Natural Resources for Human Health

visagaa publishing house

Review

View Article Online

Check for updates

Received 02 September 2021
Revised 27 October 2021
Accepted 02 November 2021
Available online 08 November 2021

Edited by Haipeng Lv

KEYWORDS:
Metabolic engineering
Biosynthetic
Microorganisms
Genetically modified
Nutritional

Natr Resour Human Health 2022; 2 (2): 121-128
https://doi.org/10.53365/nrfhh/143540
eISSN: 2583-1194

Cracking the Metabolic engineering of bacteria: Review of methods involved in organic acid Production

Manam Walait [1,*], Huda Rehman mir [1], Zainab Hassan [1], Javed Iqbal Wattoo [1]

[1]Department of Biotechnology, Faculty of Life Sciences, University of Central Punjab, Lahore, Pakistan

ABSTRACT: Metabolic engineering is defined as recombinant DNA technology to improve specific biochemical reactions for product formation. We modify the metabolic processes of bacteria to get our desired food by metabolic engineering. Metabolic engineering will enhance these microorganisms' properties and their ability to produce a diverse number of products cost-effectively. To produce amino acids, we modify the central metabolic pathway, biosynthetic pathway, and transport pathway. In many food industries, the production of organic acids through different processes and techniques have proved very beneficial because of their widespread applications. In line with this information, the present review aimed to provide background information for researchers about genetically modified foods for increased food yield to fulfil the nutritional values for average body growth.

1. INTRODUCTION

Metabolic engineering uses genetic manipulation methods to improve the productivity and yield of native products that are novel to biological systems synthesized by microorganisms and improve cell adaptations to adverse conditions (Leonard et al., 2007; Tomar & De, 2013). The genetic alteration of the cell is a common factor in almost all types of metabolic engineering.

Recombination involving plasmid preparations was one of the first techniques used, and it is still used today. As a result, metabolic engineering can be viewed as a practical manifestation and natural progression of methods first developed in the biological field. It moves away from the idea of individual enzyme and toward integrating entire systems to accomplish the objectives set out at the start of any project. Recombinant DNA technology can now be used to design and execute more rational metabolic alterations (Lixin, 2000).This allows for more logical metabolic modifications to be designed and implemented using recombinant DNA technology (Wenzel & Müller, 2005).

Metabolic engineering places a premium on the modification pathway and analysis. This is a field of metabolic engineering that has overgrown in the last few years, and computing power continues to increase exponentially. The analytical part comprises the experiment and modelling techniques, e.g., labelling diagrams and metabolic flux analysis (MFA). MFA enables the formal investigation of cellular responses to genetic and environmental perturbations in terms of metabolic fluxes (Antoniewicz, 2020).

Due to the essential fermentation role in food processing and preparation, microorganisms are continually encountered in these processes. Bacteria play an essential role in these microorganisms: they are used as a bio-catalyst in forming organic acids, amino acids, vitamins, and carbohydrates, either as bacteria or as products synthesized by bacteria. Bacterial food additives may be used in dietary supplements, texturizers, acidulates, preservatives, emulsifiers, surfactants, thickeners, or functional food ingredients (Partridge et al., 2019). Food that has the potential to enhance one's health is known as a functional food. Amino acids are used as a feed of animals, pharmaceuticals, and food industries (Mayra-Makinen et al., 2004). They can also be used as sweeteners and flavor enhancers.

2. IMPROVEMENT IN CENTRAL METABOLIC PATHWAYS

In these pathways, rerouting of carbon is required to produce the desired product. This can be done by raising the accessibility of precursor metabolites. There are various valuable strategies for redirecting the flow of carbon towards the synthesis of the desired product. Central metabolic pathways are modified to produce a higher amount of histidine and other amino acids with the help of planned modification of cellular metabolism to produce desired products (Bar-Even et al., 2012). By increasing, the accessibility of ribose-5- phosphate by transketolase deficient

---

* Corresponding author.
E-mail address: manam.walait@ucp.edu.pk (Manam Walait)

strain. By raising the amount of erythrose-4 phosphate, we can raise amino acid production. This can be achieved by two main factors; over expression of transketolases and inactivation of phosphoenolpyruvate system dependent on phosphotransferase system. This can inflate the phosphoenol synthase.

3. MODIFICATION OF BIOSYNTHETIC SHIKIMATE PATHWAYS

Over-expression of chorismate mutase enzyme as important biochemical intermediate plants and microorganisms is required in this pathway to increase phenylalanine expression (Russ et al., 2020). it is also termed the Shikimate pathway because it has one substrate (Chorismate) and one product (prephenate) (Tzin & Galili, 2010) . To increase the tyrosine and phenylalanine, simultaneous inflation of chorismate mutase and prephenate dehydrates is done. The phenomenon of Chorismate in the synthesis of aromatic amino acids and secondary metabolites has shown in Figure 1.

