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Review article

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Systems metabolic engineering of microorganisms for food and cosmetics production

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Abstract

The demand for sustainable and environmentally friendly sources of foods and cosmetics is increasing owing to concerns about the rapid growth of the global population and climate change. Microorganisms are promising cell factories in which to produce various food and cosmetic ingredients. However, the commercialization of microbial-based food and cosmetic compounds is still limited by the insufficient performances of microbial strains and processes. Systems metabolic engineering can improve the performance of microorganisms. In this Review, we highlight food and cosmetic compounds that can be produced using microorganisms. We then discuss systems metabolic engineering, with its different phases that include production mode and host selection, metabolic pathway reconstruction, tolerance enhancement, metabolic flux and fermentation process optimization, downstream process integration and scale-up, which can be optimized to improve the development of high-performance microbial processes. Finally, we overview the current limitations and future directions of industrializing microbial processes for the production of food and cosmetic compounds.

Sections

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Key points

- Microorganisms can serve as green factories for the production of food and cosmetic products.
- A growing number of food and cosmetic compounds are commercially produced from renewable substrates using microorganisms.
- Systems metabolic engineering could facilitate the development of high-performance microbial cell factories.
- Various tools and strategies are powering up systems metabolic engineering, such as synthetic/systems biology tools and process/evolutionary engineering strategies.
- Raw materials, performance of microbial strains, fermentation and downstream processes, process scale-up, economic aspects, and societal demands and perception should be collectively considered for the successful commercialization of microbial processes.

Introduction

The world's rapidly growing population, which is expected to reach ten billion people by 2057 (ref. 1), raises concerns about food shortage and nutritional inequality, especially given the increasing concerns about climate change[1]. In addition, the war in Ukraine since 2022 continues to disrupt the supply chain, thus exacerbating the food crisis[2]. At the same time, there is a growing demand for high-quality green food and cosmetics products, owing to increased awareness of environmental and sustainability issues.

Microorganisms can contribute to the alleviation of food shortages, for example, by consuming non-edible materials or lower-value biomass to generate microbial biomass rich in valuable nutrients, such as proteins and vitamins[1]. In particular, culturing microorganisms results in lower levels of carbon and water footprints and requires a smaller area of land to produce the same amount of protein compared to farming feedstock animals, fish and even plants[1]. Moreover, microbial metabolism can be engineered to produce various compounds, such as macronutrients (carbohydrates, fats and proteins), antioxidants, flavours, fragrances, colourants and skincare ingredients (Table 1). These microbial production hosts and processes have been transferred to industry for the commercial production of food and cosmetic compounds (Supplementary Table 1). However, the performance of many metabolically engineered microbial strains and their bioprocesses still needs to be improved to compete with conventional products on an industrial level. In particular, the production titre, yield and productivity of these microbial strains and processes must be improved to reduce the overall production costs because the costs of renewable raw materials and the operation costs for fermentation and downstream processes are relatively higher than those in the petrochemical industry.

Systems metabolic engineering (SysME) (Box 1) is a multidisciplinary strategy that integrates synthetic biology, systems biology and evolutionary engineering with metabolic engineering[3,4] to develop high-performance microbial cell factories suitable for industrial commercialization. For example, SysME combines systems biology tools and strategies with metabolic engineering to reduce the number of trial-and-error cycles during strain development. Additionally, SysME integrates synthetic biology tools and evolutionary engineering strategies with metabolic engineering to develop high-performance biological parts, modules and chassis strains, and enables engineering of metabolic networks. The potential of SysME was first demonstrated by developing rationally engineered microbial strains that produce L-valine[5] and L-threonine[6], whereas development of many contemporary strains had relied on random mutagenesis followed by screening mutants overproducing L-valine and L-threonine. Since then, SysME has been increasingly employed in various studies over the last 15 years to improve the performance of diverse production hosts, although most studies have exploited only a few strategies, rather than systematically applying the entire SysME strategies throughout the studies.

In this Review, we provide the basic principles of producing biocompounds using microorganisms and review the food and cosmetic compounds produced using microorganisms. In addition, SysME strategies in microbial processes are discussed with example cases. Finally, we discuss future directions for developing industrially competitive microbial strains and processes for the production of food and cosmetic compounds.

Microbial production of biocompounds

A microbial cell consumes D-glucose and/or other renewable substrates and synthesizes metabolites through its complex network of metabolic pathways, including the glycolysis pathway, the pentose phosphate pathway, the tricarboxylic acid cycle, amino acid biosynthesis pathways and lipid biosynthesis pathways. Moreover, various microbial species have evolved in and adapted to different niches; they have particular metabolic networks consisting of different sets of reactions and metabolites. As a result, many useful compounds or their analogues can be produced through the microbial metabolism.

Some microorganisms naturally overproduce useful compounds, such as *Corynebacterium glutamicum*[7], *Mannheimia succiniciproducens*[8], *Bacillus subtilis*[9] and *Lactococcus lactis*[10], producing L-glutamic acid, succinic acid, poly-γ-glutamic acid (γ-PGA) and nisin, respectively. Alternatively, metabolic engineering can exploit the diversity of microbial metabolism of non-overproducers, such as *Escherichia coli*, to overproduce compounds of interest and suppress the biosynthesis of undesired biocompounds by engineering metabolic and regulatory genes and hence the metabolic reactions and networks of the selected strains. The production of target compounds by natural overproducers can also be improved by engineering their native metabolic networks. Moreover, heterologous and artificial metabolic reactions can be introduced to produce compounds that do not exist in the endogenous metabolism of the host strain by introducing the relevant heterologous (including animal- and plant-derived) or engineered metabolic genes. Furthermore, the use of fermentors (bioreactors) to provide strain-specific culture conditions (including temperature, pH and dissolved oxygen level) and application of fermentation strategies can help to maximize the performance of the microbial overproduction strains. These strategies can be applied to produce a variety of food and cosmetic compounds using microorganisms (Fig. 1 and Table 1), through different metabolic routes (Figs. 2,3) and using renewable carbon sources (Supplementary Box 1).

Food and cosmetic compounds

Among the food compounds produced by microbial fermentation are macronutrients, (pro)vitamins, flavours, colourants, alcohols, dietary supplements and other food additives (Fig. 1 and Table 1). Similarly,

Table 1 | Recent examples of food and cosmetic compounds production using microorganisms

Product	Use	Host	Titre ($g\ l^{-1}$)	Yield ($g\ g^{-1}$)[a]	Productivity ($g\ l^{-1}\ h^{-1}$)	Carbon source	Ref.
Carbohydrates and derivatives							
C6 monosaccharides and derivatives							
D-Allulose	Sweetener	*Bacillus subtilis*	196	0.28	8.17	D-Fructose	12
L-Fructose	Sweetener	*Corynebacterium glutamicum*	20.8	0.41	0.22	Glycerol	19
L-Fucose	Skincare ingredient	*Escherichia coli*	16.7	NR	0.1	Glycerol	20
L-Sorbose	Precursor of vitamin C	*Escherichia coli*	4.1	46%	6.15	D-Sorbitol	169
		Gluconobacter oxydans	135	91%	5.63	D-Sorbitol	161
D-Tagatose	Sweetener	*Corynebacterium glutamicum*	180	60.60%	60	D-Glucose	13
Allitol	Sweetener	*Escherichia coli*	48.62	53.4%	3.24	D-Fructose	11
myo-Inositol	Food additive	*Escherichia coli*	1.77	0.177	0.028	D-Glucose	176
D-Glucaric acid	Food additive	*Escherichia coli*	0.85	0.085	0.012	D-Glucose	176
Ascorbic acid	Vitamin C	*Xanthomonas campestris*	20.4	NR	0.408	D-Glucose	180
C5/C4/C3 monosaccharides and derivatives							
Arabitol	Sweetener and dental caries reducer	*Rhodosporidium toruloides*	49	0.33	0.20	D-Xylose	14
Xylitol	Sweetener and dental caries reducer	*Escherichia coli*	204	0.873	1.2	D-Xylose	15
			162	2.50	2.13	Corncob hydrolysate	16
D-Xylonic acid	Food additive	*Escherichia coli*	199.44	NR	7.12	D-Xylose and D-glucose	193
Erythritol	Sweetener	*Yarrowia lipolytica*	194.3	0.49	0.95	Glycerol	18
Dihydroxyacetone	Sun-free tanning agent	*Gluconobacter oxydans*	134	97.9%	9.57	Glycerol	17
			153	86.9%	8.5	Glycerol	17
Amino sugars							
Glucosamine	Dietary supplement	*Escherichia coli*	17	NR	0.24	D-Glucose	137
N-Acetylglucosamine	Dietary supplement	*Bacillus subtilis*	82.5	0.39	1.47	D-Glucose	167
		Escherichia coli	110	0.45	1.57	D-Glucose	137
N-Acetylneuraminic acid	Dietary supplement	*Escherichia coli*	8.31	0.1	0.19	D-Glucose	165
Oligosaccharides							
α-D-Glucosylglycerol	Humectant	*Corynebacterium glutamicum*	2.1	0.13	0.037	Sucrose	194
Trehalose	Humectant	*Escherichia coli*	8.2	0.86 (Glucose)	0.085	D-Glucose and glycerol	177
		Yarrowia lipolytica	219	73%	4.5	Maltose	185
2′-Fucosyllactose	HMO	*Escherichia coli*	66.8	1.27 (Lactose)	0.95	Glycerol and lactose	21
Curdlan oligosacchride	Gelling agent	*Pichia pastoris* and *Agrobacterium* sp. (co-culture)	18.77	NR	0.26	D-Glucose	183
Polysaccharides							
Cellulose	Food additive	*Gluconacetobacter xylinus*	4.3	0.1847	0.012	D-Glucose	146
Curdlan	Gelling agent	*Agrobacterium* sp.	67	0.57	0.98	D-Glucose	144
Pullulan	Edible film	*Aureobasidium pullulans*	109	NR	0.956	D-Glucose	145
Xanthan	Food additive	*Xanthomonas campestris*	62	0.82	0.72	D-Glucose	147
Hyaluronic acid (hyaluronan)	Humectant, anti-wrinkle agent and dermal filler	*Corynebacterium glutamicum*	74.1	0.17	1.03	D-Glucose	22
			34.2	0.206	0.713	D-Glucose	22
		Bacillus subtilis	7	0.3	0.3	Glycerol	23
			19.38	NR	0.22	Sucrose	24

Table 1 (continued) | Recent examples of food and cosmetic compounds production using microorganisms

Product	Use	Host	Titre ($g\ l^{-1}$)	Yield ($g\ g^{-1}$)[a]	Productivity ($g\ l^{-1}\ h^{-1}$)	Carbon source	Ref.
Polysaccharides (continued)							
Chondroitin	Dietary supplement	*Bacillus subtilis*	7.15	NR	0.102	Sucrose	25
Chondroitin sulfate	Dietary supplement	*Bacillus subtilis*	NA	96.09–98.26%	NA	Sucrose	25
Lipids and fatty acids							
Total lipids	Macronutrient	*Schizochytrium* sp.	113.5	NR	0.946	D-Glucose	31
			120	NR	0.71	D-Glucose	30
Free fatty acids	Macronutrient	*Rhodococcus opacus*	50.2	NR	0.479	D-Glucose	26
		Saccharomyces cerevisiae	33.4	NR	0.133	D-Glucose	27
Polyunsaturated fatty acids							
Eicosatetraenoic acid	Dietary supplement	*Mortierella alpina*	0.61	NR	0.0025	D-Glucose	28
Eicosapentaenoic acid	Dietary supplement	*Mortierella alpina*	1.8	NR	0.0125	D-Glucose	29
		Schizochytrium sp.	1.65	NR	0.0077	D-Glucose	30
Docosapentaenoic acid	Dietary supplement	*Schizochytrium* sp.	11.94	NR	0.056	D-Glucose	30
Docosahexaenoic acid	Dietary supplement	*Schizochytrium* sp.	44.3	NR	0.369	D-Glucose	31
			47.39	NR	0.221	D-Glucose	30
Amino acids and peptides							
L-Alanine	NEAA	*Escherichia coli*	120.8	0.883	3.09	D-Glucose	41
		Corynebacterium glutamicum	275.3	0.82	3.82	D-Glucose	42
L-Valine	Essential amino acid	*Corynebacterium glutamicum*	172.2	0.41	7.18	D-Glucose	43
			227.3	NR	4.73	D-Glucose	43
L-Leucine	Essential amino acid	*Corynebacterium glutamicum*	38.1	0.306	0.794	D-Glucose	44
L-Isoleucine	Essential amino acid	*Corynebacterium glutamicum*	32.4	0.116	0.45	D-Glucose	45
L-Threonine	Essential amino acid	*Escherichia coli*	22.9	NR	0.477	D-Glucose	54
			116.62	0.486	2.43	D-Glucose	55
L-Methionine	Essential amino acid	*Escherichia coli*	16.86	NR	0.35	D-Glucose	56
β-Alanine	Dietary supplement	*Escherichia coli*	85.18	0.24	1.05	D-Glucose	57
L-Lysine	Essential amino acid	*Corynebacterium glutamicum*	221.3	0.71	5.53	D-Glucose	46
L-Serine	NEAA	*Corynebacterium glutamicum*	43.9	0.44	0.37	Sucrose	47
		Escherichia coli	34.8	0.32	0.97	D-Glucose	48
L-Cysteine	CEAA	*Escherichia coli*	7.5	NR	0.34	D-Glucose	64
L-Glutamic acid	NEAA	*Corynebacterium glutamicum*	125	NR	4.81	D-Glucose	7
L-Arginine	CEAA	*Corynebacterium crenatum*	87.3	0.431	1.21	D-Glucose	58
		Corynebacterium glutamicum	92.5	0.40	1.28	D-Glucose and sucrose	59
			81.2	0.353	0.91	Corn starch hydrolysate and decomposed raw sugars (D-glucose and D-fructose) from sugarcane	59
		Corynebacterium crenatum	95.5	0.273	0.99	D-Glucose	60
L-Citrulline	Dietary supplement	*Corynebacterium glutamicum*	8.51	0.11	0.10	D-Glucose	62
L-Ornithine	Dietary supplement	*Corynebacterium glutamicum*	43.6	0.34	0.727	D-Glucose	63
			18.9	0.40	0.263	D-Xylose	63

Table 1 (continued) | Recent examples of food and cosmetic compounds production using microorganisms

