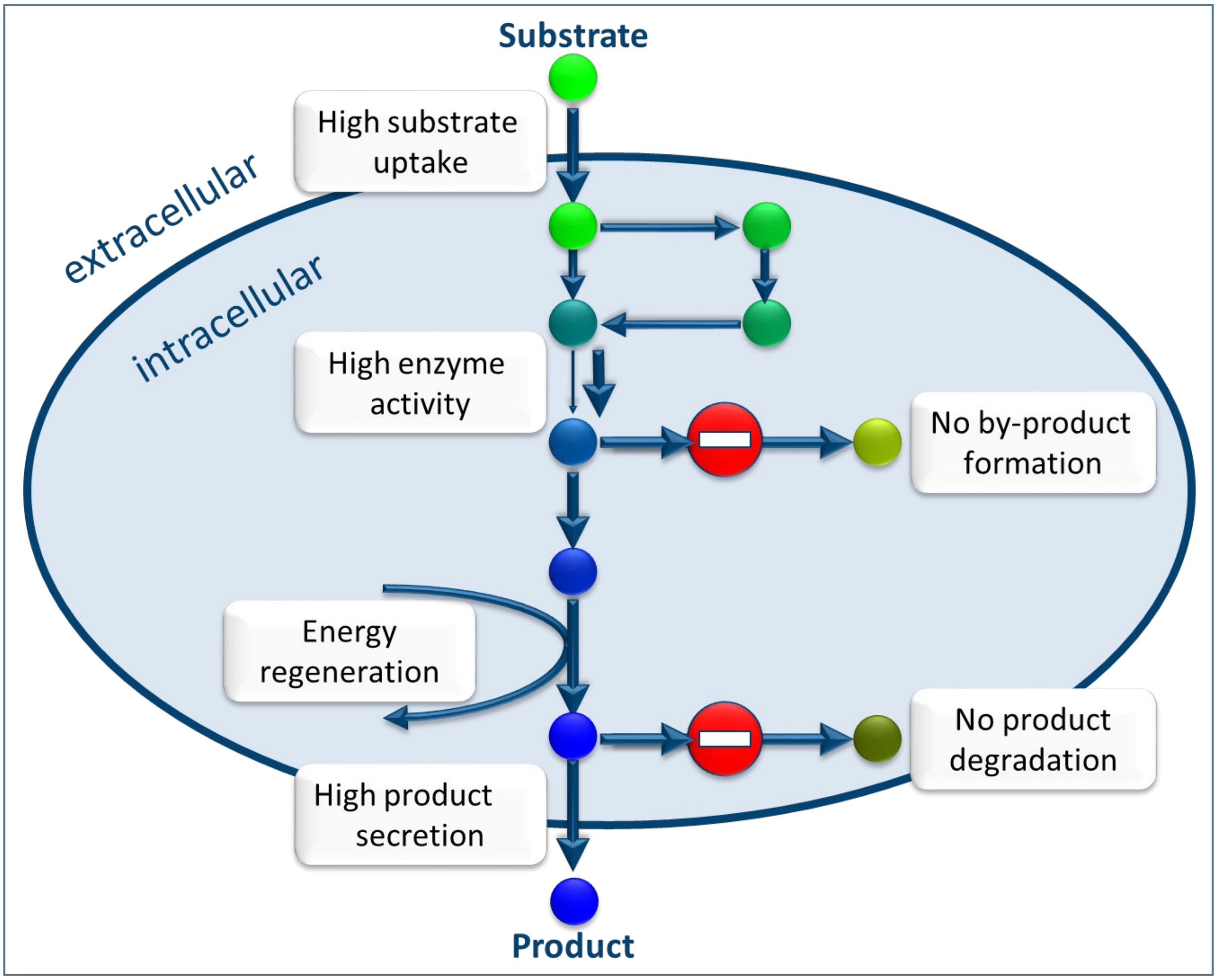


Fig. 1: Important features that have to be considered in metabolic engineering projects aiming at the development of efficient microbial production strains, e.g. for amino acids.

Table 1: Overview of biotechnologically-produced amino acids and vitamins, the microbes used for production, their natural habitat, and companies involved in that business.

Product (major application)	Microbes used for production [#]	Habitat of production hosts	Companies
L-Glutamate (flavour enhancer)	*Corynebacterium glutamicum* (B)	Soil	• Ajinomoto • COFCO Group (China National Cereals, Oils and Foodstuffs Corporation) • Fufeng Group • Lotus Health Industry Holding Group Company • Meihua Group
L-Lysine (feed additive)	*Corynebacterium glutamicum* (B)	Soil	• Ajinomoto • ADM (Archer Daniels Midland) • Changchun Dacheng • Cheil Jedang • Global Biochem Technology • COFCO Biochemical
	Escherichia coli (B)	Lower intestine of humans and mammals	
Vitamin C = ascorbic acid (food and feed additive)	*Gluconobacter oxydans* (B)	Sugar-rich habitats such as fruits or flowers	DSM Nutritional Products Weisheng NCPC Northeast
	Ketogulonicigenium vulgare (B)	Soil	
Vitamin B_2 = riboflavin (food and feed additive)	*Ashbya gosypii* (F)	Pathogen of cotton plants	• DSM Mutritional Products • Hubei Guanji • Desano • BASF
	Bacillus subtilis (B)	Soil and vegetation	
Vitamin B_{12} = cobalamin (food additive, drug)	*Pseudomonas denitrificans* (B)	Variety of habitats, including soil and surface waters	• BASF • Hebei Yuxing Bio-Engineering • Hebei Huarong Pharmaceutical • Pharmavit