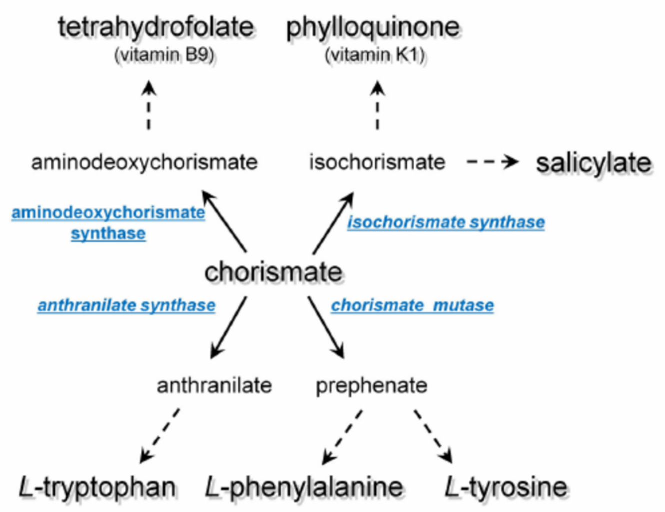

Figure 1. Chorismate, a central branch point metabolite insynthesising aromatic amino acids and secondary metabolites (Tzin & Galili, 2010)

4. MODIFICATION OF TRANSPORT SYSTEM

The intracellular concentration of amino acids and feedback inhibition can be decreased or avoided by altering the amino acids transport system (Maser et al., 2020). Two examples can understand the significance of this strategy. When C-glutamicum produces the L- tryptophan, which results in the accumulation of L-tryptophan in the extracellular medium, there is a backflow of it into cells. It results in the production of severe feedback inhibition during the biosynthesis of tryptophan. Ikeda and Katsumata solve this problem by lowering the level of tryptophan uptake due to creating the mutants, which result in 10 to 20% accumulation of tryptophan more than in their parent. There can be an increase in cysteine's fermentation yield, which is another excellent example of transport engineering (Antoniewicz, 2020). This can be obtained by the overexpression of multidrug efflux genes in the strain of *E.coli*, which produce cysteine.

5. USE OF ANALYTICAL TOOLS IN A TOOLBOX

The MFA has been used to modify the production of many amino acids, including the two essential amino acids (Chotani et al., 2000; Ivanov et al., 2013). Both amino acids have the most significant production volume in the world. For example, L-glutamic acid has 800,000 tons/year, and L-lysine has 600,000 tons/year.

5.1. Glutamate

Several processes and pathways have been contributed to the improvement of the glutamate producing by C-glutamicum, such as modification and analysis of biosynthetic and central metabolic pathways. The MFA shows the estimation of fluxes by the label experiments (Antoniewicz, 2020). It explains the relative contribution of different pathways. During the metabolic branch point of 2- oxoglutarate glutamate, there is a relationship established by MFA between the decline of the oxoglutarate dehydrogenase complex and the flux distribution. It suggests that they could be limiting the production of glutamate by metabolic flux through anaplerotic pathways.

5.2. L-lysine

One other example of labelling experiments based on MFA is the L-Lysine (Van Ooyen et al., 2012) . The comprehensive approaches have been used to assess all the significant effluxes in the C-glutamicum metabolism. It reveals the pattern which is used for designing ME strategies. For this PPP the high capacity for NADPH reoxidation, and the futile cycle between the C4 compound of the TCA cycle and the C3 compound of glycolysis. This metabolic fluxislow (Holms, 1987). The NADPH is required in high amount byheterologous NADPH dependent glutamate dehydrogenase mutant, and it results in the increasing flux of TCA cycle and here the isocitrate step of TCA cycle results in the HencedecreasesPPP flux because of the NADPH requirements, which are lower. For the homologous NADPH dependent glutamate-dependent glutamate dehydrogenase mutant, the inverse is true. So, the NADPH requirements are high, so the PPP flux is also higher, and there is a lower TCA cycle.

6. USE OF LACTIC ACID BACTERIA IN THE PRODUCTION OF AMINO ACID

The different types of amino acids can also be produced using the LAB (Toe et al., 2019) . The metabolic engineering of *Lactococcus lactis* produces L- alanine which is the product of fermentation. In the lactate dehydrogenase deficient strain, the *Bacillus sphaericus*. Alanine dehydrogenase results in the rerouting of the carbon flux towards the alanine. Finally, by disrupting the gene encoding the alanine racemase, the stereospecific production of L- alanine was achieved (Ferreira & Guido, 2018).

7. MULTIFUNCIONAL ORGANIC ACIDS

Organic acids are organic compounds having acidic properties. Carboxylic acids are the most common organic acids having a carboxyl group (-COOH). Some are strong organic acids, and some are weak organic acids, but both are helpful in food production. Examples of organic acids are lactic acid, acetic acid, formic acid, etc (Clark, 1989). In many food industries, the production of organic acids through different processes and techniques such as fermentation proved very beneficial because of their widespread applications in beverage industries, food preservation, and sweeteners and flavor enhancers. For this purpose, many types of bacteria, such as *E.coli*, go through different mixed acid fermentation of many sugars, for example, glucose, and produce good products like formate, acetate, ethanol, and succinate et (Clark, 1989). Anaerobic fermentative pathways have the following functions: It produces energy in this anaerobic environment through phosphorylation at a substrate level. It provides a source to regenerate NAD+.

8. METABOLIC PATHWAYS

Following are some examples of metabolic pathways of *E.coli* for the production of organic acids include succinate, lactate, and acetic acid (Yamauchi et al., 2014).