Product	Use	Host	Titre (g l^{-1})	Yield (g g^{-1})[a]	Productivity (g l^{-1} h^{-1})	Carbon source	Ref.
Amino acids and peptides (continued)							
L-Proline	CEAA	*Corynebacterium glutamicum*	120.18	NR	1.581	D-Glucose	61
		Escherichia coli	54.1	NR	2.25	D-Glucose	54
L-Phenylalanine	Essential amino acid	*Escherichia coli*	72.9	0.26	1.40	D-Glucose	49
L-Tyrosine	Essential amino acid	*Escherichia coli*	62	0.30–0.32	1.29	D-Glucose	50
			43.14	0.107	0.52	D-Glucose	51
L-Tryptophan	Essential amino acid	*Escherichia coli*	49	0.186	1.36	D-Glucose	52
L-Histidine	Essential amino acid	*Escherichia coli*	66.5	0.23	1.5	D-Glucose	53
Glutathione	Antioxidant, dietary supplement and skincare ingredient	*Saccharomyces cerevisiae*	4.88	0.026	0.055	Molasses (CSL)	39
			5.76	0.031	0.053	Molasses and citrate (CSL)	39
Nisin	Preservative	*Lactococcus lactis*	0.3835	NR	0.0213	D-Glucose	10
γ-PGA	Food additive and humectant	*Bacillus subtilis*	101.1	0.57	2.19	D-Glucose and L-glutamate	9
		Bacillus licheniformis	39.85	NR	0.926	D-Glucose and citrate	40
L-Phenylalanine/L-tyrosine derivatives							
Cinnamic acid	Flavour, fragrance and skincare ingredient	*Escherichia coli*	6.9	0.028	0.138	D-Glucose	195
Cinnamaldehyde	Flavour and fragrance	*Escherichia coli*	0.075	NR	0.0016	D-Glucose	65
Benzoic acid	Preservative	*Escherichia coli*	2.37	0.0312	0.0165	D-Glucose	66
Pinocembrin	Antioxidant	*Escherichia coli*	0.5258	NR	0.01878	D-Glucose	182
Melanin	Sunscreen, antioxidant and colourant	*Streptomyces kathirae*	28.8	NR	0.225	Amylodextrine and L-tyrosine	67
		Escherichia coli	3.223	0.093	0.0268	D-Glucose	68
Betanin	Colourant	*Saccharomyces cerevisiae*	0.017	NR	0.00035	D-Glucose	69
Coniferyl alcohol	Antioxidant	*Saccharomyces cerevisiae*	0.2011	NR	0.002793	D-Glucose	73
Rosmarinic acid	Flavour and dietary supplement	*Escherichia coli* (co-culture)	0.172	NR	0.00287	D-Xylose and D-glucose	154
Phloretin	Antioxidant	*Saccharomyces cerevisiae*	0.0427	NR	0.000593	D-Glucose	173
Phlorizin	Antioxidant and skincare ingredient	*Saccharomyces cerevisiae*	0.065	NR	0.00090	D-Glucose	173
Trilobatin	Sweetener	*Saccharomyces cerevisiae*	0.0328	NR	0.000456	D-Glucose	173
Flavonoids							
Resveratrol	Antioxidant	*Yarrowia lipolytica*	12.355	0.0544	0.14	D-Glucose	162
Naringenin	Skincare ingredient	*Escherichia coli*	0.588	NR	0.007	D-Glucose	70
Kaempferol	Antioxidant	*Saccharomyces cerevisiae* (co-culture)	0.1681	NR	0.00233	D-Glucose	71
Quercetin	Antioxidant	*Saccharomyces cerevisiae* (co-culture)	0.1542	NR	0.00214	D-Glucose	71
Myricetin	Antioxidant	*Saccharomyces cerevisiae* (co-culture)	0.1450	NR	0.00201	D-Glucose	71
Eriodictyol	Bitter-masking agent	*Saccharomyces cerevisiae*	0.152	NR	0.00211	D-Glucose	72
Catechin	Dietary supplement	*Escherichia coli*	0.9109	0.9109	0.01898	Eriodictyol	151
Taxifolin	Anti-ageing and hair repigmenting agent	*Saccharomyces cerevisiae*	0.3368	NR	0.003119	D-Glucose	73

Table 1 (continued) | Recent examples of food and cosmetic compounds production using microorganisms

Product	Use	Host	Titre ($g\ l^{-1}$)	Yield ($g\ g^{-1}$)[a]	Productivity ($g\ l^{-1}\ h^{-1}$)	Carbon source	Ref.
Flavonoids (continued)							
Silybin (silibinin)	Dietary supplement	*Saccharomyces cerevisiae*	0.10485	NA	NA	D-Glucose	73
Isosilybin	Dietary supplement	*Saccharomyces cerevisiae*	0.19626	NA	NA	D-Glucose	73
Apigenin 7-*O*-glucuronide	Dietary supplement	*Saccharomyces cerevisiae*	0.185	NR	0.00154	D-Glucose	148
Anthocyanins							
Pelargonidin 3-*O*-glucoside (callistephin)	Colourant	*Saccharomyces cerevisiae*	0.00085	NR	0.000012	D-Glucose	72
		Escherichia coli (co-culture)	0.0095	NR	0.00018	D-Glucose and malonate	74
			0.0083	NR	0.00016	D-Glucose	74
Cyanidin 3-*O*-glucoside	Colourant	*Saccharomyces cerevisiae*	0.00155	NR	0.0000215	D-Glucose	72
Delphinidin 3-*O*-glucoside	Colourant	*Saccharomyces cerevisiae*	0.00186	NR	0.0000258	D-Glucose	72
Other derivatives of amino acids/intermediates							
Vanillin	Flavour	*Escherichia coli*	38.3	82.3%	4.79	Isoeugenol	135
		Amycolatopsis sp.	22.3	NR	0.858	D-Glucose and ferulic acid	134
		Schizosaccharomyces pombe	0.065	NR	0.0014	D-Glucose	75
Vanillin β-glucoside	Flavour	*Schizosaccharomyces pombe*	0.118	NR	0.00245	D-Glucose	75
Salicylic acid	Skin exfoliant	*Escherichia coli*	0.520	NR	0.0217	Glycerol and D-glucose	77
Methyl anthranilate	Flavour and fragrance	*Escherichia coli*	4.47	0.045	0.062	D-Glucose	76
		Corynebacterium glutamicum	5.74	0.020	0.052	D-Glucose	76
Violacein	Colourant	*Escherichia coli*	4.07	NR	0.0885	D-Glucose	54
			6.69	NR	0.0542	D-Glucose	79
Deoxyviolacein	Colourant	*Escherichia coli*	1.23	NR	0.318	D-Glucose	54
			11.3	NR	0.105	D-Glucose	79
Indigo	Colourant	*Escherichia coli*	0.64	NR	0.0089	D-Glucose	78
Indirubin	Colourant	*Escherichia coli*	0.056	NR	0.00078	D-Glucose	78
Indigoidine	Colourant	*Corynebacterium glutamicum*	49.3	0.14	0.96	D-Glucose	83
Haem	Flavour and colourant	*Escherichia coli*	1.0	0.0041	0.021	Glycerol	80
		Corynebacterium glutamicum	0.309	0.0021	0.00644	D-Glucose	81
Zinc protoporphyrin IX	Colourant	*Escherichia coli*	2.2	0.0091	0.040	Glycerol	82
Ectoine	Skincare ingredient and sunscreen	*Corynebacterium glutamicum*	65.3	0.19	1.16	D-Glucose and molasses	84
Terpenoids							
Monoterpenoids							
Geraniol	Fragrance	*Escherichia coli*	2.0	NR	0.029	D-Glucose	85
			2.1241	NR	0.04425	Glycerol	86
		Saccharomyces cerevisiae	5.52	NR	0.00743	D-Galactose and raffinose	87
Limonene	Fragrance	*Escherichia coli*	3.63 (*S*)	NR	0.151	Glycerol	88
		Saccharomyces cerevisiae	2.58 (*R*)	NR	0.00347	D-Galactose and raffinose	87
Linalool	Fragrance	*Pantoea ananatis*	5.60 (*S*)	0.0933	0.117	D-Glucose	157
			3.71 (*R*)	0.0618	0.773	D-Glucose	157
Citronellol	Fragrance	*Saccharomyces cerevisiae*	8.30	NR	0.0716	D-Glucose	170
β-Phellandrene	Fragrance	*Synechocystis* sp.	0.0181	NR	0.000126	CO_2	142

Table 1 (continued) | Recent examples of food and cosmetic compounds production using microorganisms

Product	Use	Host	Titre ($g\ l^{-1}$)	Yield ($g\ g^{-1}$)[a]	Productivity ($g\ l^{-1}\ h^{-1}$)	Carbon source	Ref.
Terpenoids (continued)							
Monoterpenoids (continued)							
Sabinene	Flavour and fragrance	*Saccharomyces cerevisiae*	0.1549	NR	0.001614	Ethanol	164
			0.16738	NR	0.0011624	D-Galactose and raffinose	158
Sesquiterpenoids							
α-Santalene	Flavour and fragrance	*Escherichia coli*	2.916	NR	0.0247	Glycerol	150
Santalols	Fragrance	*Saccharomyces cerevisiae*	1.2955	NR	0.015	D-Galactose	196
α-Santalol	Fragrance	*Saccharomyces cerevisiae*	1.1769	NR	0.014	D-Galactose	196
α-Farnesene	Fragrance	*Yarrowia lipolytica*	25.55	NR	0.0887	D-Glucose	89
β-Farnesene	Fragrance	*Saccharomyces cerevisiae*	130	NR	0.417	Cane syrup	90
Farnesol	Fragrance	*Escherichia coli*	0.5261	NR	0.008768	Glycerol	197
Nootkatone	Insect repellant and cosmetic ingredient	*Saccharomyces cerevisiae*	1.02	NR	0.00515	D-Glucose	91
Valencene	Fragrance	*Saccharomyces cerevisiae*	3.73	NR	0.0188	D-Glucose	91
Bisabolene	Fragrance And flavour	*Escherichia coli*	1.15	NR	0.016	D-Glucose	179
α-Bisabolene	Fixative in perfumery	*Yarrowia lipolytica*	0.9731	NR	0.00811	Waste cooking oil	139
β-Bisabolene	Flavour	*Yarrowia lipolytica*	0.0682	NR	0.00056	Waste cooking oil	139
γ-Bisabolene	Flavour and fragrance	*Yarrowia lipolytica*	0.0202	NR	0.00017	Waste cooking oil	139
α-Bisabolol	Fragrance and skin-healing agent	*Yarrowia lipolytica*	4.4	NR	0.0262	D-Glucose	153
Nerolidol	Flavour and fragrance	*Saccharomyces cerevisiae*	5.5	NR	0.057	Sucrose	175
Diterpenoids							
Rebaudioside A	Sweetener	*Saccharomyces cerevisiae*	0.7037	NR	0.0056	D-Glucose	92
Rebaudioside D	Sweetener	*Saccharomyces cerevisiae*	2.786	NR	0.0221	D-Glucose	92
Rebaudioside M	Sweetener	*Saccharomyces cerevisiae*	2.673	NR	0.0212	D-Glucose	92
Triterpenoids							
Squalene	Dietary supplement	*Saccharomyces cerevisiae*	21.1	NR	0.149	D-Glucose	93
Oleanolic acid	Skincare ingredient	*Yarrowia lipolytica*	0.5407	0.005407	0.00659	D-Glucose	94
Ergosterol	Provitamin D_2	*Saccharomyces cerevisiae*	2.9867	NR	0.024481	D-Glucose and ethanol	95
7-Dehydrocholesterol	Provitamin D_3	*Saccharomyces cerevisiae*	0.3606	NR	0.003606	D-Glucose and D-galactose	96
Tetraterpenoids and derivatives							
Lycopene	Dietary supplement	*Escherichia coli*	3.52	NR	0.0352	Glycerol	97
		Yarrowia lipolytica	4.2	NR	0.016	D-Glucose, palmitic acid and isoprenol	98
β-Carotene	Dietary supplement	*Yarrowia lipolytica*	6.5	0.036	0.053	D-Glucose	99
Zeaxanthin	Dietary supplement	*Escherichia coli*	0.72246	NR	0.0129	D-Glucose	100
Astaxanthin	Dietary supplement	*Escherichia coli*	1.18	NR	0.0197	Glycerol	101
Lutein	Dietary supplement	*Escherichia coli*	0.218	NR	0.00501	Glycerol	102
Retinal	Dietary supplement and skincare ingredient	*Saccharomyces cerevisiae*	2.094	NR	0.0131	D-Xylose	103

Table 1 (continued) | Recent examples of food and cosmetic compounds production using microorganisms

Product	Use	Host	Titre (g l^{-1})	Yield (g g^{-1})[a]	Productivity (g l^{-1} h^{-1})	Carbon source	Ref.
Tetraterpenoids and derivatives (continued)							
Retinoic acid	Dietary supplement and skincare ingredient	*Escherichia coli*	0.0082	0.00041	0.00055	Glycerol	104
Retinol	Dietary supplement and skincare ingredient	*Saccharomyces cerevisiae*	1.338	NR	0.00836	D-Xylose	103
			2.47934	NR	0.02066	D-Glucose and ethanol	105
Small organic acids							
Acetic acid	Food ingredient	*Acetobacter aceti*	111.7	NR	0.60	Ethanol	155
Glycolic acid	Skin exfoliant	*Escherichia coli*	65.5	0.765	0.85	D-Glucose	114
			44	0.44	0.92	D-Xylose	115
			43.6	0.46	0.91	D-Xylose	116
Pyruvic acid	Dietary supplement and skincare ingredient	*Saccharomyces cerevisiae*	135	0.54	1.35	D-Glucose	106
Lactic acid	Skin exfoliant	*Rhizopus oryzae*	231 (L)	0.925	1.83	D-Glucose	110
		Corynebacterium glutamicum	215 (L)	0.96	4.48	D-Glucose	198
			212 (L)	0.979	2.65	D-Glucose	112
			264 (D)	0.95	3.30	D-Glucose	112
		Sporolactobacillus inulinus	221 (D)	0.96	1.55	D-Glucose (HPAR)	111
Propionic acid	Preservative	*Propionibacterium freudenreichii*	136.23	0.5	0.57	D-Glucose	156
Butyric acid	Flavour	*Clostridium tyrobutyricum*	86.9	0.46	1.1	D-Glucose	113
Succinic acid	Acidulant and flavour	*Yarrowia lipolytica*	209.7	NR	0.65	Crude glycerol	108
		Corynebacterium glutamicum	152.2	0.85	0.95	D-Glucose	109
		Mannheimia succiniciproducens	134.25	0.819	5	D-Glucose and glycerol	8
Malic acid	Food additive	*Aspergillus niger*	201.13	1.22	1.05	D-Glucose	163
Citramalic acid	Acidulant and skin wrinkle reducer	*Escherichia coli*	82	0.48	1.85	D-Glucose	181
Fumaric acid	Acidulant	*Rhizopus oryzae*	103	0.79	1.43	Commercial corn sugar (CSL)	199
Citric acid	Flavour and preservative	*Yarrowia lipolytica*	140	0.82	0.73	Sucrose	107
Alcohols							
Ethanol	Food ingredient	*Kluyveromyces marxianus*	118	0.44	2.19	Cassava chip hydrolysates and molasses	138
1-Propanol	Personal care ingredients	*Escherichia coli*	10.8	0.107	0.144	D-Glucose	166
			10.3	0.259	0.083	Glycerol	166
2-Propanol	Solvent and fragrance	*Escherichia coli*	143	0.225	0.596	D-Glucose	184
1-Butanol	Solvent and fragrance	*Clostridium acetobutylicum*	146	0.31	1.32	D-Glucose	160
Isobutanol	Solvent and fragrance	*Corynebacterium glutamicum*	72.7	0.321	2.65	D-Glucose	119
Acetoin	Flavour	*Saccharomyces cerevisiae*	100.1	0.44	1.82	D-Glucose	117
		Bacillus amyloliquefaciens	65.9	0.31	1.57	Bakery waste hydrolysate	141
2-Methyl-1-butanol	Fragrance	*Corynebacterium crenatum*	4.87	NR	0.0507	NR	120
		Saccharomyces cerevisiae	2.38	0.01417	0.00902	D-Glucose	121