[#] B = Bacterium; F = Fungus

11. Microbial production of amino acids and vitamins relies on renewable raw materials. Microbial production processes in industrial biotechnology use renewable carbon sources as substrates (Fig. 2). In many cases, these are sugars like glucose obtained from starch derived from maize or wheat, or sucrose derived from sugar beet or sugar cane. In some cases, like for example in vitamin B_2 production with *A. gossypii*, plant fats are used as substrate.

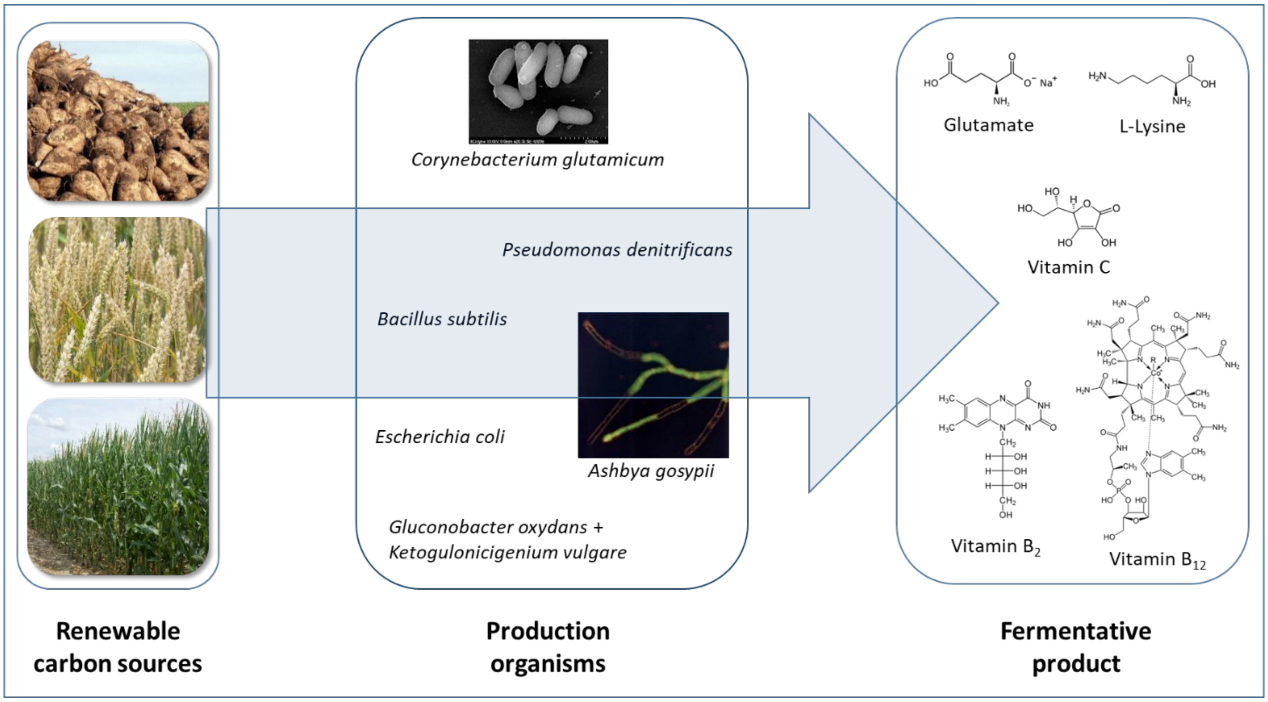


Fig. 2: Schematic overview on the production of selected amino acids and vitamins from renewable carbon sources with microbes.

12. Advantages and challenges of biotechnological production of amino acids and vitamins. In industrial amino acid production, microbial processes are used with few exceptions. One of these is the production of DL-methionine, an essential amino acid used as feed additive that is currently still produced chemically from petrochemical feedstocks like propylene. The production process involves highly toxic compounds such as hydrogen cyanide and methyl mercaptan. However, also biotechnological production routes for methionine were developed in recent years and might substitute the chemical process in the future.

The protection of the environment is a very strong argument for the use of biotechnology in the chemical industry. However, not only ecological aspects characterize biotechnological processes, but they can also offer economic and functional benefits. For example, the conversion from chemical to biotechnological production of vitamin B_2 drastically reduced (i) production costs, (ii) use of resources (reduction by 60 %), (iii) waste (reduction by 95 %), and (iv) CO_2 emissions (reduction by 30 %). Another important advantage is that fermentative production typically results in only one enantiomer of a chiral substance, e.g. in microbial amino acid production only the proteinogenic L-enantiomers are formed. Therefore, a subsequent separation of the racemate (mixture of D and L-amino acid) into L- and D-enantiomers is not necessary.