8.1. Succinic acid

Succinic acid can be produced from different strains of bacteria like obligate anaerobe *Anaerobiospirillum succiniciproducens*, *Enterococcus sp.* and *Actinobacillus*, which have been investigated (Stols et al., 1997). However, the metabolic engineering of *Escherichia coli* is focused on producing succinate in the form of the primary fermentation product. Wild type strains of *E.coli* produce succinate as a minor product in fermentation, but after some genetic manipulations, one can increase succinate production and reduce its by-product generation. For example, in the first step of succinate production, increasing the flux overexpressing PEP carboxylase can increase succinate production from 12% to 45% (Millard et al., 1996). Another technique is done when pyruvate to succinate form by transforming a wild type strain of *E.coli* with plasmid pTrc99A-PYC, which shows rhizobium pyruvate carboxylase. This step also increases the yield of succinate production by 17% (Gokarn et al., 1998). For its production, glucose is converted into 2 molecules of PEP by gene ptsG (a gene that encodes the enzyme IICBGlc). Then, PEP is converted into OAA (Oxaloacetic acid) by amplification in its gene ppc (gene which encodes PEP carboxylase). OAA by reaction with 2 hydrogens is converted into malate by the mdh gene (which encodes malate dehydrogenase). Malate is converted into fumarate and then into succinate (Millard et al., 1996).

8.2. Lactic acid

Many organisms generate D-lactic acid, and some lactic acid bacteria, like *Lactobacillus bulgaricus*, produce a large amount of pure D-Lactic acid (Benthin et al., 1995). L-Lactic acid can also be produced from lactic acid bacteria such as *Lactobacillus amylophilus*, *Lactobacillus helveticus* and *Lactobacillus delbrueckii* (Vickroy et al., 1985) . Nevertheless, lactic acid bacteria are not considered beneficial to produce lactic acid because they have complex nutritional needs, have less growth rate, and do not utilize pentose or its incomplete utilization (Hoet & Stainer, 1970). So, other bacterial strains suppose *E.coli* (Chang et al., 1999) , is then considered efficient to produce pure lactic acid by metabolic engineering. For example, the strain of *E.coli* RR1 metabolized glucose into D-lactic acid and produced less succinate as by product under anaerobic conditions. For L-lactic acid production, a gene named L-lactate dehydrogenase from the *Lactobacillus casei* bacteria was introduced into a strain ldhA. This recombinant strain metabolises glucose into L-lactic acid, a primary product of fermentation in this strain (Y.C. Wang et al., 2002). Glucose is converted into 2PEP and then into pyruvate by ppc gene, which encodes PEP carboxylase, then it is converted into lactic acid by modification in gene ldhA (gene which encodes lactate dehydrogenase).

8.3. Acetic acid

Acetic acid is produced as the main product of fermentation used in food markets like meat preservation, vinegar, etc. Microbes that are used for its production are *Saccharomyces cerevisiae*, *Clostridium spp.*, and *Acetobacter aceti*. The strain of *E.coli* W3110 was metabolically engineered for the production of acetic acid from glucose (Causey et al., 2003). Firstly, glucose is converted into 2PEP, and then by the involvement of the ppc gene, it is converted into two molecules of pyruvate. Pyruvate is then converted into an intermediate called Acetyl Co-A by pflB (gene which encodes pyruvate-formate lyase) converted into 2Acetyl-P by pta gene involvement encodes the enzyme phosphotransacetylase. Acetyl-P has done the dephosphorylation by removing ATP and converted into acetate, which is our product of interest (Wolfe, 2005) .

8.4. Vitamin

Vitamins are nutritious or practical foods (Ottaway, 2012). They function as cofactors of various enzymes concerned in numerous biochemical reactions, and they are essential additives inside the diet of humans. A few examples of the use of metabolic engineering to supply three vitamins (riboflavin, folate, and ascorbic acid) in microorganism strains (Leblanc et al., 2010) .

8.5. Riboflavin

Both *Bacillus subtilis* and *Lactococcus lactis* were designed to create riboflavin (Hao et al., 2013). In *Bacillus subtilis*, greater expression of the genes called rib (engaged with manufacturing of Vitamin B), prepared in a group, is finished by supplanting the two regulated promoters with constitutive ones were given from a phage. Although several duplicates of these four gene improvements had embedded specific websites inside the genome, an exclusive overexpression of ribs became crucial to attaining the most satisfactory productivity. The rib A gen. 83 encodes a bifunctional protein with dihydroxy-butanone phosphate synthetase pastime on the N-terminal and GTP cyclo hydrolase II motion on the C-terminal (Friedman et al., 2004). This confirmed that the beginning steps of riboflavin manufacturing restrained profitability within side the closing dispensed degree of pressure improvement. A metabolic exam of the wild-kind and riboflavin-turning in *B. subtilis* lines tracked down that the flux of the oxidative part of the pentose phosphate pathway turned into accelerated withinside the created pressure. Therefore, this department might also be in charge of prescribing, and new metabolic engineering structures are probably required for destiny pressure improvement. *Lactococcus lactis* is also being engineered to synthesis riboflavin through greater expressing the gene (rib A) encoding the catalyst (GTP cyclo hydrolase, catalyzing the number one reaction for its synthesis from GTP. These days, this catalyst spoke back to limitation of movement and overexpression of rib A delivered approximately a 3-fold greater in riboflavin synthesis.

8.6. Vitamin B11

Lactococcus lactis designed to synthesize vitamin B11 (W. Wang et al., 2021) . Techniques include the appearance of some genes engaged with the synthesis of vitamin B11 from GTP both independently and dependently and the appearance of heterologous y-glutamyl hydrolase (from people and mice). These methodologies have delivered approximately 3-6 fold expansions within the synthesis of folate and an amendment within the folate spatial dispersion (for instance, a pass from essentially intracellular to the extracellular gathering).