Table 1 (continued) | Recent examples of food and cosmetic compounds production using microorganisms

Product	Use	Host	Titre ($g\ l^{-1}$)	Yield ($g\ g^{-1}$)[a]	Productivity ($g\ l^{-1}\ h^{-1}$)	Carbon source	Ref.
Alcohols (continued)							
3-Methyl-1-butanol (Isoamyl alcohol)	Fragrance	*Escherichia coli*	9.5	0.11	0.158	D-Glucose	159
		Corynebacterium crenatum	3.57	NR	0.0372	NR	120
Diols							
1,2-Propanediol	Foam stabilizer and humectant	*Escherichia coli*	9.3 (*S*)	0.098	0.39	D-Glucose	149
			17.3 (*R*)	0.178	0.72	D-Glucose	149
1,3-Propanediol	Humectant	*Corynebacterium glutamicum*	110.4	0.42	2.3	D-Glucose	118
1,3-Butanediol	Humectant	*Escherichia coli*	15.75 (*R*)	0.186	0.1641	D-Glucose	178
		Cupriavidus necator	2.97 (*R*)	NR	0.0248	CO_2	143
2,3-Butanediol	Humectant	*Saccharomyces cerevisiae*	178	0.335	1.88	D-Glucose	168
			132	0.324	1.92	Cassava hydrolysate	168
		Enterobacter cloacae	152	0.489	3.5	D-Glucose and D-xylose	140
			119.4	0.475	2.3	Corn stover hydrolysate	140
Others							
Butyl butyrate	Fragrance and flavour	*Clostridium* sp.	22.4	NR	0.184	D-Xylose and butyrate	122
Isobutyl acetate	Fragrance and flavour	*Escherichia coli*	36	0.18	0.50	D-Glucose	200
Isoamyl acetate	Fragrance and flavour	*Escherichia coli*	0.78	0.039	0.016	D-Glucose	200
Carminic acid	Colourant	*Escherichia coli*	0.00065	NR	0.000006	D-Glucose	123
Inosine monophosphate	Flavour enhancer	*Corynebacterium ammoniagenes*	70.3	NR	NR	D-Fructose, D-glucose and molasses	124
Guanosine monophosphate	Flavour enhancer	*Corynebacterium ammoniagenes*	23	NR	0.23	D-Glucose	125
Thiamine pyrophosphate	Vitamin B_1 derivative	*Escherichia coli*	0.00080	NR	0.000017	D-Glucose	129
Riboflavin	Vitamin B_2	*Bacillus subtilis*	26.8	NR	0.38–0.45	D-Glucose (CSL)	126
Nicotinamide	Vitamin B_3	*Escherichia coli*	508	NR	508	D-Glucose, lactose and 3-cyanopyridine	136
Cobalamin	Vitamin B_{12}	Mesophilic methane bacteria	0.185	NR	NR	CO_2	128
Tocotrienols	Vitamin E	*Saccharomyces cerevisiae*	0.32	NR	0.00213	D-Glucose and ethanol (CSP)	130
Menaquinone 4	Vitamin K_2	*Bacillus subtilis*	0.217	NR	0.00181	D-Glucose	131
Menaquinone 7	Vitamin K_2	*Escherichia coli*	1.35	NR	0.026	D-Glucose	132
Coenzyme Q_{10}	Dietary supplement	*Rhodobacter sphaeroides*	1.95	NR	0.0257	D-Glucose	133

CEAA, conditionally essential amino acid; CSL, corn steep liquor; CSP, corn steep powder; HMO, human milk oligosaccharide; HPAR, hydrolysate of protein-rich agricultural residues; NA, not applicable; NEAA, non-essential amino acid; NR, not reported. [a]For biotransformation studies, conversion yields are provided as a percentage; for studies utilizing multiple carbon sources, the reference carbon source for calculating yields is provided in parentheses.

microbial fermentation can be applied to produce cosmetic compounds, including fragrances, colourants, solvents, humectants and other skincare compounds. These microbial products can be classified by their molecular and biosynthetic features.

Saccharides and derivatives

Microorganisms can be used to convert lower-value sugars into rare carbohydrates with beneficial features, such as low calorific content, anti-dental caries activity and gelling properties. In particular, six- and five-carbon (C6/C5) monosaccharides and derivatives are often produced through isomerization, oxidation and/or reduction of lower-value (in terms of both price and nutrition) monosaccharides with the same number of carbon units (Fig. 2a), using microbial strains that overexpress relevant enzymes. For example, common hexoses (sucrose, D-glucose and D-fructose) are used to produce allitol[11], D-allulose[12] and D-tagatose[13], which are C6 sugar substitutes with higher sweetness or lower calories. In addition, C5 sugars and their derivatives, including arabitol[14] and xylitol[15,16], used as sugar substitutes with anti-dental caries properties, can be produced from D-xylose abundant in lignocellulosic biomass.

Box 1

SysME strategies

Systems metabolic engineering (SysME) streamlines developing high-performance strains through recurrent flows among eight phases: production mode selection; host selection; metabolic pathway reconstruction; tolerance enhancement; metabolic flux optimization; fermentation process optimization; downstream process integration; and scale-up.

Production mode selection
In the first phase, the overall scheme of a microbial process is drawn up by determining a target product, a carbon source for the microbial process and a way of converting the substrate to the target product.

Host selection
A production host is selected from a pool of model organisms that have comprehensively understood physiology and well established engineering tools, native overproducers of target products or their precursors/analogues and a wider range of microorganisms with remarkable characteristics (such as harmlessness, tolerance and autotrophy).

Metabolic pathway reconstruction
For production hosts lacking metabolic routes towards the target compound, a heterologous biosynthetic pathway from other organisms or a synthetically designed pathway is introduced in this phase.

Tolerance enhancement
Some target compounds, intermediates and exogenous stimuli generated during fermentation may impose cytotoxicity or metabolic burden on the production host. Tolerance of these factors is enhanced in this phase to achieve a high production titre by applying a rational, random or process engineering approach.

Metabolic flux optimization
The metabolic flux towards the target product is maximized by applying diverse strategies, such as eliminating feedback regulations, enhancing functional expression and catalytic activities of biosynthetic enzymes, optimizing coenzyme supply, channelling substrates and electrons, the divide-and-conquer strategy, adjusting the intracellular environment and securing precursors/intermediates/final products.

Fermentation process optimization
In addition to genetically modulating production strains, adjusting culture conditions (such as temperature, dissolved oxygen level and medium composition) and applying external stimuli (such as nutrient limitation and pH shift) as well as various modes of fermentation (such as batch, fed-batch and multi-stage fermentations) are examined in this phase to maximize the production performance.

Downstream process integration
Multiple strategies are applied during strain development and fermentation (for example, maximizing product titre, lowering by-product concentration and in situ isolation of products) to streamline the recovery and purification of target compounds from the fermentation broth and substantially reduce the purification cost.

Scale-up
In the last phase, design of a seed train, optimization of the mass-transfer rate and minimization of the raw material cost are conducted to establish a scale-up fermentation process with high production capacity and reduced operational cost.

Similarly, three-carbon (C3) sugars and derivatives can be produced through biotransformation of lower-value C3 compounds (Fig. 2a). For example, dihydroxyacetone (DHA), used as a sun-free tanning agent[17], can be produced from glycerol, a low-priced by-product of biodiesel production from lipids. Four-carbon (C4) sugars infrequently found in nature can be produced using the pentose phosphate pathway of microorganisms, which can transform C3/C5/C6 sugars to C4 sugars and their derivatives by transferring the two-carbon (C2) or C3 moiety between three-to-seven-carbon (C3–C7) substrates and intermediates (Fig. 2a). For example, erythritol, a C4 sweetener, can be produced from glycerol[18]. Similarly, C6 sugars including L-fructose and L-fucose, used as a non-nutritive sweetener[19] and a skincare additive[20], respectively, can be produced from glycerol.

These metabolic routes can also be applied to produce oligosaccharides and polysaccharides. For example, the human milk oligosaccharide 2′-fucosyllactose[21], hyaluronic acid (used as a humectant, anti-wrinkle agent and dermal filler)[22–24] and chondroitin and chondroitin sulfate (consumed as dietary supplements)[25] can be produced from D-glucose, D-galactose, lactose, maltose, sucrose or glycerol (Fig. 2a).

Fatty acids and lipids

Microbial fatty acids and lipids are mostly used for biodiesel bioproduction, yet also as food resources, similar to plant oils or animal fats[26,27]. They can be produced through endogenous fatty acid biosynthesis routes using acetyl coenzyme A (acetyl-CoA) as a precursor (Fig. 2b). Microorganisms also produce essential fatty acids such as ω-3 and ω-6 polyunsaturated fatty acids, which are well recognized for their broad benefits to the cardiovascular system, to mental health and in reducing inflammation. Hence, microbial eicosatetraenoic acid[28], eicosapentaenoic acid[29], docosapentaenoic acid[30] and docosahexaenoic acid[30,31] are being exploited to replace less sustainable fish oils.

Amino acids and proteins

Proteins and amino acids are important macronutrients that are scarce in many parts of the world[1]. Microorganisms can be used to produce

protein-rich cell biomass or single-cell proteins from lower-price and less nutritious substrates[32,33]. Microorganisms are also used to produce non-nutritional proteins for flavouring, food processing, skin and hair conditioning and scavenging reactive oxygen species[34–38]. Moreover, non-ribosomal peptides produced by microbial fermentation, including glutathione[39], nisin[10] and poly-γ-glutamic acid (γ-PGA)[9,40] are used as an antioxidant, a natural food preservative and a super water-absorbing polymer, respectively.

Many amino acids are synthesized by microorganisms from central carbon metabolites (Fig. 3a), such as metabolites of the glycolysis pathway, the TCA cycle and the pentose phosphate pathway. For example, pyruvate serves as a precursor of a non-essential amino acid L-alanine[41,42] and the essential amino acids L-valine[43], L-leucine[44], L-isoleucine[45] and L-lysine[46], whereas acetyl-CoA is a co-precursor of L-leucine[44]. In addition, 3-phosphoglycerate and 2-ketoglutarate (from the glycolysis pathway and the TCA cycle) are used to produce L-serine[47,48] and L-glutamic acid[7], respectively. Moreover, phosphoenolpyruvate and erythrose 4-phosphate (from the glycolysis and pentose phosphate pathways) are precursors of the essential aromatic amino acids L-phenylalanine[49], L-tyrosine[50,51] and L-tryptophan[52]. Ribose 5-phosphate (from the pentose phosphate pathway) is a precursor of the essential amino acid L-histidine[53].

Some amino acids are key intermediates in the synthesis pathways of other amino acids (Fig. 3a). For example, L-aspartatic acid is an intermediate metabolite in the synthesis of the essential amino acids L-threonine[54,55], L-methionine[56], L-lysine[46] and β-alanine[57]. In addition, L-glutamic acid is an intermediate for the conditionally essential amino acids L-arginine[58–60] and L-proline[54,61] and the dietary supplements L-citrulline[62] and L-ornithine[63]. L-Serine and L-threonine are used for the biosynthesis of the conditionally essential amino acid L-cysteine[64] and the essential amino acid L-isoleucine[45], respectively.

Amino acid derivatives

Various natural compounds with aromatic ring(s), including phenolic acids, phenylpropanoids, flavonoids, anthocyanins and polyphenols, can be synthesized from L-phenylalanine or L-tyrosine (Fig. 3a). For example, cinnamoyl-CoA (derived from L-phenylalanine) serves as a precursor of cinnamaldehyde[65] and benzoic acid[66], which are used as a flavour and a preservative, respectively. In addition, L-dihydroxyphenylalanine (L-DOPA, derived from L-tyrosine) is a key intermediate of melanin that is used as a sunscreen ingredient, antioxidant and colourant[67,68], and betalains that are used as colourants[69]. Moreover, feruloyl-CoA, caffeoyl-CoA and *p*-coumaroyl-CoA (derived from L-phenylalanine or L-tyrosine) serve as key precursors for various functional compounds. In particular, naringenin (derived from *p*-coumaroyl-CoA)[70] is a key intermediate for flavonoid production, such as quercetin with an antioxidant activity[71], eriodictyol with a bitter-taste-masking property[72], (iso)silybin (a major constituent of silymarin, milk thistle seed extract), which is useful for treating liver diseases[73], and anthocyanins including pelargonidin 3-*O*-glucoside, cyanidin 3-*O*-glucoside and delphinidin 3-*O*-glucoside[72,74].

Intermediates in the biosynthesis of aromatic amino acids are important precursors of food and cosmetic compounds (Fig. 3a). For example, dehydroshikimate, an early intermediate in aromatic amino acid (L-phenylalanine, L-tyrosine and L-tryptophan) biosynthesis, is used for the de novo synthesis of vanillin, a key flavour ingredient of vanilla[75]. In addition, methyl anthranilate, which gives grape flavour[76], and salicylic acid, which is used as a skin exfoliant[77], are produced throughout the pathways involving chorismate, the last common intermediate of aromatic amino acids (Fig. 3a).

Other amino acids also act as key precursors for food and cosmetics production (Fig. 3a). For example, blue-to-purple colourants such as indigo[78], deoxyviolacein[54,79] and violacein[54,79] can be derived from L-tryptophan. Moreover, haem, a flavour and colourant for alternative meat production[80,81], and zinc protoporphyrin IX (ZnPPIX)[82], a red colourant for nitrite- and nitrate-free meat products, are derivatives of L-glutamic acid. In addition, another blue colourant, indigoidine[83], and a skincare ingredient, ectoine[84], are derived from L-glutamine and L-aspartic acid, respectively.

Terpenoids

Various terpenoids can be produced using microorganisms. Isopentenyl pyrophosphate (IPP) and dimethylallyl pyrophosphate (DMAPP), derived from acetyl-CoA (the mevalonate (MVA) pathway) or glyceraldehyde 3-phosphate and pyruvate (the 2-*C*-methylerythritol 4-phosphate (MEP) pathway), are building blocks of terpenoids (Fig. 2b). Combining IPP and DMAPP generates geranyl pyrophosphate (GPP), a precursor of monoterpenoids, and addition of IPP to GPP generates farnesyl pyrophosphate (FPP), a precursor of sesquiterpenoids. Monoterpenoids and sesquiterpenoids are volatile owing to their relatively small molecular weight, and thus they are used as fragrances and flavours such as geraniol[85–87], limonene[87,88], α-/β-farnesene[89,90], valencene[91] and nootkatone[91].

Diterpenoids, triterpenoids and tetraterpenoids are less volatile than monoterpenoids and sesquiterpenoids and, therefore, are used in applications other than fragrances. Geranylgeranyl pyrophosphate (GGPP), generated by combining IPP and FPP, is a key precursor of diterpenoids (Fig. 2b). Steviol glycosides, major components of the natural sweetener stevia, are diterpenoids produced by fermentation[92]. Triterpenoids including squalene, a dietary supplement[93], are synthesized by combining two FPP molecules (Fig. 2b). Subsequent modification of squalene, including oxidation, reduction, methylation and/or decarboxylation, enables the production of oleanolic acid[94], ergosterol[95] and 7-dehydrocholesterol[96], which are used as a skincare additive, and the provitamins D_2 and D_3, respectively.