Relevance for sustainable development goals and grand challenges

- **Goal 2. End hunger, achieve food security and improved nutrition and promote sustainable agriculture.** Addition of those amino acids that are underrepresented in the vegetable feed used for pigs and poultry improves the utilization of the entire feed by the animals and thereby reduces the required feed quantity. Consequently, more animals can be fed with the same amount of feed, making the growing request for animal-based protein sources affordable for more people. However, it is very important to realize that the consumption of animal protein by mankind should be reduced, as it comes with a huge ecological burden.

- **Goal 3. Ensure healthy lives and promote well-being for all at all ages.** Vitamin products in the form of dietary supplements, medicines or dietetic foods are taken (i) to prevent a deficiency caused e.g. by special diets, (ii) to treat a vitamin deficiency, or (iii) to meet an increased vitamin requirement due to illness or particular life situations. In addition, amino acids and vitamins are essential components in products for artificial nutrition.
- **Goal 6. Ensure availability and sustainable management of water and sanitation for all.** Supplementation of feed for pigs and poultry with essential amino acids underrepresented in the feed improves the utilization of all amino acids by the animals and thereby reduces the faecal matter produced and in particular its nitrogen load. This in turn reduces faecal pollution of water bodies with nitrates and expenditures for nitrogen removal in wastewater treatment plants. In addition, the reduced feed demand enabled by amino acid supplementation also reduces the water required for growing the feed crops.

Potential Implications for Decisions

1. Individual

a. Consider how often you eat animal protein and if there is potential for reduction.
b. Should you pop vitamin pills or not?

2. Community policies

a. Education about amino acid and vitamin needs, the consequences of dietary habits on deficiencies, and the whys and wherefores of supplementation

3. National policies

a. Policies relating to sustainability involving land use for animal feed and the efficient use of such feed
b. Policies relating to environment protection require consideration of policies regulating farming practices (animal feed efficiency/waste/etc.)

Pupil participation

1. Class discussion of the issue associated with microbial production of food and feed supplements

Discuss the advantages of amino acid supplementation of animal feed, but also the many negative effects of increasing livestock farming for reaching the sustainable development goals.

2. Pupil stakeholder awareness

a. What advantages do microbial production processes offer? Can you also imagine disadvantages?

3. Exercises

a. Check at home which foods contain amino acids or vitamins

4. Class experiments *(select appropriate experiment from the Class Experiment list)*

a. Quantitative vitamin C detection with test sticks

b. Biological protection - Ascorbic acid as antioxidant
c. Protein detection in food using copper(ii) sulfate and sodium hydroxide
d. Protein detection in food using vinegar essence
e. Glutamate determination using thin-layer chromatography

The evidence base, further reading and teaching aids

1. https://www.ajinomoto.com/aboutus/umami/5_facts
2. Karau A, Grayson I. Amino acids in human and animal nutrition. Adv Biochem Eng Biotechnol. 2014;143:189-228. doi:10.1007/10_2014_269
3. Wilson, D. B., Sahm, H., Stahmann, K.-P., & Koffas, M. (2020). Industrial Microbiology. Newark: John Wiley & Sons, Incorporated.
4. Pietzsch, J. (2020) Bioecomy for Beginners. Berlin: Springer-Verlag.

Glossary

Amino acids: 20 different amino acids are the building blocks of proteins, they are called proteinogenic amino acids. They all have at least two functional groups, an amino group (-NH_2) and a carboxyl group (-COOH). The simplest amino acid is glycine (NH_2-CH_2-COOH). In the other amino acids an aliphatic or aromatic side chain replaces a hydrogen of the CH_2 group. Except for glycine, all proteinogenic amino acids are optically active. In proteins, only the L-form occurs.

Enantiomer: Enantiomer (or optical isomer) is the name for the two stereoisomers of an asymmetric molecule. This asymmetric molecule has a chiral carbon with four different atoms or group of atoms attached. The two enantiomers are non-superimposable mirror images of each other, like the left and the right hand.

Fermentation: In microbiology, fermentation describes a type of metabolism in which sugars are metabolized without oxygen or other external electron acceptors to end products like ethanol or lactic acid that accumulate in the culture medium. In biotechnology, fermentation describes any microbial production processes for chemicals or proteins in bioreactors, independent of whether oxygen is present or not.

Industrial biotechnology: Uses microorganisms or enzymes to synthesis a variety of different products, usually from renewable carbon sources. Biotechnological processes are key players in the industrial production of e.g. bioethanol, amino acids, vitamins, organic acids, or antibiotics.

Racemate: A racemate is a mixture of enantiomers. Such mixtures, such as L- and D-methionine, are difficult to separate.

Wild-type strain: A microorganism isolated from nature is called a wild-type strain.