8.7. Vitamin C

Practically all natural cycles for generating vitamin C quit with manufacturing 2-keto-L-gulonic acid, which is later changed into ascorbic acid with the aid of using traditional chemical techniques innovation (i.e., esterification and lactonization). Consequently, the aim was targeted round designing strains organized to hand over 2-keto-L-gulonic acid from numerous sugars productively. Two strains of Gluconobacter oxydans had been designed to extrude over sorbitol into 2-keto-L-gulonic acid. Genes that encode the sorbitol dehydrogenase and sorbose dehydrogenase was cloned from glucose. Oxydans T-one hundred into glucose. Orydans G624, allowing the advent of 2- keto-L-gulonic acid in 3 stages. An Erwinia herbicola has been designed to supply 2-keto- L-gulonic acid from glucose in one fermentation step (Ikemi, 1994). *E. coli* typically creates 2,5-diketo- D-gluconic acid (2, five- DKG) through glucose oxidation but no longer have the chemical 2,5-diketo-D- gluconic acid reductase extra de over 2,5-diketo-D- gluconic acid into 2-keto-L- gulonic acid. Along with these modifications, *E. coli* became designed to communicate the gene encoding 2, 5-diketo-D-gluconic acid reductase from *Corynebacterium spp*. bringing approximately a recombinant strain that might change over glucose into 2-keto-L-gulonic acid in a one fermentation step. Moreover, this interplay has been streamlined, and 2-keto-L-gulonic acid concentrations > 120 g/L were accomplished (Levine et al., 1999).

8.8. Carbohydrates

A few non-digestible polysaccharides positively affect the improvement of gainful intestinal microflora, and along these lines are referred to by their capacity as prebiotics. Among them are galactooligosaccharides, glucooligosaccharides, transgalactooligosaccharides, and xylooligosaccharides. Lactic acid bacteria are a decent wellspring of non-digestible polysaccharides, basically, exopolysaccharides, which are additionally perceived by their commitment to the surface, taste insight, mouth feel and security of the last food item (Vilela et al., 2020). Given the accessible genetic data on qualities needed for exopolysaccharides biosynthesis in lactic acid bacteria, heterologous creation of exopolysaccharides has been accomplished in a lactic acid bacteria strain that could not deliver them. The comparative test has shown that exopolysaccharides with various pieces can be created from articulating a similar group of qualities due to one or the other selectivity in the fare and polymerization framework or restriction in the stock of forerunners. Exopolysaccharide creation in *Lactococcus lactis* can be expanded by overexpressing the quality of epsD, encoding a preparing glucosyltransferase (Looijesteijn et al., 2001). The two different instances of utilizing metabolic designing systems to improve exopolysaccharide creation and alter exopolysaccharide pieces in Lactococcus bacteria. Upregulation of fbp quality encoding fructose bisphosphatase brought about expanded Lactococcus bacteria development and exopolysaccharide combination utilizing fructose as a sugar source. Uridine diphosphate glucose pyrophosphorylase and uridine diphosphate galactose epimerase qualities of *L. lactis* to research their association in the biosynthesis of uridine diphosphate glucose and uridine diphosphate galactose, which are forerunners of glucose-and galactose- containing exopolysaccharide in Lactococcus lactis. Overexpression of the two qualities expanded the intracellular pools of Uridine diphosphate glucose and Uridine diphosphate galactose. These models delineate the part that metabolic design plays in the bacterial creation of polysaccharides (Mazmanian & Kasper, 2006).

8.9. Low calories sugar

These are similar to sorbitol and trehalose, etc., add for weight reduction, due to which there is high shopping demand for them (Looijesteijn et al., 1999). Tagatose is a low-calorie sugar that is ineffectively corrupted by the human body (Oh, 2007). It is viewed as both an antiplaque and prebiotic specialist. As of late, Lactoccus bacteria have been designed to create tagatose by upsetting the lacC or potentially lacD qualities, which brought about the creation of tagatose 6-phosphate or tagatose 1,6-diphosphate. Further hereditary adjustments brought about the creation of tagatose 1,6- diphosphate as the sole final result. Current endeavours are centered around the dephosphorylation of tagatose 1,6-diphosphate and discharge of the result, tagatose.

8.10. Aroma compounds

Diacetyl is a significant flavor compound in numerous dairy items like margarine, buttermilk, and cheeses (it gives a rich flavor) (Sales et al., 2018) . *L. lactis* has been designed to orchestrate diacetyl as the fundamental maturation item (Longo et al., 2006). Proficient diacetyl creation came about because of the inactivation of the aldB quality, which encodes acetolactate decarboxylase and overproduction of NADH-oxidase action. This methodology brought about the change of 80% of the carbon source into diacetyl. Another significant gathering of smell compounds is acquired from enzymatic corruption of amino acids, including isobutyrate, phenylacetaldehyde and indole, etc. Researchers have seen that overexpression of a heterologous glutamate dehydrogenase quality in *L. lactis* expanded the change of amino acids into smell compounds. In this manner, rather than adding ketoglutarate to cheddar, a GDH-delivering lactococcal strain could be utilized to upgrade amino corrosive corruption in fragrance compounds (Tananuwong et al., 2010).