Tetraterpenoids, also known as carotenoids, can be synthesized by microorganisms by combining two GGPP molecules forming phytoene (Fig. 2b). For example, lycopene[97,98], β-carotene[99], zeaxanthin[100], astaxanthin[101] and lutein[102] are red-to-yellow antioxidant carotenoids widely consumed as dietary supplements and colourants. In addition, β-carotene[99] and its derivatives, such as retinal[103], retinoic acid[104] and retinol[103,105], known as (pro)vitamins A, are used as skin anti-ageing agents.

Small organic acids

Many low-molecular-weight organic acids are derived from central carbon metabolites (Fig. 3b). For example, pyruvic acid, used as a dietary supplement and skincare ingredient[106], is a glycolytic product, and citric acid[107] and succinic acid[8,108,109], the food acidulants, flavours and preservatives, are TCA cycle metabolites. Additionally, lactic acid[110–112], used as a skincare ingredient, is derived from pyruvate, and butyric acid, a flavouring acid, is produced from acetoacetyl-CoA, which is generated by combining two molecules of acetyl-CoA[113]. Alternatively, some products are produced using multiple precursors and pathways. For example, glycolic acid, used as a skin-peeling agent, is produced from isocitrate through the glyoxylate shunt[114–116], ribulose 1-phosphate is produced through the ribulose 1-phosphate pathway[115] and/or xylonate is produced through the Dahms pathway[116].

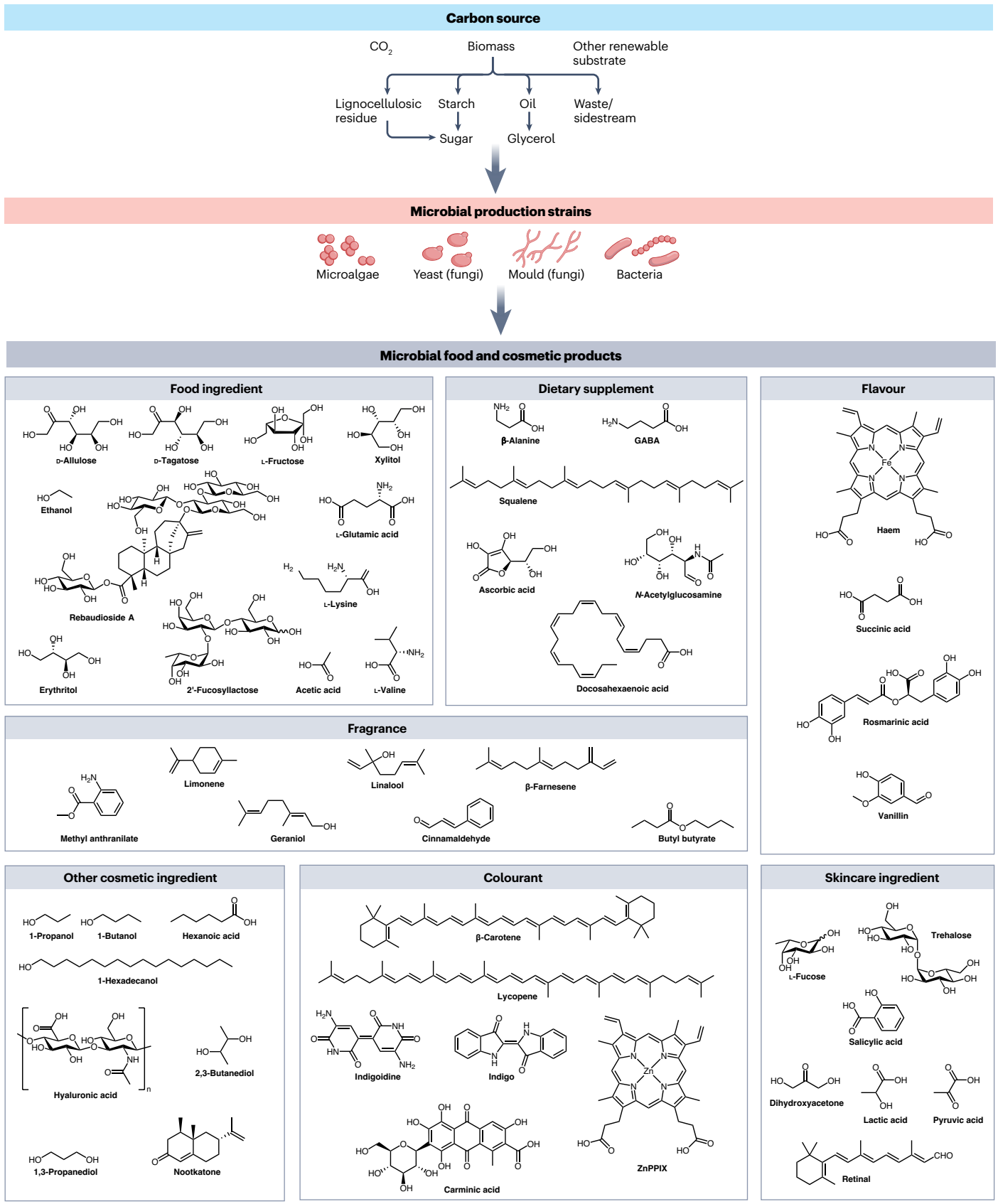
Carbon source
CO_2
Biomass
Other renewable substrate
Lignocellulosic residue
Starch
Oil
Waste/ sidestream
Sugar
Glycerol
Microbial production strains
Microalgae
Yeast (fungi)
Mould (fungi)
Bacteria
Microbial food and cosmetic products
Food ingredient
D-Allulose
D-Tagatose
L-Fructose
Xylitol
Ethanol
L-Glutamic acid
Rebaudioside A
L-Lysine
Erythritol
2'-Fucosyllactose
Acetic acid
L-Valine
Dietary supplement
β-Alanine
GABA
Squalene
Ascorbic acid
N-Acetylglucosamine
Docosahexaenoic acid
Flavour
Haem
Succinic acid
Rosmarinic acid
Vanillin
Fragrance
Limonene
Linalool
β-Farnesene
Methyl anthranilate
Geraniol
Cinnamaldehyde
Butyl butyrate
Other cosmetic ingredient
1-Propanol
1-Butanol
Hexanoic acid
1-Hexadecanol
Hyaluronic acid
2,3-Butanediol
1,3-Propanediol
Nootkatone
Colourant
β-Carotene
Lycopene
Indigoidine
Indigo
ZnPPIX
Carminic acid
Skincare ingredient
L-Fucose
Trehalose
Salicylic acid
Dihydroxyacetone
Lactic acid
Pyruvic acid
Retinal

Fig. 1 | Microbial production of food and cosmetic products. Various microbial strains can produce food and cosmetic products. Utilizing compounds derived from biomass, carbon dioxide and other renewable substrates as carbon sources, microbial strains can produce food ingredients, dietary supplements, flavours, fragrances, colourants, skincare ingredients and other cosmetic ingredients. Example compounds in each category and their chemical structures are presented. GABA, γ-aminobutyric acid; ZnPPIX, zinc protoporphyrin IX.

Short-chain alcohols

Various short-chain alcohols are also derived from central carbon metabolites (Fig. 3b). For example, acetolactate (generated by combining two pyruvate molecules) can produce acetoin[117], used as a flavour reminiscent of butter. Glycerol, derived from dihydroxyacetone phosphate (DHAP) of the upper glycolysis pathway, is used to produce the humectant 1,3-propanediol[118]. Intermediates of amino acid biosynthesis can also produce alcohols. For example, 2-ketoisobutyrate and 2-keto-3-methylvalerate from L-valine and L-isoleucine biosynthesis are used to produce isobutanol[119] and 2-methyl-1-butanol[120,121], used as perfuming agents.

Other compounds

Many other microbially produced compounds are applied in the food and cosmetic industries. For example, butyl butyrate, reminiscent of pineapple scent, is produced from butyric acid and butanol as precursors[122]. Also, carminic acid, a widely used red polyketide colourant, is produced from acetyl-CoA, malonyl-CoA and D-glucose[123]. Among the nucleotides and their derivatives, inosine monophosphate (IMP)[124] and guanosine monophosphate (GMP)[125], used as flavour enhancers, are produced through biosynthetic routes starting from 5-phosphoribose1-pyrophosphate (PRPP) and glycine (Fig. 3b). Moreover, riboflavin[126], total folates[127] and cobalamin[128] (vitamins B_2, B_9 and B_{12}) are produced by microorganisms using precursors derived from IMP and GMP (Fig. 3b). Other vitamins, including thiamine pyrophosphate (a physiologically active form of vitamin B_1)[129], tocotrienols (vitamin E)[130] and menaquinone 4/7 (vitamers of vitamin K_2)[131,132] and the dietary supplement coenzyme Q_{10} (ref. 133), are produced from various precursors derived from the central carbon metabolism, amino acid biosynthesis and/or terpenoid biosynthesis (Figs. 2b and 3b).

Systems metabolic engineering

Some microbial processes to produce food and cosmetic compounds have been commercialized (Supplementary Table 1), but there are still many that have not been translated owing to economic infeasibility (Box 2). SysME can help to develop high-performance strains (in terms of production titre, yield and productivity) for industrially competitive processes, following a series of strategies grouped into eight phases: production mode selection, host selection, metabolic pathway reconstruction, tolerance enhancement, metabolic flux optimization, fermentation process optimization, downstream process integration and scale-up (Box 1). Notably, recurrent flows, including feedforward and feedback operations, among the steps allow more effective development of industrially competitive processes.

Production mode selection

De novo biosynthesis of food and cosmetic compounds from renewable sugars, such as D-glucose and sucrose, is a favourable process owing to their abundance in nature, low price and compatibility with microbial fermentation (Table 1). Establishing an efficient de novo production process, however, may be difficult owing to high complexity of the biosynthetic pathway, absence of efficient catalytic enzymes or lack of knowledge about the complete biosynthetic pathway. If a low-priced compound that is structurally similar to the target compound or an intermediate of the biosynthesis is available, a biotransformation process can be devised to convert such a compound to the desired form. For example, microbial production of vanillin from D-glucose achieves a low titre (<0.1 g l^{-1})[75]. However, biotransformation of low-priced precursors, such as ferulic acid[134] and isoeugenol[135], is more efficient, producing 22.3 and 38.34 g l^{-1} of vanillin, respectively. Similarly, 508 g l^{-1} of nicotinamide (vitamin B_3) can be produced through biotransformation of 3-cyanopyridine, whereas microbial production of this compound from D-glucose or other renewable sugar has not been reported[136].

Alternatively, renewable sugars can be converted to an intermediate molecule through microbial fermentation and further transformed to the target product through additional chemical or enzymatic conversion steps. For example, chondroitin sulfate can be produced by enzymatic introduction of a sulfate group to the de novo biosynthesized chondroitin from sucrose[25]. In addition, (iso)silybin can be microbially produced by combining de novo biosynthesis of taxifolin and coniferyl alcohol from D-glucose, and adding an enzymatic step to join the two intermediates[73].

If the target product is unstable or toxic to the production host, a derivative of the compound with higher stability or lower toxicity can first be produced through fermentation and then converted back to the desired form through additional steps. For instance, glucosamine can be produced in the form of *N*-acetylglucosamine, a more stable derivative that hydrolyses back to glucosamine under acidic conditions[137]. Similarly, betanin is produced as a glycosylated derivative for its protection from oxidation[69].

In addition to utilizing sugars, glycerol is widely used as a precursor (Table 1). Waste from the food and agricultural industries, such as molasses[39,84,138], waste cooking oil[139] and hydrolysates of corn cob/stover[16,140] and bakery waste[141], are also used as carbon sources. Moreover, CO_2 can be used to produce various compounds by engineered autotrophs or heterotrophs[128,142,143].

Host selection

Along with traditional model organisms (for example, *Escherichia coli*, *Bacillus subtilis* and *Saccharomyces cerevisiae*), other natural host strains can produce target compounds or their precursors or intermediates with higher production performances (Table 1). For example, *Corynebacterium glutamicum* is a major workhorse that can produce various amino acids, such as L-alanine[42], L-valine[43], L-leucine[44], L-serine[47], L-proline[61], L-isoleucine[45], L-glutamic acid[7], L-lysine[46] and L-arginine[59]. Oleaginous microorganisms including *Rhodococcus opacus*[26], *Mortierella alpina*[28,29] and *Schizochytrium* species[30,31] are frequently employed to produce lipids, fatty acids and derivatives. Moreover, *Agrobacterium* species[144], *Aureobasidium pullulans*[145], *Gluconobacter xylinus*[146] and *Xanthomonas campestris*[147] are used to produce the polysaccharides curdlan, pullulan, cellulose and xanthan, respectively. Moreover, *Lactococcus lactis*[10], *Rhodobacter sphaeroides*[133] and *Mannheimia succiniciproducens*[8] can naturally overproduce nisin, coenzyme Q_{10} and succinic