9. FUTURE TRENDS AND POTENTIAL

Nature makes use of microorganisms to maintain our fermentation processes, and for lots of years, humankind has used yeasts, molds, and microorganisms to shape meals products like bread, beer, wine, vinegar, meats and cheese, furthermore as complex fish, meat and vegetables (Gonzalez et al., 2003). The key to the long run meals lies in microbiomes, the groups of microorganisms that colonize every viable circumstance in the world. By exploiting these ecosystems' ability, it is viable to offer better pleasant meals, growing the productivity, protection and belongings of meals provide chains. As the daily increase in population, we require more, quick and easy food that produces quickly and in massive amounts to get it according to their needs. Microorganisms have been a great help in getting such foods as many GMOs have been developed with the help of microorganisms for increased yield and fast growth (Hanlon & Sewalt, 2021).

9.1. Smart food

Smart food is highly nutrient food made with particular GMO crops that lead to quick growing and healthy nutritious and safe food that helps farmers for a daily wage. Producing, accessing, and consuming nutritionally rich and diverse foods is central to overcoming malnutrition and micronutrient deficiencies. A strong agricultural sector is vital to supporting rural livelihoods, and smart foods have a significant role to play in improving food security and protecting the environment (Maye et al., 2019) .

9.2. Protein sources

Protein is a very nutritional compound for the body and is mainly in the daily routine of people (Zhang et al., 2017). However, many people who cannot afford or do not have the resources to get meat cannot even consume it daily for that many proteins added things had been made using various microorganisms and similar techniques. One of the prominent examples is PROTEIN BARS; these are small bars containing a high level of proteins and other nutrients and can be obtained easily with low price than meat. Also, some more products, such as some cereals with high protein content, are now made for people who have any issue consuming meat. One of the common examples of cereals is Kellogs cereal that is protein-based (Tas et al., 2021).

9.3. Lactic acid bacteria

Lactic acid bacteria have been beneficial in the making and preserving of food, and many more benefits regarding food have been discovered by using lactic acid bacteria (Agbor-Egbe & Mbome, 2006). Several daily use food products have been produced by lactic acid bacteria such as yoghurt, cheese and many more milk products that are used and a need in daily food also it is a cheap source too (Boels et al., 2001). This bacterium has been used to make products that help make whole food, such as yeast, which helps produce the bread of different flavors. For a long time, milk is being fermented by using these bacteria and further shortly, there are changes expected in milk as we know there is already lactose-free milk, but there are to be milk produced that will be high dose in calcium and any required component as milk would be manipulated in different ways through different processes. Probiotic and based farm products are likewise hired inside the closest destiny to deal with various fitness disorders. Current research recommends that carboxylic acid bacterium own anti-weight problems and anti-diabetic propensities on their hosts and consequently will play a crucial function in human fitness care (Annunziato et al., 2021) . Analysis inside the physics and chemical technological know-how homes of frozen dessert and its programs is also rising. These programs face certain hurdles collectively with technological (for much less advanced countries), consumer satisfactoriness of new realistic meals is likewise stimulated via means of culture, ethics, or faith. There would be love for added research at the genetic foundation for probiotic homes that can provide any knowledge regarding novel manipulation capabilities and pertinence in nutrients and fitness sectors (Alkay et al., 2021) considerable influence of microorganisms in them, but they would be beneficial and not harmful.

9.4. Plants and crops

The poor outcomes at the surroundings and human fitness resulting from the contemporary farming structures primarily based totally on the overuse of chemical fertilizers had been pronounced in lots of research. By contrast, bacterial inoculations produce superb outcomes on yields without inflicting this kind of harm (Muhammed et al., 2013). In recent years, the commercialization of bio-fertilizers has increased, and the variety of businesses and merchandise to be had is increasing international each year. In addition to the beautiful enhancement of crop manufacturing, much research has proven how the utility of microorganisms has good outcomes on meals exceptional including progressed vitamin, flavonoid and antioxidant content, amongst different benefits. This benefit is thrilling with admire to meals this is fed on raw, including result and plenty of vegetables, as those bioactive molecules are maintained up till the instant the meal is fed on. This overview specializes in gathering research that exhibits that microorganisms can act as plant probiotics of fruit and horticultural crops, vital forms of meals that shape a part of a healthful diet. It is consequently vital that agricultural productiveness be drastically improved with inside the following couple of decades. To this end, agricultural exercise is transferring closer to an extra sustainable and environmentally pleasant approach (Chowdhury & Yasmin, 2018). This consists of using transgenic vegetation and plant increase-selling microorganisms as part of mainstream agricultural exercise. It is anticipated that with inside the now no longer too remote future, plant increase selling microorganisms (PGPB) will start to update using chemical compounds in agriculture, horticulture, silviculture, and environmental clean-up strategies. While there won't be one easy method that may efficiently sell the increase of all vegetation beneath neath all conditions.

10. CONCLUSION

In this advanced era, microbial production of food products, nutrients, food supplements and food additives has been improved using gene manipulation techniques. In this challenge, genetic and metabolic engineering are playing an essential role in producing different types of genetically modified foods in the food industry. Human beings have greatly embraced the information of engineering of microorganisms to improve their lives. The products produced by genetically modified microorganisms are used for health benefits and the prevention of malnutrition problems. Furthermore, genetically modified foods such as food additives and some organic acids have been employed to develop a variety of foods, pharmaceuticals, chemicals, etc. This chapter focuses on the role of microbes in the food industry with particular reference to genetic and metabolically modified microorganisms. By overcoming the current challenges, we can increase the benefits.

CONFLICTS OF INTEREST

There is no conflict of interest associated with this work.

ORCID

Manam Walait	0000-0002-9982-7117
Huda Rehman mir	0000-0003-3283-0490
Zainab Hassan	0000-0002-7201-4853
Javed Iqbal Wattoo	0000-0003-3819-4755

AUTHOR CONTRIBUTIONS

MW - Research concept and design; MW - Collection and/or assembly of data; MW - Data analysis and interpretation; MW, HRM - Writing the article; HRM, ZH, JIW - Critical revision of the article.

REFERENCES

Agbor-Egbe, T., Mbome, I.L.J.J.O.F.C., 2006. The effects of processing techniques in reducing cyanogen levels during the production of some Cameroonian cassava foods. Journal of Food Composition and Analysis. 19, 354–363. https://doi.org/10.1016/j.jfca.2005.02.004

Alkay, Z., Dertli, E., Durak, M.J.A.A., 2021. Investigation of probiotic potential of yeasts isolated from sourdoughs from different regions of Turkey. Acta Alimentaria. https://doi.org/10.1556/066.2021.00150

Annunziato, G., Spadini, C., Franko, N., Storici, P., Demitri, N., Pieroni, M., Flisi, S., Rosati, L., Iannarelli, M., Marchetti, M.J.A.I.D., 2021. Investigational Studies on a Hit Compound Cyclopropane-Carboxylic Acid Derivative Targeting O-Acetylserine Sulfhydrylase as a Colistin Adjuvant. ACS Infectious Diseases. 7, 281–292. https://doi.org/10.1021/acsinfecdis.0c00378

Antoniewicz, M.R.J.M.E., 2020. A guide to metabolic flux analysis in metabolic engineering: Methods, tools and applications. Metabolic Engineering. 63, 2–12. https://doi.org/10.1016/j.ymben.2020.11.002

Bar-Even, A., Flamholz, A., Noor, E., Milo, R.J.N.C.B., 2012. Rethinking glycolysis: on the biochemical logic of metabolic pathways. Nature Chemical Biology. 8, 509–517. https://doi.org/10.1038/nchembio.971

Benthin, S., Villadsen, J.J.A.M., Biotechnology., 1995. Production of optically pure D-lactate by Lactobacillus bulgaricus and purification by crystallisation and liquid/liquid extraction. Applied Microbiology and Biotechnology. 42, 826–829. https://doi.org/10.1007/BF00191176

Boels, I.C., Van Kranenburg, R., Hugenholtz, J., Kleerebezem, M., Vos, W.M.J.I.D.J.D., 2001. Sugar catabolism and its impact on the biosynthesis and engineering of exopolysaccharide production in lactic acid bacteria. International Dairy Journal. 11, 723–732. https://doi.org/10.1016/S0958-6946(01)00116-9

Causey, T., Zhou, S., Shanmugam, K., J, L., 2003. Engineering the metabolism of Escherichia coli W3110 for the conversion of sugar to redox-neutral and oxidized products: Homoacetate production. Proceedings of the National Academy of Sciences. 100, 825–832. https://doi.org/10.1073/pnas.0337684100

Chang, D.E., Jung, H.C., Rhee, J.S., J, J.-G., 1999. Homofermentative production of D- or L-lactate in metabolically engineered Escherichia coli RR1. Applied and Environmental Microbiology. 65, 1384–1389. https://doi.org/10.1128/aem.65.4.1384-1389.1999

Chotani, G., Dodge, T., Hsu, A., Kumar, M., Laduca, R., Trimbur, D., Weyler, W., B, K.J.B.E., Sanford, A.-P.S., Enzymology, M., 2000. The commercial production of chemicals using pathway engineering. Biochimica et Biophysica Acta (BBA) - Protein Structure and Molecular Enzymology. 1543, 434–455. https://doi.org/10.1016/S0167-4838(00)00234-X

Chowdhury, N., Yasmin, J., 2018. A study on sustainable manufacturing practices in RMG industries of Bangladesh. Munich Personal RePEc Archive. 87104, 1–17.

Clark, D.P.J.F., 1989. The fermentation pathways of Escherichia coli. FEMS Microbiology Reviews. 5, 223–234. https://doi.org/10.1016/

0168-6445(89)90033-8

Ferreira, I.M., Guido, L.F.J.F., 2018. Impact of wort amino acids on beer flavour: A review. Fermentation. 4(2), 23. https://doi.org/10.3390/fermentation4020023

Friedman, C., Shagina, L., Lussier, Y., Hripcsak, G.J.J.O.T.A.M.I.A., 2004. Automated encoding of clinical documents based on natural language processing. Automated encoding of clinical documents based on natural language processing. 11, 392–402. https://doi.org/10.1197/jamia.m1552

Gokarn, R., Eiteman, M., Altman, E.J.B., 1998. Expression of Pyruvate Carboxylase Enhances Succinate Production in Escherichia coli without Affecting Glucose Uptake. Biotechnology Letters. 20, 795–798. https://doi.org/10.1023/B:BILE.0000015925.52287.1f

Gonzalez, R., Andrews, B.A., Molitor, J., Asenjo, J.A.J.B., Bioengineering., 2003. Metabolic analysis of the synthesis of high levels of intracellular human SOD in Saccharomyces cerevisiae rhSOD 2060 411 SGA122. Biotechnology & Bioengineering. 82, 152–169. https://doi.org/10.1002/bit.10556

Hanlon, P., Sewalt, V., 2021. _genetically engineered microorganisms and the regulatory oversight of their uses in modern food production. Critical Reviews in Food Science and Nutrition. 61, 959–970. https://doi.org/10.1080/10408398.2020.1749026

Hao, T., Han, B., Ma, H., Fu, J., Wang, H., Wang, Z., Tang, B., Chen, T., Zhao, X.J.M.B., 2013. In silico metabolic engineering of Bacillus subtilis for improved production of riboflavin, Egl-237, (R,R)-2,3-butanediol and isobutanol. Molecular BioSystems. 9, 2034–2044. https://doi.org/10.1039/c3mb25568a

Hoet, P., Stainer, R.J.E., 1970. Existence and functions of two enzymes with beta-ketoadipate: succinyl-CoA transferase activity in Pseudomonas florescens. European Journal of Biochemistry. 13, 71–76. https://doi.org/10.1111/j.1432-1033.1970.tb00900.x

Holms, W., 1987. Control of flux through the citric acid cycle and the glyoxylate bypass in Escherichia coli. Biochemical Society symposium. 54, 17–31.