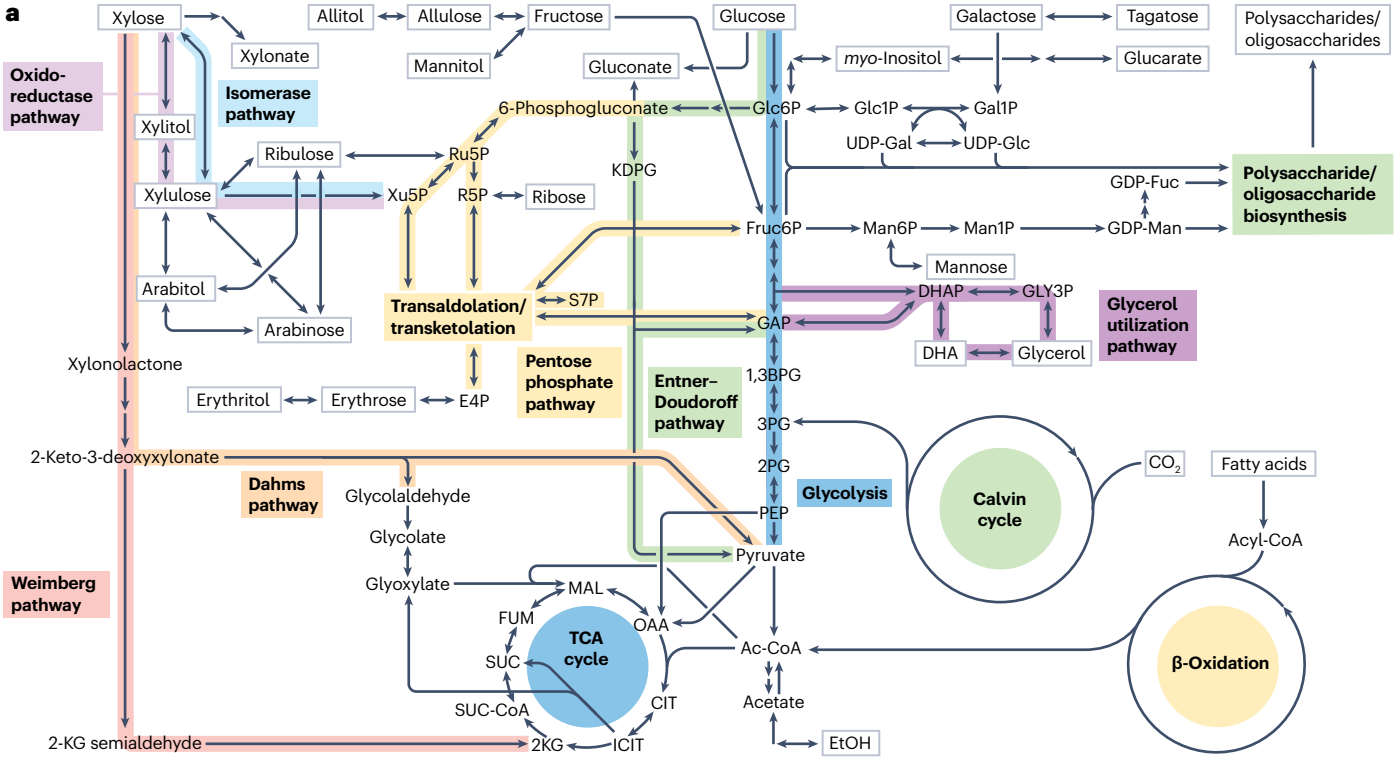
a
Xylose
Xylonate
Allitol
Allulose
Fructose
Mannitol
Glucose
Gluconate
Galactose
Tagatose
myo-Inositol
Glucarate
Polysaccharides/ oligosaccharides
Oxido-reductase pathway
Isomerase pathway
Xylitol
6-Phosphogluconate
Glc6P
Glc1P
Gal1P
UDP-Gal
UDP-Glc
Ribulose
Ru5P
KDPG
Xylulose
Xu5P
R5P
Ribose
GDP-Fuc
Polysaccharide/ oligosaccharide biosynthesis
Fruc6P
Man6P
Man1P
GDP-Man
Mannose
Arabitol
Arabinose
Transaldolation/ transketolation
S7P
DHAP
GLY3P
GAP
Glycerol utilization pathway
DHA
Glycerol
Xylonolactone
Pentose phosphate pathway
Entner–Doudoroff pathway
1,3BPG
Erythritol
Erythrose
E4P
3PG
2-Keto-3-deoxyxylonate
2PG
Dahms pathway
Glycolaldehyde
Glycolysis
PEP
CO2
Fatty acids
Calvin cycle
Glycolate
Pyruvate
Acyl-CoA
Weimberg pathway
Glyoxylate
MAL
FUM
OAA
TCA cycle
Ac-CoA
β-Oxidation
SUC
SUC-CoA
CIT
Acetate
2-KG semialdehyde
2KG
ICIT
EtOH
b
Xylose
Glucose
Xylitol
6-Phosphogluconate
Glc6P
Ru5P
KDPG
Xylulose
Xu5P
R5P
Fruc6P
Transaldolation/ transketolation
S7P
DHAP
GLY3P
GAP
DHA
Glycerol
Xylonolactone
E4P
1,3BPG
MEP pathway
VitE
Tetraterpenoids
3PG
VitK2
GGPP
Diterpenoids
2-Keto-3-deoxyxylonate
2PG
DXP
CoQ10
FPP
Triterpenoids
MEP
Glycolaldehyde
PEP
DMAPP
Sesquiterpenoids
Glycolate
Pyruvate
IPP
GPP
Monoterpenoids
Glyoxylate
MAL
FUM
OAA
TCA cycle
Ac-CoA
AcAc-CoA
Mevalonate
SUC
MVA pathway
Fatty alcohols
Lipids
SUC-CoA
CIT
2-KG semialdehyde
2KG
ICIT
Malony-CoA
Fatty acid synthesis
Fatty acids
Lipid synthesis
Fat-soluble vitamins and related compounds
Terpenoids
Fatty acids and derivatives
Renewable carbon sources, saccharides and derivatives

Fig. 2 | Metabolic pathways for producing carbohydrates, fatty acids, lipids, terpenoids and fat-soluble vitamins. a, Metabolic pathways for biosynthesis of carbohydrates utilizing renewable carbon sources. **b**, Biosynthetic pathways for fatty acids, lipids, terpenoids and fat-soluble vitamins. Solid and dashed arrows indicate single- and multiple-step reactions, respectively. 1,3BPG, 1,3-bisphosphoglycerate; 2KG, 2-ketoglutarate; 2PG, 2-phosphoglycerate; 3PG, 3-phosphoglycerate; Ac-CoA, acetyl-CoA; AcAc-CoA, acetoacetyl-CoA; CIT, citrate; CoQ_{10}, coenzyme Q_{10}; DHA, dihydroxyacetone; DHAP, dihydroxyacetone phosphate; DMAPP, dimethylallyl pyrophosphate; DXP, 1-deoxyxylulose 5-phosphate; E4P, erythrose 4-phosphate; EtOH, ethanol; FPP, farnesyl pyrophosphate; Fruc6P, fructose 6-phosphate; FUM, fumarate; Gal1P, galactose 1-phosphate; GAP, glyceraldehyde 3-phosphate; GDP, guanosine diphosphate; GDP-Fuc, GDP-fucose; GDP-Man, GDP-mannose; GGPP, geranylgeranyl pyrophosphate; Glc1P, glucose 1-phosphate; Glc6P, glucose 6-phosphate; GLY3P, glycerol 3-phosphate; GPP, geranyl pyrophosphate; ICIT, isocitrate; IPP, isopentenyl pyrophosphate; KDPG, 2-keto-3-deoxy-6-phosphogluconate; MAL, malate; Man1P, mannose 1-phosphate; Man6P, mannose 6-phosphate; MEP, 2-*C*-methylerythritol 4-phosphate; OAA, oxaloacetate; PEP, phosphoenolpyruvate; R5P, ribose 5-phosphate; Ru5P, ribulose 5-phosphate; S7P, sedoheptulose 7-phosphate; SUC, succinate; SUC-CoA, succinyl-CoA; TCA cycle, tricarboxylic acid cycle; UDP, uridine diphosphate; UDP-Gal, UDP-galactose; UDP-Glc, UDP-glucose; VitE, vitamin E (tocotrienols); $VitK_2$, vitamin K_2 (menaquinone 4/menaquinone 7); Xu5P, xylulose 5-phosphate.

acid, respectively, and *Bacillus subtilis*[9] and *Bacillus licheniformis*[40] can overproduce γ-PGA.

Other microorganisms can also be applied according to their characterisitsics. For example, *Saccharomyces cerevisiae*, *Bacillus subtilis* and *Corynebacterium glutamicum* are generally recognized as safe (GRAS) and used to produce compounds for direct consumption by humans, such as amino acids, sweeteners and dietary supplements (Table 1). As another example, *Amycolatopsis* species are used to produce vanillin owing to their high tolerance to the compound[134]. In addition, *Synechococcus* species[142] and *Cupriavidus necator*[143] utilize CO_2 through their native photosynthetic and hydrogenotrophic metabolisms, respectively. Moreover, the thermotolerant bacterium *Kluyveromyces marxianus* enables non-sterile fermentation at 42 °C without contamination and helps to reduce operational costs associated with sterilizing the medium, carbon sources and fermentation facilities while still maintaining fermentation performance[138]. Furthermore, *Yarrowia lipolytica*, which possesses hydrophobic intracellular compartments called lipid bodies, can help to produce hydrophobic carotenoids[98,99].

Metabolic pathway reconstruction

For production hosts lacking metabolic routes towards the target compound, heterologous biosynthetic pathways from other organisms or a synthetically designed pathway can be introduced. For example, microbial production of apigenin-7-*O*-glucuronide and scutellarin, two active ingredients of breviscapine (a total flavonoid extract of the medicinal plant *Erigeron breviscapus*) has been attempted using *Saccharomyces cerevisiae* as a heterologous production host, yet their biosynthetic routes were unclear[148]. To identify missing enzymes, the draft genome and transcriptome of *Erigeron breviscapus* were analysed to search for enzymes homologous to other reported enzymes showing similar catalytic activities. Following the process, two novel enzymes, flavonoid-7-*O*-glucuronosyltransferase and flavone-6-hydroxylase, were identified. Subsequent reconstruction of a heterologous biosynthetic pathway from *Erigeron breviscapus* involving the two enzymes in *Saccharomyces cerevisiae* enables production of 0.185 g l^{-1} of apigenin-7-*O*-glucuronide and 0.108 g l^{-1} of scutellarin[148]. Enzyme promiscuity, allowing catalysis of multiple structurally related substrates by a single enzyme, was exploited to devise heterologous biosynthetic pathways. For example, a synthetic de novo biosynthetic pathway of carminic acid was devised by integrating the promiscuous actions of aklavinone 12-hydroxylase from *Streptomyces peucetius* and *C*-glucosyltransferase from *Gentiana triflora* on flavokermesic acid[123]. Also, the promiscuous insertion of a zinc ion, instead of a ferrous ion, by ferrochelatase allows for the repurposing a haem-producing strain[80] into a zinc protoporphyrin IX (ZnPPIX) producer[82].

Some food and cosmetic compounds can be synthesized through multiple metabolic routes. For example, glyoxylic acid can be produced through the glyoxylate shunt[114–116], the ribulose-1-phosphate pathway[115] or the Dahms pathway[116], whereas 1,2-propanediol can be synthesized by lactate or methylglyoxal, derived from pyruvate or DHAP, respectively[149] (Fig. 3b). The optimal metabolic route can be selected through computational and experimental assessments. For example, in silico flux response analysis followed by experimental analysis helps to identify the best pathway for benzoic acid production among three routes derived from plants and one route synthetically designed[66]. As another example, for β-farnesene production, acetyl-CoA can be generated without carbon loss using the non-oxidative phosphoketolase-phosphotransacetylase bypass instead of the glycolysis pathway[90]. Alternatively, for terpenoid production, the MVA and MEP pathways are applied together to maximize generation of the key precursors IPP/DMAPP[86,100,150] (Fig. 2b). Similarly, the C4/C5 pathways for 5-aminolevulinate biosynthesis and the canonical/non-canonical pathways that convert coproporphyrinogen III derived from 5-aminolevulinate to haem can be combined to enhance the metabolic fluxes towards haem[81].

The rapid and modular cloning of plasmids can help in the genetic reconstruction of the designed metabolic pathways. For example, three flavanone 3β-hydroxylases, three dihydroflavonol 4-reductases and two leucoanthocyanidin reductases are used to produce catechin by constructing 18 plasmids that cover all possible combinations of the three enzymes. The best combinations can be screened for by culturing 18 recombinant *Yarrowia lipolytica* strains, each containing one of the 18 plasmids[151]. In another example, four compatible plasmids have been used to overexpress twelve endogenous genes in *Escherichia coli* and improve the metabolic flux of haem by optimizing the expression levels[152].

Chromosomal integration of the reconstructed genetic modules results in higher genetic stability during fermentation, although it requires more development time compared with the plasmid-based system. For example, heterologous genes encoding acetyl-CoA acetyltransferase, nicotinamide adenine dinucleotide (NADH)-dependent β-hydroxy-β-methylglutaryl (HMG)-CoA reductase and α-farnesene synthase are integrated to the *Yarrowia lipolytica* chromosome throughout non-homologous end joining (NHEJ)-mediated random integration, enabling α-farnesene production[89]. Moreover, combining the advantages of both plasmid- and integration-based approaches improves the modular cloning of up to 10 DNA fragments followed by NHEJ-dependent random integration of the assembly product into the chromosome, generating an α-bisabolol-producing strain[153].

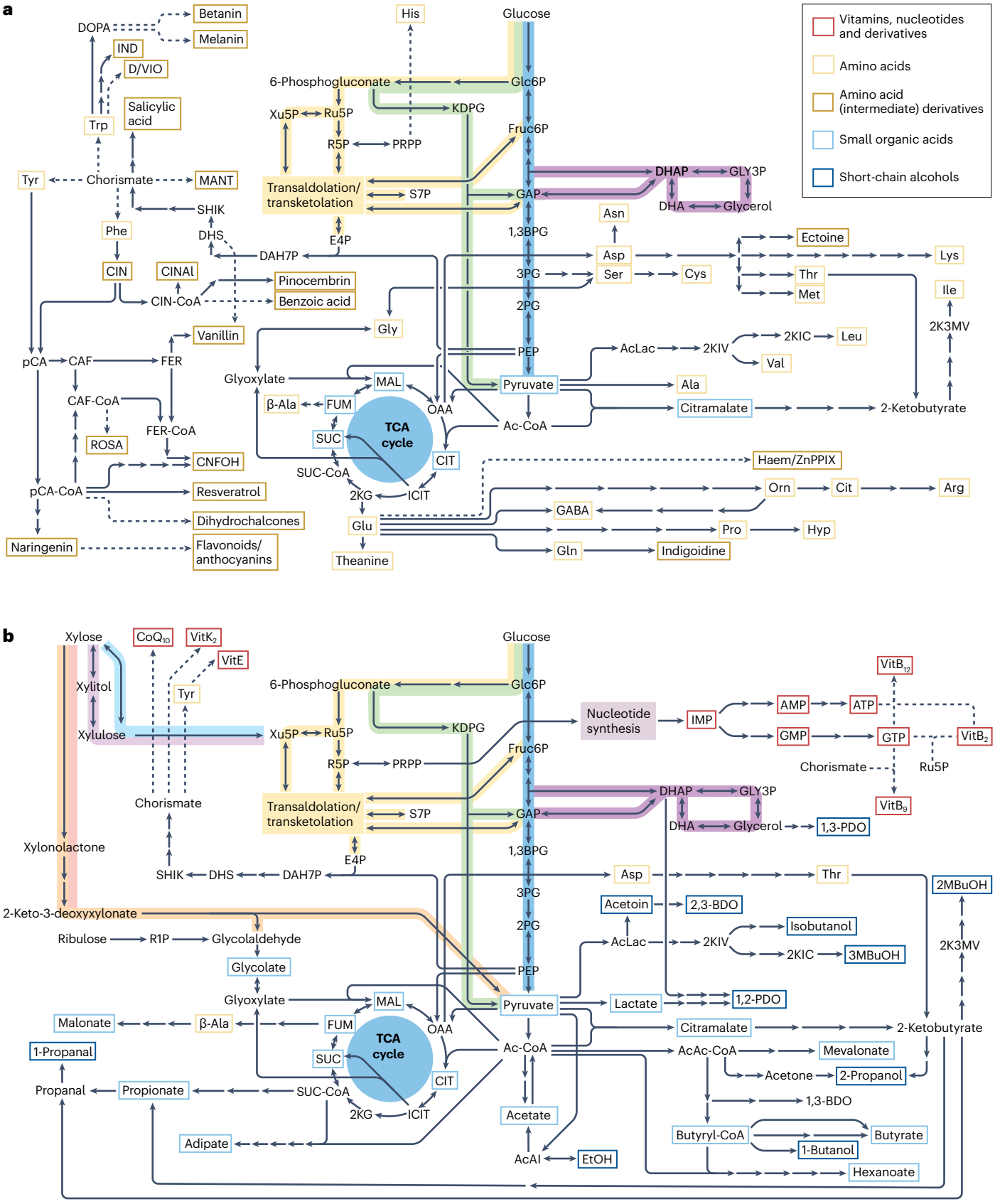
a
Vitamins, nucleotides and derivatives
Amino acids
Amino acid (intermediate) derivatives
Small organic acids
Short-chain alcohols
Glucose
Glc6P
Fruc6P
6-Phosphogluconate
KDPG
Xu5P
Ru5P
R5P
PRPP
Transaldolation/ transketolation
S7P
E4P
GAP
DHAP
GLY3P
DHA
Glycerol
1,3BPG
3PG
2PG
PEP
Pyruvate
Ac-CoA
TCA cycle
OAA
CIT
ICIT
2KG
SUC
SUC-CoA
FUM
MAL
Glyoxylate
β-Ala
Gly
Asn
Asp
Ser
Cys
Thr
Met
Lys
Ectoine
Ile
2K3MV
AcLac
2KIV
2KIC
Leu
Val
Ala
Citramalate
2-Ketobutyrate
Haem/ZnPPIX
Orn
Cit
Arg
GABA
Pro
Hyp
Gln
Indigoidine
Glu
Theanine
His
DOPA
Betanin
Melanin
IND
D/VIO
Trp
Salicylic acid
Tyr
Chorismate
MANT
SHIK
DHS
DAH7P
Phe
CIN
CINAl
CIN-CoA
Pinocembrin
Benzoic acid
Vanillin
pCA
CAF
FER
CAF-CoA
FER-CoA
ROSA
CNFOH
pCA-CoA
Resveratrol
Dihydrochalcones
Naringenin
Flavonoids/ anthocyanins
b
Xylose
Xylitol
Xylulose
Xylonolactone
2-Keto-3-deoxyxylonate
Ribulose
R1P
Glycolaldehyde
Glycolate
CoQ10
VitK2
VitE
Tyr
Chorismate
SHIK
DHS
DAH7P
Glucose
Glc6P
6-Phosphogluconate
KDPG
Xu5P
Ru5P
R5P
PRPP
Fruc6P
Transaldolation/ transketolation
S7P
E4P
GAP
DHAP
GLY3P
DHA
Glycerol
1,3-PDO
1,3BPG
3PG
2PG
PEP
Pyruvate
Ac-CoA
TCA cycle
OAA
CIT
ICIT
2KG
SUC
SUC-CoA
FUM
MAL
Glyoxylate
β-Ala
Malonate
1-Propanal
Propanal
Propionate
Adipate
Acetate
AcAl
EtOH
Nucleotide synthesis
IMP
AMP
GMP
ATP
GTP
VitB12
VitB2
VitB9
Ru5P
Chorismate
Asp
Thr
2MBuOH
Acetoin
2,3-BDO
AcLac
2KIV
Isobutanol
2KIC
3MBuOH
2K3MV
Lactate
1,2-PDO
Citramalate
2-Ketobutyrate
AcAc-CoA
Mevalonate
Acetone
2-Propanol
1,3-BDO
Butyryl-CoA
Butyrate
1-Butanol
Hexanoate