Ikemi, M.J.R.M.F.I., 1994. Industrial chemicals: enzymatic transformation by recombinant microbes. Bioprocess Technology. 19, 797–813.

Ivanov, K., Stoimenova, A., Obreshkova, D., Saso, L.J.B., Equipment, B., 2013. Biotechnology in the Production of Pharmaceutical Industry Ingredients: Amino Acids. Biotechnology & Biotechnological Equipment. 27, 3620–3626. https://doi.org/10.5504/BBEQ.2012.0134

Leblanc, J.G., Taranto, M.P., Molina, V., J, F., 2010. B-Group Vitamins Production by Probiotic Lactic Acid Bacteria, In: Second (Eds.); F. Mozzi, R.R. Raya, G.M. Vignolo, et al., (Eds.), Biotechnology of Lactic Acid Bacteria: Novel Applications. John Wiley & Sons, Ltd, pp. 211–232. https://doi.org/10.1002/9781118868386.ch17

Leonard, E., Lim, K.H., Saw, P.N., Koffas, M.A.J.A., Microbiology, E., 2007. Engineering central metabolic pathways for high-level flavonoid production in Escherichia coli. Applied and Environmental Microbiology. 73, 3877–3886. https://doi.org/10.1128/AEM.00200-07

Levine, M., Rumsey, S.C., Daruwala, R., Park, J.B., Wang, Y.J.J., 1999. Criteria and recommendations for vitamin C intake. JAMA. 281, 1415–1423. https://doi.org/10.1001/jama.281.15.1415

Lixin, S.J.J.O.M., 2000. Metabolic engineering: A rising filed in biochemical engineering. Journal of Microbiology. 3.

Longo, M.A., Sanromán, M.A.J.F.T., Biotechnology., 2006. Production of Food Aroma Compounds: Microbial and Enzymatic Methodologies. Food Technology and Biotechnology. 44, 335–353.

Looijesteijn, P.J., Boels, I.C., Kleerebezem, M., Hugenholtz, J.J.A., Microbiology, E., 1999. Regulation of exopolysaccharide production by Lactococcus lactis subsp. cremoris by the sugar source. Applied and Environmental Microbiology. 65, 5003–5008. https://doi.org/10.1128/aem.65.11.5003-5008.1999

Looijesteijn, P.J., Trapet, L., Vries, E., Abee, T., J, J., 2001. Physiological function of exopolysaccharides produced by Lactococcus lactis.. 64, 71–80. https://doi.org/10.1016/S0168-1605(00)00437-2

Maser, A., Peebo, K., Vilu, R., Nahku, R.J.R.I.M., 2020. Amino acids are key substrates to Escherichia coli BW25113 for achieving high specific growth rate. Research in Microbiology. 171, 185–193. https://doi.org/10.1016/j.resmic.2020.02.001

Maye, D.J.C., Culture, Society., 2019. ‘Smart food city’: Conceptual relations between smart city planning, urban food systems and innovation theory. City, Culture and Society. 16, 18–24. https://doi.org/10.1016/j.ccs.2017.12.001

Mayra-Makinen, A., Bigret, M.J.F.S., Dekker, T.N.Y.M., 2004. Industrial Use and Production of Lactic Acid Bacteria, S. Salminen, A. von Wright, A. Ouwehand, et al., (Eds.), Lactic Acid Bacteria Microbiological and Functional Aspects. Marcel Dekker, Inc, New York, pp. 175–198.

Mazmanian, S.K., Kasper, D.L.J.N.R.I., 2006. The love–hate relationship between bacterial polysaccharides and the host immune system. Nature Reviews Immunology. 6, 849–858. https://doi.org/10.1038/nri1956

Millard, C.S., Chao, Y.P., Liao, J.C., Donnelly, M.I.J.A., Microbiology, E., 1996. Enhanced production of succinic acid by overexpression of phosphoenolpyruvate carboxylase in Escherichia coli. Applied and Environmental Microbiology. 62, 1808–1810. https://doi.org/10.1128/aem.62.5.1808-1810.1996

Muhammed, A.S., Memon, H., Baloch, S., Osman, K.A., Ali, S.A.J.P.G.C.P., 2013. Effect of Bio-organic And Inorganic Fertilizers On The Growth And Yield Of Wheat. Current Investigations in Agriculture and Current Research.

Oh, D.-K., 2007. Tagatose: properties, applications, and biotechnological processes. Applied Microbiology and Biotechnology. 76, 1–8. https://doi.org/10.1007/s00253-007-0981-1

Ottaway, P.B., 2012. The Technology of Vitamins in Food, In: 1st (Eds.); and others, (Eds.). Springer Science & Business Media, Boston, MA, p. 270. https://doi.org/10.1007/978-1-4615-2131-0

Partridge, D., Lloyd, K., Rhodes, J., Walker, A., Johnstone, A., Campbell, B.J.N.B., 2019. Food additives: Assessing the impact of exposure to permitted emulsifiers on bowel and metabolic health – introducing the FADiets study. Nutrition Bulletin. 44, 329–349. https://doi.org/10.1111/nbu.12408

Russ, W.P., Figliuzzi, M., Stocker, C., Barrat-Charlaix, P., Socolich, M., Kast, P., Hilvert, D., Monasson, R., Cocco, S., Weigt, M.J.S., 2020. An evolution-based model for designing chorismate mutase enzymes. Science. 369, 440–445. https://doi.org/10.1126/science.aba3304

Sales, A., Paulino, B.N., Pastore, G.M., Bicas, J.L., 2018. Biogeneration of aroma compounds. Current Opinion in Food Science. 19, 77–84. https://doi.org/10.1016/j.cofs.2018.03.005

Stols, L., Donnelly, M.I.J.A., Microbiology, E., 1997. Production of succinic acid through overexpression of NAD(+)-dependent malic enzyme in an Escherichia coli mutant. Applied and Environmental Microbiology. 63, 2695–2701. https://doi.org/10.1128/aem.63.7.2695-2701.1997

Tananuwong, K., Lertsiri, S.J.J.O.T.S.O.F., Agriculture., 2010. Changes in volatile aroma compounds of organic fragrant rice during storage under different conditions. Journal of the Science of Food and Agriculture. 90, 1590–1596. https://doi.org/10.1002/jsfa.3976

Tas, A., Shah, A.J.T.I.F.S., Technology., 2021. The replacement of cereals by legumes in extruded snack foods: Science, technology and challenges. Trends in Food Science & Technology. 116, 701–711. https://doi.org/10.1016/j.tifs.2021.08.016

Toe, C.J., Foo, H.L., Loh, T.C., Mohamad, R., Rahim, R., J, Z.,

2019. Extracellular Proteolytic Activity and Amino Acid Production by Lactic Acid Bacteria Isolated from Malaysian Foods. International Journal of Molecular Sciences. 20(7), 1777. https://doi.org/10.3390/ijms20071777

Tomar, N., De, R.K.J.G., 2013. Comparing methods for metabolic network analysis and an application to metabolic engineering. Gene. 521, 1–14. https://doi.org/10.1016/j.gene.2013.03.017

Tzin, V., Galili, G., 2010. The biosynthetic pathways for shikimate and aromatic amino acids in Arabidopsis thaliana. Arabidopsis Book. 8, e0132. https://doi.org/10.1199/tab.0132

Van Ooyen, J., Noack, S., Bott, M., Reth, A., B, L.J., 2012. Improved L-lysine production with Corynebacterium glutamicum and systemic insight into citrate synthase flux and activity. Biotechnology & Bioengineering. 109, 2070–2081. https://doi.org/10.1002/bit.24486

Vickroy, T.W., Watson, M., Leventer, S.M., Roeske, W.R., Hanin, I., Yamamura, H.I.J.J.O.P., Therapeutics, E., 1985. Regional differences in ethylcholine mustard aziridinium ion (AF64A)-induced deficits in presynaptic cholinergic markers for the rat central nervous system. Journal of Pharmacology and Experimental Therapeutics. 235, 577–582.

Vilela, A., Cosme, F., Inês, A.J.F., 2020. Wine and Non-Dairy Fermented Beverages: A Novel Source of Pro-and Prebiotics. Fermentation. 6, 113. https://doi.org/10.3390/fermentation6040113

Wang, W., Ma, H., Zhu, Y., Ni, K., Qin, G., Tan, Z., Wang, Y., Wang, L., J, H., 2021. Screening of Lactic Acid Bacteria with Inhibitory Activity against ETEC K88 as Feed Additive and the Effects on Sows and Piglets. Animals. 11, 1719. https://doi.org/10.3390/ani11061719

Wang, Y.C., Yu, R.C., Chou, C.-C.J.F.M., 2002. Growth and survival of bifidobacteria and lactic acid bacteria during the fermentation and storage of cultured soymilk drinks. Food Microbiology. 19, 501–508. https://doi.org/10.1006/fmic.2002.0506

Wenzel, S.C., Müller, R.J.C., 2005. Recent developments towards the heterologous expression of complex bacterial natural product biosynthetic pathways. Current Opinion in Biotechnology. 16, 594–606. https://doi.org/10.1016/j.copbio.2005.10.001

Wolfe, A.J.J.M., 2005. The acetate switch. Microbiology and Molecular Biology Reviews. 69, 12–50. https://doi.org/10.1128/mmbr.69.1.12-50.2005

Yamauchi, Y., Hirasawa, T., Nishii, M., Furusawa, C., T, H.J., 2014. Enhanced acetic acid and succinic acid production under microaerobic conditions by Corynebacterium glutamicum harboring Escherichia coli transhydrogenase gene pntAB. The Journal of General and Applied Microbiology. 60, 112–118. https://doi.org/10.2323/jgam.60.112

Zhang, B., Tian, W., Wang, S., Yan, X., Jia, X., Pierens, G.K., Chen, W., Ma, H., Deng, Z., J, X., 2017. Activation of Natural Products Biosynthetic Pathways via a Protein Modification Level Regulation. ACS Chemical Biology. 12, 1732–1736. https://doi.org/10.1021/acschembio.7b00225