Fig. 3 | Metabolic pathways for producing amino acids, short-chain alcohols, small organic acids, nucleotides and vitamins. a, Metabolic pathways for biosynthesis of amino acids and derivatives. **b**, Biosynthetic pathways for short-chain alcohols, small organic acids, nucleotides and vitamins. Solid and dashed arrows indicate single- and multiple-step reactions, respectively. 1,2-PDO, 1,2-propanediol; 1,3-BDO, 1,3-butanediol; 1,3BPG, 1,3-bisphosphoglycerate; 1,3-PDO, 1,3-propanediol; 2,3-BDO, 2,3-butanediol; 2K3MV; 2-keto-3-methylvlaerate; 2KG, 2-ketoglutarate; 2KIC, 2-ketoisocaproate; 2KIV, 2-ketoisovalerate; 2MBuOH, 2-methylbutanol; 2PG, 2-phosphoglycerate; 3MBuOH, 3-methylbutanol; 3PG, 3-phosphoglycerate; β-Ala, β-alanine; Ac-CoA, acetyl-CoA; AcAc-CoA, acetoacetyl-CoA; AcAl, acetaldehyde; AcLac, acetolactate; Ala, L-alanine; AMP, adenosine monophosphate; Arg, L-arginine; Asn, L-asparagine; Asp, L-aspartic acid; ATP, adenosine triphosphate; CAF, caffeate; CAF-CoA, caffeoyl-CoA; CIT, citrate; Cit, L-citrulline; CIN, cinnamic acid; CINAl, cinnamaldehyde; CIN-CoA, cinnamoyl-CoA; CNFOH, coniferyl alcohol; CoQ_{10}, coenzyme Q_{10}; Cys, L-cysteine; DAH7P, 3-deoxyarabinoheptulosonate 7-phosphate; DHA, dihydroxyacetone; DHAP, dihydroxyacetone phosphate; DHS, 3-dehydroshikimate; DMAPP, dimethylallyl pyrophosphate; DOPA, L-dihydroxyphenylalanine; D/VIO, deoxyviolacein/violacein; E4P, erythrose 4-phosphate; EtOH, ethanol; FER, ferulate; FER-CoA, feruloyl-CoA; Fruc6P, fructose 6-phosphate; FUM, fumarate; GABA, γ-aminobutyric acid; GAP, glyceraldehyde 3-phosphate; Glc6P, glucose 6-phosphate; Gln, L-glutamine; Glu, L-glutamic acid; Gly, glycine; GLY3P, glycerol 3-phosphate; GMP, guanosine monophosphate; GTP, guanosine triphosphate; His, L-histidine; Hyp, L-hydroxyproline; ICIT, isocitrate; Ile, L-isoleucine; IMP, inosine monophosphate; IND, indigo/indirubin; KDPG, 2-keto-3-deoxy-6-phosphogluconate; Leu, L-leucine; Lys, L-lysine; MAL, malate; MANT, methyl anthranilate; Met, L-methionine; OAA, oxaloacetate; Orn, L-ornithine; pCA, *p*-coumaric acid; pCA-CoA, *p*-coumaroyl-CoA; PEP, phosphoenolpyruvate; Phe, L-phenylalanine; Pro, L-proline; PRPP, phosphoribosyl pyrophosphate; R1P, ribose 1-phosphate; R5P, ribose 5-phosphate; ROSA, rosmarinic acid; Ru5P, ribulose 5-phosphate; S7P, sedoheptulose 7-phosphate; Ser, L-serine; SHIK, shikimate; SUC, succinate; SUC-CoA, succinyl-CoA; TCA cycle, tricarboxylic acid cycle; Thr, L-threonine; Trp, L-tryptophan; Tyr, L-tyrosine; Val, L-valine; $VitB_2$, vitamin B_2 (riboflavin); $VitB_9$, vitamin B_9 (total folates); $VitB_{12}$, vitamin B_{12} (cobalamin); VitE, vitamin E (tocotrienols); $VitK_2$, vitamin K_2 (menaquinone 4/menaquinone 7); Xu5P, xylulose 5-phosphate.

The titres and yields can be improved by dividing the biosynthetic pathway into multiple modules and allocating them to multiple strains. For example, co-culturing two strains, which have naringenin and naringenin-to-flavonoid pathways, can produce over 100 mg l^{-1} of a flavonoid such as kaempferol, quercetin and myricetin[71]. Another example is dividing the biosynthetic pathway of rosmarinic acid into three modules (xylose to caffeic acid, D-glucose to salvianic acid A, caffeic acid and salvianic acid A to rosmarinic acid) and individually introducing them to three strains, followed by co-culturing to facilitate de novo biosynthesis of rosmarinic acid[154]. Moreover, biosynthesis of pelargonidin 3-*O*-glucoside, a red anthocyanin from strawberries, is achieved by allocating the relevant biosynthetic enzymes to four *Escherichia coli* strains and co-culturing[74].

Another strategy is the addition of external enzymes to replace some part of the metabolic pathway. For example, raw-starch-degrading enzyme and glucoamylase can be added during fermentation to produce ethanol from raw cassava chips using *Kluyveromyces marxianus*, which cannot consume raw starch[138].

Tolerance enhancement

Overexpressing exporters of toxic compounds can improve tolerance to such compounds. For example, a putative ATP-binding cassette transporter encoded by the *aatA* gene of *Acetobacter aceti* is involved in acetic acid tolerance[155]. Thus, plasmid-based overexpression of the *aatA* gene improves acetic acid tolerance and production in *Acetobacter aceti*[155]. Similarly, overexpressing *Vitreoscilla* haemoglobin in the obligate aerobe *Gluconobacter xylinus* improves cellulose production by enhancing oxygen supply under the hypoxic conditions induced during cellulose accumulation[146]. Another useful strategy is to eliminate the source of toxicity. For example, the generation of methylglyoxal, which is a cytotoxic intermediate of 1,2-propanediol biosynthesis through DHAP, can be eliminated by implementing an alternative methylglyoxal-free metabolic route through lactate[149].

Along with rational strategies, random approaches can help to improve tolerance against toxic compounds for which the stress mechanisms are unclear, as has long been practised in industry. For example, mutant *Corynebacterium glutamicum* strains exhibiting higher L-arginine tolerance and production can be isolated using *N*-methyl-*N*′-nitro-*N*-nitrosoguanidine and ultraviolet light treatment followed by screening for colonies tolerant to L-arginine analogues (L-arginine hydroxamate or L-canavanine)[59]. Alternatively, 18 rounds of repeated fed-batch fermentation enable the isolation of an adapted *Clostridium tyrobutyricum* strain tolerant to butyric acid[113].

Process engineering approaches also help to overcome product toxicities. Immobilizing host strains on solid particles can enhance tolerance to the production of organic acids. For example, adding sugarcane bagasse allows *Propionibacterium freudenreichii* and *Yarrowia lipolytica* to attach to the debris and thus enhance their tolerance to propionic acid[156] and succinic acid[108], respectively. Similarly, introducing sponge-like polyurethane foam can enhance the tolerance of *Rhizopus oryzae* to lactic acid[110]. Another way to reduce product toxicity is the in situ extraction of hydrophobic products using organic solvents. For example, the toxicity of various terpenoids, including geraniol[85], limonene[88], linalool[157], sabinene[158] and nootkatone[91], can be reduced by in situ extraction using isopropyl myristate, diisononyl phthalate or n-dodecane. In addition, tributyrin[76] and oleyl alcohol[159] are used to extract methyl anthranilate and 3-methyl-1-butanol, respectively, in situ. Additionally, volatile compounds such as 1-butanol can be recovered in situ by gas stripping[160].

Metabolic flux optimization

The metabolic flux towards target product can be optimized and maximized by applying diverse strategies, such as improving gene expression by modulating multiple factors such as promoters[91], 5′/3′ untranslated regions (UTRs)[104,161], ribosome binding sites (RBSs)[97], codons[69] and gene copy number[42,89,92,162,163] (Fig. 4a). For example, overexpressing chaperone genes encoding GroEL/ES and DnaK/DnaJ/GrpE enhances functional expression of genes involved in astaxanthin[101] and carminic acid[123] production by assisting their proper folding. Additionally, modifying target enzymes by fusion to short peptide tags[86] or endogenous or heterologous proteins[142,150] can increase expression levels through enhanced transcription and translation[86,142] or folding and solubility of proteins[150]. Moreover, removing N-terminal signal peptides of some plant-based enzymes, such as milk thistle peroxidase, sabinene synthase, and tocopherol cyclase, can enhance the expression levels as well as the catalytic activities of the enzymes and improve the microbial production of (iso)silybin[73], sabinene[164], lutein[102] and tocotrienols[130], respectively.

Box 2

Technology transfer considerations

Accomplishing high titre, yield and productivity is one of the most critical and fundamental requirements for the successful transfer of a microbial manufacturing process to industry. Of the three metrics describing the performance of a microbial process, the titre infers how concentrated the product is in the fermentation broth, whereas the yield indicates how much product is produced from a given amount of raw materials. The productivity implies how fast the product is produced, and both specific productivity (gram product per gram dry cell weight per hour) and volumetric productivity (gram product per litre per hour) are important factors to consider. Various advantages are associated with achieving high titre, yield and productivity; for example, high titre generally results in reduced product purification costs, and high yield allows lower substrate costs. High productivity allows the use of smaller-scale process units to produce the same amount of product, thus lowering the direct fixed capital costs and consequently the annual operating costs, including the depreciation costs. These strategies are important for the commercialization of microbial processes, considering that most commercially available food and cosmetic compounds, derived from petrochemicals or agricultural products, are low-priced. For new and difficult-to-produce products, the production cost can be a less critical factor compared to other well established products. In addition, the higher price of the product produced by microbial fermentation might still be competitive if the conventional production method of the compound is considered unethical, unhealthy for humans or harmful to the environment.

Legal regulations are another important aspect to consider in the commercialization of microbial processes for food and cosmetics production. The governments of many countries regulate the use of genetically modified organisms for the production of compounds for food and other compounds for human consumption. To streamline approval processes, requirements from the target governments (for example, the complete elimination of endotoxins, exclusion of genes from virulent organisms or prohibited antibiotics and their resistance genes) should be carefully addressed during strain engineering and process development.

Finally, understanding consumer and market demand is a prerequisite. Microbial strains and processes with high production performances become useless if their products have insufficient market demand or are not of interest to consumers. Hence, consumer and market demand should be carefully surveyed and analysed from the beginning of a project. Additionally, acceptance of microbial products by consumers should be promoted through a science-based approach to ensure their safety and efficacy. Obviously, compounds with high global demand are good targets, but products of small volume and high price are also good candidates for industrial-level microbial production.

High-throughput screening technologies, such as omics, in silico modelling, *trans*-regulating systems and biosensors facilitate the identification of engineering targets. For example, proteome analysis is used to identify 3-dehydroquinate dehydratase (encoded by the *aroD* gene) as the rate-limiting enzyme in *Escherichia coli*-based L-phenylalanine production[49], whereas transcriptome analysis helps to find *Corynebacterium glutamicum* genes that have been feedback-repressed by succinic acid[109]. In addition, sRNA-based high-throughput screening suggests various knockdown targets that cannot be rationally identified[54]. Furthermore, biosensors composed of reporter genes (for example, fluorescent protein genes[165], antibiotic resistance genes[129,165] or toxic genes[60]) and biological parts sensing target metabolites and modulating reporter gene expression (for example, aptazymes[165], riboswitches[129] and transcriptional regulators and their target promoters[60]) expedite the high-throughput screening studies (Fig. 4b). The identified target genes are then subjected to upregulation/downregulation for metabolic flux optimization.

Repressor or activator genes can be engineered to overcome transcription-level feedback regulation to improve the metabolic flux towards target products (Fig. 4c). For example, the repressor genes *argR*[59,62], *tyrR*[51,70] and *trpR*[78] are routinely deleted to facilitate overproduction of L-arginine, L-tyrosine and L-tryptophan and their intermediates and derivatives, respectively. Alternatively, overexpressing the *rcsA* and *rcsB* genes encoding transcription activators enables upregulation of multiple genes involved in GDP-fucose formation during 2′-FL production[21]. Some regulators function as both repressors and activators, depending on the target genes. For example, L-tyrosine repressor encoded by the *tyrR* gene can repress L-phenylalanine/L-tyrosine biosynthesis genes and also activate some other genes, including the *folA* and *mtr* genes. The knockout of this dual regulator reduces L-phenylalanine production, and thus modulation of its DNA-binding domain enhances L-phenylalanine production[49]. In addition, feedback inhibition of an enzyme is overcome by introducing feedback-resistant mutations based on previously obtained results[43,44,51,58,70,162,166] or newly identified through random mutagenesis or screening[137]. Knockout of the *rpoS* gene encoding stationary phase sigma factor also helps to improve metabolic flux towards 1-propanol in the stationary phase[166].

Metabolic flux can also be improved by employing enzymes with higher catalytic properties compared to conventional ones as identified through genome mining and by searching enzyme databases. For example, malate dehydrogenase from *Corynebacterium glutamicum* shows the highest catalytic activity and the least substrate inhibition among eight enzymes from various organisms to improve the production of succinic acid[8]. L-Tyrosine production can also be improved by employing the *tyrC* gene from *Zymomonas mobilis*, which encodes an enzyme catalytically identical to that of the endogenous *tyrA* gene but uninhibited by L-tyrosine[51]. Similarly, an allulose 3-epimerase identified from the metagenome of a thermal spring can substitute for conventional, thermally unstable enzymes and enables biotransformation of D-fructose to D-allulose at 60 °C (ref. 12).

Additionally, the catalytic properties of enzymes can be improved through enzyme engineering assisted by multiple tools, including protein modelling and high-throughput screening. For example, the catalytic activities of aklavinone 12-hydroxylase and *C*-glucosyltransferase can be improved by protein engineering based on homology modelling and docking simulation to improve the metabolic flux towards carminic acid[123]. Moreover, site-directed mutagenesis of a flexible region of santalene synthase from *Clausena lansium* allows the creation of a mutant with increased solubility that enhances conversion of FPP to α-santalene[150]. Enzyme engineering can also help to prevent enzyme inactivation. For instance, a pyruvate-tolerant mutant of glucosamine-6-phosphate *N*-acetyltransferase from *Caenorhabditis elegans* can be screened from a mutant library generated by error-prone polymerase chain reaction[167]. Moreover, rational engineering of aspartate α-decarboxylase can prevent the inactivation induced by aspartate and enhance β-alanine production[57]. In addition, oxygen sensitivity of *Escherichia coli* aldehyde-alcohol dehydrogenase can be alleviated, improving 1-propanol production under aerobic conditions[166].

Balancing the coenzyme pools and streamlining the coenzyme supply are also crucial for flux optimization (Fig. 4d). Employing enzymes with altered coenzyme preference to balance NADH/NADPH pools[43,89,119], and adjusting central carbon metabolism can modulate the supply of NAD(P)H[58,61,97]. Conversely, water-forming NAD(P)H oxidases from *Lactococcus lactis*[117,168] and *Lactobacillus reuteri*[169] can facilitate regeneration of $NAD(P)^+$ and balancing $NAD(P)^+$/NAD(P)H pools. Additionally, augmenting supply of other coenzymes, such as *S*-adenosyl-L-methionine[76] and haem[102], improves production of the related metabolites.

Substrate channelling, which brings relevant enzymes into proximity to facilitate transfer of intermediates to the next enzymes, can expedite a series of conversions and secure intermediates from competitive reactions (Fig. 4e). To devise a substrate channelling strategy, the enzymes responsible for consecutive metabolic reactions are colocalized by fusing them together using a short linker peptide[170], or fusing them to protein–protein interaction domains or ligands[151,171] or to proteins forming stable protein crystalline inclusions (for example, crystalline inclusion proteins CipA and CipB from *Photorhabdus luminescens*)[102,172]. Also, direct fusion or CipA/CipB-mediated colocalization of cytochrome P450 enzymes and their partner reductases enables the channelling of electrons from NADPH to the substrate metabolites, such as oxidosqualene, naringenin and L-tyrosine[94,102]. In eukaryotes, terpenoid production can be facilitated by compartmentalizing (sequestering the target object into a desired, isolated subcellular space) the relevant enzymes to organelles, such as peroxisomes[87,93], mitochondria[164], endoplasmic reticulum[96] and lipid bodies[96].

Preventing degradation of products, intermediates and precursors and their conversion into by-products, which can be achieved by deleting and repressing the genes responsible for such metabolic reactions[20,134] and employing enzymes with high specificity and low promiscuity (Fig. 4f), is an important strategy to improve the metabolic flux. For example, chalcone synthase from barley, which exhibits a high substrate specificity to *p*-dihydrocoumaroyl-CoA and low activity on *p*-coumaroyl-CoA (an intermediate of *p*-dihydrocoumaroyl-CoA), can reduce formation of naringenin (a by-product) and improve production of phloretin, phlorizin and trilobactin[173]. Similarly, two anthocyanin synthases from *Magnolia sprengeri* and *Ginkgo biloba* with low promiscuity on dihydroflavonols (intermediates of leucoanthocyanidins) can catalyse the conversion of leucoanthocyanidins to anthocyanidins and decrease the formation of flavonols as by-products[72]. Engineering the substrate specificity of the bifunctional Erg20 enzyme from *Saccharomyces cerevisiae*, which natively joins DMAPP/IPP and GPP/IPP to GPP and FPP, respectively, improves citronellol[170] and sabinene[174] production by reducing Erg20-mediated FPP formation while increasing GPP formation. In some cases, unwanted by-products can be transformed to target products. For example, geranyl acetate, an acetylated by-product of geraniol production, can be converted back to geraniol by inducing D-glucose starvation at the end of fermentation[85].

Unfavourable — but necessary for generating biomass — reactions can be knocked out and minimal amounts of the auxotrophic metabolites, which must be externally supplied for survivor and growth of a cell, can be supplemented. This is an easy, yet not ideal, way to secure metabolic flux towards target compounds[50,56,62]. Sophisticated controls of metabolic fluxes must be applied to balance the formation of target compounds and biomass without inducing auxotrophy (Fig. 4f). Natural and synthetic regulatory circuits (a set of subcellular components that regulate the expression of other genes) controlled by various signals, such as carbon source[95,175], autoinducer[77,131,176] and metabolite[55,158] levels, light[121] and temperature[41,130], enable the dynamic regulation of target genes and thus improve production of target compounds. For polyploid organisms, which have multiple sets of chromosomes per cell, partial disruption of target genes is also effective in attenuating undesired yet essential metabolic fluxes for biomass formation. For example, partial disruption of the *PDC* and *ADH* genes in a polyploid *Saccharomyces cerevisiae* strain reduces, but does not prevent, biomass formation and enhances metabolic fluxes towards the target product, enabling improved production of 2,3-butanediol without growth defects and by-product formation[168]. Orthogonal metabolic routes, which do not interfere with the native metabolic pathways, can also help to improve metabolic flux towards target compounds without compromising essential metabolism. For example, a synthetic route throughout neryl pyrophosphate, an optical isomer of GPP, can enhance production of sabinene and the use of GPP to produce various essential metabolites[158]. Moreover, carbon utilization pathways of *Escherichia coli* can be engineered to generate biomass from glycerol while using D-glucose for trehalose production[177].

Dividing the biosynthetic pathway into multiple modules and optimizing flux within the modules are also methods exploited to enhance the metabolic flux of given compounds (Fig. 4g). For example, the menaquinone 7 biosynthesis pathway can be divided into three modules, responsible for supplying IPP/DMAPP, generating 1,4-dihydroxy-2-naphthoate (DNHA) and incorporating FPP/IPP/DHNA[132]. Similarly, the L-methionine[56] and 1,3-propanediol[118] biosynthesis pathways are divided into multiple modules. Furthermore, the libraries of various biological parts including promoters, RBSs, UTRs and feedback regulators help to balance metabolic fluxes between the modules and improve the overall flux towards target products[100,118].

Optimizing the intracellular environment, using strategies such as transporter engineering, can also improve metabolic fluxes (Fig. 4h). First, upregulating importers of carbon sources can improve the intracellular pool of the carbon sources[58,63,163]. Similarly, importers of intermediates can be upregulated to re-uptake metabolic intermediates from the extracellular space, which were undesirably secreted to the medium[52]. Alternatively, the secretion of intermediates can be prevented by knocking out their exporters[59,163]. Second, upregulation of exporters[47,80,81] and/or knockout of importers of target products[48,51] can reduce intracellular concentrations of the target products.

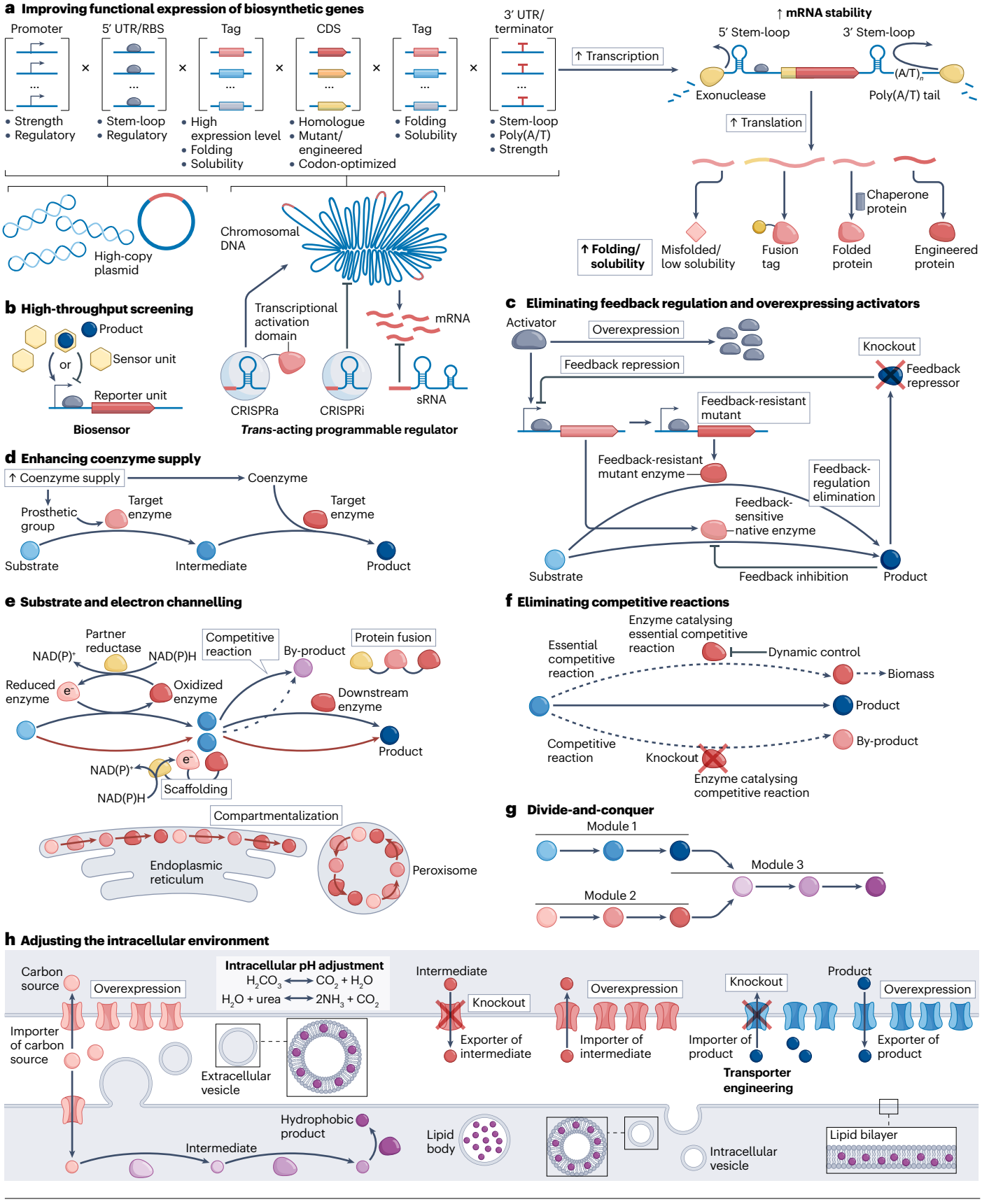
a Improving functional expression of biosynthetic genes
Promoter
5′ UTR/RBS
Tag
CDS
Tag
3′ UTR/ terminator
• Strength • Regulatory
• Stem-loop • Regulatory
• High expression level • Folding • Solubility
• Homologue • Mutant/ engineered • Codon-optimized
• Folding • Solubility
• Stem-loop • Poly(A/T) • Strength
↑ Transcription
↑ mRNA stability
5′ Stem-loop
3′ Stem-loop
Exonuclease
Poly(A/T) tail
↑ Translation
Chaperone protein
↑ Folding/ solubility
Misfolded/ low solubility
Fusion tag
Folded protein
Engineered protein
High-copy plasmid
Chromosomal DNA
b High-throughput screening
Product
Sensor unit
or
Reporter unit
Biosensor
Transcriptional activation domain
mRNA
CRISPRa
CRISPRi
sRNA
Trans-acting programmable regulator
c Eliminating feedback regulation and overexpressing activators
Activator
Overexpression
Feedback repression
Knockout
Feedback repressor
Feedback-resistant mutant
Feedback-resistant mutant enzyme
Feedback-regulation elimination
Feedback-sensitive native enzyme
Substrate
Feedback inhibition
Product
d Enhancing coenzyme supply
↑ Coenzyme supply
Coenzyme
Prosthetic group
Target enzyme
Target enzyme
Substrate
Intermediate
Product
e Substrate and electron channelling
Partner reductase
NAD(P)+
NAD(P)H
Competitive reaction
By-product
Protein fusion
Reduced enzyme
e−
Oxidized enzyme
Downstream enzyme
Product
NAD(P)+
e−
NAD(P)H
Scaffolding
Compartmentalization
Endoplasmic reticulum
Peroxisome
f Eliminating competitive reactions
Enzyme catalysing essential competitive reaction
Essential competitive reaction
Dynamic control
Biomass
Product
By-product
Competitive reaction
Knockout
Enzyme catalysing competitive reaction
g Divide-and-conquer
Module 1
Module 2
Module 3
h Adjusting the intracellular environment
Carbon source
Overexpression
Importer of carbon source
Intracellular pH adjustment
H2CO3 ⟷ CO2 + H2O
H2O + urea ⟷ 2NH3 + CO2
Extracellular vesicle
Intermediate
Knockout
Exporter of intermediate
Overexpression
Importer of intermediate
Knockout
Importer of product
Transporter engineering
Product
Overexpression
Exporter of product
Hydrophobic product
Intermediate
Lipid body
Intracellular vesicle
Lipid bilayer

Fig. 4 | Applying systems metabolic engineering tools and strategies to optimize metabolic fluxes. a, Enhancing functional expression of biosynthetic genes. To enhance the expression of functional enzymes and metabolic fluxes towards target products, transcription and translation levels, mRNA stability, protein folding efficiency and enzyme solubility can be improved by exploiting biological parts libraries (such as for the promoter, the 5′/3′ untranslated region (5′/3′ UTR), the ribosome binding site (RBS), the fusion tag, the coding sequence (CDS) and the terminator) **b**, High-throughput screening of upregulation and downregulation targets can be facilitated by *trans*-acting programmable regulators, such as synthetic small regulatory RNA (sRNA), CRISPR interference (CRISPRi) and CRISPR activation (CRISPRa), and biosensors. **c**, Eliminating feedback regulation and overexpressing activators. Knocking out relevant feedback repressors and introducing feedback-resistant mutations can eliminate feedback regulation. Overexpressing relevant activators also enhances the expression level. **d**, Enhancing coenzyme supply. **e**, Substrate and electron channelling (for example, protein fusion, scaffolding and compartmentalization) can further streamline metabolic fluxes. **f**, Eliminating competitive reactions. Competitive reactions can be knocked out or attenuated by substrate channelling to direct metabolic fluxes towards target compounds. Alternatively, control of metabolic fluxes (for example, dynamic control) can also be applied to attenuate competitive yet essential reactions (for example, biomass formation). **g**, Divide-and-conquer. Extensive or complicated metabolic pathways can be divided into smaller modules, individually optimized and re-integrated. **h**, Adjusting the intracellular environment. Overexpressing importers of carbon source or intermediates and exporters of target products as well as knocking out exporters of intermediates and importers of target products can improve the biosynthetic reactions thermodynamically. For hydrophobic compounds, expanding hydrophobic environments (for example, generating lipid bodies or intra- and extracellular vesicles) sequesters hydrophobic products and enhances the overall production. Intracellular pH can also be adjusted to provide an optimal environment for the biosynthetic enzymes.

For the production of hydrophobic compounds, increasing the intracellular lipid contents is helpful to sequester the products to the lipid bodies and enhance metabolic flux throughout the biosynthetic routes[98,99]. Generating intracellular and extracellular vesicles to increase the surface area of hydrophobic lipid membranes also improves the production of hydrophobic compounds[79] (Fig. 4h). In addition, adjusting intracellular pH, by for example, overexpressing urease to keep the intracellular pH near neutral[167], can maintain enzyme activities and improve overall metabolic fluxes for *N*-acetylglucosamine production by *Bacillus subtilis*.

Fermentation process optimization

To improve the performance of metabolically engineered strains during fermentation, multiple parameters, including temperature[28,29,70,104,120,135], pH[40,70,89,104,120] and dissolved oxygen (DO) level[7,10,40,42,43,89,104,119,146,148], are routinely optimized. Notably, *Corynebacterium glutamicum* strains are fermented under oxygen deprivation conditions to convert sugars into amino acids and their derivatives with high yield and to prevent biomass formation[42,43,119]. Increasing the mass-transfer rates can also be achieved by modulating bioreactor configurations[31,89,178], decreasing the viscosity of the medium[22,24] or by supplementing water-miscible organic solvents to dissolve the substrates of low solubility in the medium[135]. It is also necessary to optimize medium composition including the carbon source[70,80,82,135,162,175], the nitrogen source[14,70,135], the carbon-to-nitrogen ratio[144,162] and other salts and components[70,80,82,135]. Systematic experimental design schemes and statistical analysis methods such as factor combination design[120] and principal component analysis[179] are helpful approaches for fermentation optimization.

Target compound production can also be improved by introducing some product- and host-dependent stimuli. For example, temporary exposure of a recombinant *Escherichia coli* strain to a low pH (pH 4–6), by delaying the initiation of pH control, improves haem and ZnPPIX production[80,82], whereas an osmotic shock induced by increasing the substrate (glycerol) concentration improves erythritol production by the osmophilic *Yarrowia lipolytica*[18]. Inducing oxidative stress by supplementing potassium permanganate-sustained-release particles[39] and hypochlorous acid[180] can improve production of the antioxidants glutathione and ascorbic acid by *Saccharomyces cerevisiae*[39] and *Xanthomonas campestris*[180], respectively. In addition, phosphate limitation improves coenzyme Q_{10} production by *Rhodobacter sphaeroides* strain HY01[133]. Moreover, supplementing olive oil or kerosene induces secretion of a lipase and subsequent production of butyl butyrate by *Clostridium* species strain BOH3 (ref. 122).

Industrial fermentation is operated in batch and fed-batch modes, where fermentation is continued until the substrate in the medium is depleted (batch mode) and further extended by supplementing concentrated substrate solution afterward (fed-batch mode). Thus, optimizing the substrate feeding strategies can improve production performances. For example, DO-stat (substrate is supplemented when the dissolved oxygen level rises owing to the depletion of substrate), pH-stat (substrate is supplemented when the pH rises owing to the depletion of substrate) and constant-rate feeding can be examined to improve β-alanine production[57], and supplementing a carbon source in a growth-limiting manner can improve citramalic acid production[181]. In addition, adjusting the concentrations of carbon sources and other organic and inorganic substrates in the feeding solution is an important approach to improving production[80,82,147]. Supplying a carbon source in the form of sterilized powder, rather than a concentrated solution, can minimize dilution of the fermentation broth upon feeding and improve product titre[110].

Multi-stage fermentation often divides the overall fermentation period into growth and production phases[41,80,82,93,95,130,131,150,175], where different condition sets can be applied before and after induction to improve both cell growth and product formation[41,93,95,130]. For example, shifting the growth conditions of a recombinant *Escherichia coli* strain from an aerobic culture at 33 °C to 42 °C with limited oxygen supply can induce L-alanine production[41]. Moreover, the residual D-glucose level can be elevated upon induction to enhance squalene production by a recombinant *Saccharomyces cerevisiae* strain[93]. Switching the carbon source from D-glucose to ethanol upon induction can improve tocotrienol production by an engineered *Saccharomyces cerevisiae* strain[130]. Alternatively, deprivation and supplementation of copper ions (a cofactor of tyrosinase) can switch melanin production off and on[68]. For pinocembrin production, involving upstream and downstream enzymes of different optimal pH values, two pH conditions (7.0 and 6.5) are applied in the growth and production phases of fermentation to facilitate the upstream and downstream metabolic reactions, respectively[182]. Alternatively, docosahexaenoic acid production by *Schizochytrium* species is optimized by dividing the overall fermentation period into three stages based on the residual nitrogen source level and the non-oil biomass formation profile[31].

In co-culture systems, modulating the inoculum ratio[71,74,154] and the substrate preference of co-culture strains can regulate the strains' population ratios during fermentation. For example, co-culturing three *Escherichia coli* strains, of which two prefer to consume D-xylose and the third prefers D-glucose, enables dynamic and controlled growth of the strains during fermentation and allows de novo production of rosmarinic acid from D-glucose and D-xylose[154]. Alternatively, strains can be sequentially inoculated at intervals. For example, *Agrobacterium* species are first inoculated to produce curdlan polysaccharide from D-glucose, followed by a recombinant *Pichia pastoris* strain secreting a heterologous *endo*-β−1,3-glucanase to cleave the synthesized curdlan polysaccharides into shorter oligosaccharides[183].

Downstream process integration

Multiple strategies are applied during strain development and fermentation (for example, maximizing product titre, lowering by-product concentration and in situ isolation of products) to streamline recovery and purification of target compounds from the fermentation broth and substantially reduce the purification cost. Process engineering strategies, such as precipitating acidic products as salts[110], in situ extraction of hydrophobic compounds using organic solvents[76,85,88,91,157–159], gas stripping of volatile compounds[160,184] and in situ adsorption[135], can reduce toxicities and separate the products for downstream processes.

Preventing degradation and undesirable conversion (for example, oxidation) of products is also critical to increase the yield and economic competitiveness of the overall process. For example, oxidation of retinol during and after fermentation can be prevented by supplementing butylated hydroxytoluene, a low-priced antioxidant[105]. Additionally, some by-products and impurities that are physicochemically similar to target products and that are not easy to separate in downtream processes can be removed by growing another organism that selectively consumes the impurities. For example, in trehalose production, residual D-glucose and maltose (sharing similar physicochemical properties with trehalose) can be converted to ethanol by a *Saccharomyces cerevisiae* strain, which consumes D-glucose and maltose but not trehalose, and the resulting ethanol can be eliminated owing to its high volatility[185]. Finally, many target compounds can be recovered and purified through the combination of various unit operations, such as crystallization, filtration and evaporation. Assessment of multiple candidate downstream processes in terms of yield, purity, cost and other notable features can provide insights into integrating downstream processes and optimizing strains and processes[50].

Scale-up

To generate a large inoculum for scaled-up fermentation in a time- and cost-effective manner, seed trains are applied by repeated serial culture in gradually increasing volumes. In each round of serial culture, the volume is routinely increased by a factor of 10 to 20. For example, a *Bacillus subtilis* strain starting with 400 ml of medium is serially scaled up to 4-litre and 40-litre cultures for γ-PGA production[9]. Morevover, 10 ml of seed culture can be sequentially scaled up to 200 ml and 2 litres, and 48 litres of fermentation broth cultured in a 70-litre fermentor can be transferred by serial culture to a 1,500-litre fermentor containing 600 litres of medium for scaled-up production of L-arginine[59]. Similarly, L-leucine[44] and β-farnesene[90] are produced in 150,000-litre and 200,000-litre fermenters.

During the scale-up of fermentation processes, maintaining the mass-transfer rate, especially oxygen transfer rate in aerobic fermentation, is a critical factor[178]. Although high agitation speed facilitates mass transfer, the speed limit is lower for a large-scale fermentor compared to lab-scale production, as the high linear speed generated at the tip of impellers ruptures the production strains by high shear stress. Thus, agitation speed during the fermentation of *Bacillus subtilis* for γ-PGA production is reduced from 300–800 to 200–450 revolutions per minute as the fermentor scale is increased from 10 to 100 litres[9]. Similarly, the agitation speed is reduced from 400–1,000 to 200–450 revolutions per minute by increasing the scale of L-tyrosine production using *Escherichia coli* from 10 to 200 litres[50]. Moreover, for L-arginine production using *Corynebacterium glutamicum*, the agitation speed is lowered from 600 to 90 revolutions per minute as the fermentation scale is increased from 5 to 1,500 litres[59]. Additionally, the volumetric air flow rate is increased proportionally to the fermentation volume[9,59]. If the oxygen transfer rate should be further increased without increasing agitation speed, a porous-membrane impeller connected to the sparger line can increase the oxygen-transfer coefficient by generating finer air bubbles[31]. Alternatively, an airlift fermentor can be applied instead of a stirred tank fermentor. For commercial production of single cell protein in 155-m^3 airlift fermenters using the mycelial fungal species *Fusarium venenatum*, air bubbles rising from the bottom lift up cells and medium and generate impeller-independent convection with resultant lower sheer stress and operational cost[186].

Replacing refined carbon sources with lower-priced raw substances or industrial side streams is also important to reduce the material costs of scaled-up fermentation. Although production performances tend to be slightly decreased, unrefined cane sugar[59,90], molasses[39], corn starch hydrolysate[59], cassava hydrolysate[168] and lignocellulosic hydrolysate[16,140] can be used instead of refined sugars (for example, D-glucose, sucrose and D-fructose). In addition, high-priced nitrogen sources used in laboratories (for example, yeast extract, tryptone, casamino acid and beef extract) are often replaced with corn steep liquor[39,130] and hydrolysates of protein-rich agricultural residues[111]. Moreover, the systematic optimization of medium composition[9] or the stoichiometric supply of substrates[23] to minimize residual components at the end of fermentation and wasted materials can further reduce raw material costs and consequently the overall production costs.

Outlook

The economic advantages of conventional petrochemical and agricultural products represent a major obstacle to translating microbial processes to industries (Box 2). Indeed, the market price of petroleum and other raw materials influences commercial interests in microbial products and the business profits of operating microbial processes. Nevertheless, the establishment of sustainable societies and the emphasis on environmental, social and governance (ESG) criteria in industrial management are turning attention towards green microbial processes for food and cosmetic production. Thus, such green industries are expected to complement and gradually to replace the petrochemical- and agriculture-based food and cosmetic industries.

Various microbial strains and fermentation processes have been developed to produce diverse food and cosmetic compounds, such as food ingredients, additives, dietary supplements, flavours, fragrances, colourants and skincare ingredients (Table 1). Indeed, an increasing number of compounds are commercially produced through microbial fermentation (Supplementary Table 1). However, the number of commercialized microbially produced food and cosmetic compounds remains low, compared to those academically investigated. To facilitate the commercialization of microbial processes, high titre, yield and

productivity should be accomplished to guarantee the economic feasibility of the processes (Box 2). Yet most academically developed microbial strains and processes exhibit low production metrics (Table 1), hampering their translation to industry.

For the industrial development of microbial-based food and cosmetics with competitive performance, SysME can aid in the development of high-performance microbial cell factories by integrating synthetic biology, systems biology and evolutionary engineering with conventional metabolic engineering. Additionally, computational sciences and robotics, such as deep learning, machine learning[187,188] and automation systems[189,190], can be applied in combination with SysME. Although there is a growing number of studies in which SysME tools and strategies have been used to construct superior production strains with higher production metrics, there is still room for further improvement. In addition, the high-performance microbial strains developed by systematically applying SysME tools and strategies could improve the commercialization of microbial processes for food and cosmetic products (Box 2).

In addition to improving the production performances of microbial strains and processes, their potential impacts on the environment and society should also be considered during the development phase. In industrial-scale microbial production, in which fermentation is operated at a large volume (hundreds of thousands of litres per batch), there is a risk of accidentally releasing the engineered strain into the environment. Hence, the potential of the production strains to survive in the environment and invade into the ecosystem should be carefully evaluated. Additionally, a biocontainment system that kills a strain outside the fermentation condition can be implemented to actively prevent this potential hazard[191,192]. Moreover, constructing production strains free of antibiotic resistance genes can prevent the possible spread of these genes to the ecosystem and the emergence of multidrug-resistant bacteria.

It is furthermore important to build a positive public consensus on food and cosmetic compounds produced by microbial strains. In 2022, the United States Department of Agriculture introduced 'bioengineered' as a new label for foods derived from genetically modified organisms to overcome the public misunderstanding of and the negative connotation of genetically modified organisms. In addition, researchers should communicate with the public, discussing the safety of engineered microbial products. Importantly, scientific evaluation of the safety of the production strains and their final fermentation products should be conducted, strictly following the guidelines specified by the corresponding governments of the region.

Finally, academia and industry should collaborate to expedite the commercialization of microbial food and cosmetic compounds, combining cutting-edge technologies and strategies developed in academia with the industrial knowledge and experience in operating industrial-scale fermentors and downstream processes. Such early-stage collaboration could streamline the development of superior strains and fermentation processes with high production metrics by minimizing unnecessary errors during strain and bioprocess development.

Citation diversity statement

We acknowledge that papers authored by scholars from historically excluded groups are systematically under-cited. Here, we have made every attempt to reference relevant papers in a manner that is equitable in terms of racial, ethnic, gender and geographical representation.

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K.R.C. researched data for the article and contributed to the conceptualization, writing, reviewing and editing of the manuscript. S.Y.L. contributed to the conceptualization, reviewing and editing of the manuscript.

Competing interests

The authors declare no competing interests.

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