Riboflavin (RF) is an essential micronutrient of the human and animal diet [1]. It is the precursor of the flavoenzymes; flavin mononucleotide (FMN) and flavin adenine dinucleotide (FAD), which are mostly involved in redox reactions. RF FMN and FAD are also referred to as flavins. They participate in the metabolism of carbohydrates, fats, ketone bodies and proteins. RF is also closely related to the metabolism of other vitamins such as $B_6$, $B_3$ and $A$, glutathione recycling and homocysteine metabolism. The main natural sources of RF are milk, dairy products, eggs and lean meat. Green leafy vegetables, fish, legumes, cereals and nuts are also important sources of RF. RF is light-sensitive, it is therefore, important that these food sources are stored in dark environments but the vitamin is heat stable [1,2].

The Recommended Daily Allowance (RDA) of RF varies with age and gender. It is between 0.3 and 0.4 mg/day for infants, between 0.5 and 0.9 mg/day for children, 1.3 mg/day for adult males and 1.1 mg/day for adult females. The RDA for women during pregnancy and lactation is increased to 1.4 and 1.6 mg/day, respectively [2,3].

RF deficiencies in humans is called ariboflavinosis and it often occur in association with multiple nutrient deficits and also very common in developing countries [3,4]. Clinical manifestation is observed in the mucocutaneous surfaces of the mouth, through the occurrence of cracks at the corners, and inflammation of the lips and tongue [4]. The deficiencies can also be associated with increased risk of cardiovascular diseases and impairment in iron metabolism (anaemia) which can also result in developmental abnormalities and growth retardation [3]. However, because RF is a water-soluble vitamin, excess uptake is easily eliminated through the urine. Recent researches have pointed out the fact that some diseases such as cancer, cardiac disease and diabetes mellitus can aggravate RF deficiency, therefore, higher intakes of RF are recommended for these risk groups [5].

Humans and animals lack the capacity to synthesize RF, they depend solely on their diet in order to obtain this essential micronutrient. The diet can therefore be improved on by synthesizing RF biotechnologically using microorganisms [6] and using this microorganisms to biofortified commonly consumed food. Biofortification of food is an integrated approach to reduce malnutrition. The concept of in situ fortification by bacterial fermentation provides the basis to enhance the nutritional value of food products and their commercial value [7].

Most microorganisms are able to synthesize RF, but there are only a few that are able to produce high amounts of this vitamin [8]. Microorganisms that have the ability to synthesized RF are referred to as flavinogenic microorganisms, and they can be classified into three groups according to their capacities of riboflavin production: weak overproducers (produce around 10 mg/L), medium or moderate overproducers (yields up to 600 mg/L) and highly flavinogenic overproducers (producing more than 10 g/L) [8].
Among the bacteria group, *Clostridium acetobutylicum*, *Shewanella oneidensis* and some species of *Mycobacterium* and *Corynebacterium* are the highest natural overproducers [9,10].

*Bacillus subtilis* is not a high natural overproducer of RF, however, *B. subtilis* industrial strains that are overproducers of RF have been isolated and are currently used for the biotechnological production of RF [11].

Several researches have established *B. subtilis* as a good candidate to develop a bacterial process for RF production by fermentation. Several useful features of *B. subtilis* include its classification as a GRAS (generally regarded as safe) microorganism, the deep knowledge on its physiology and recombinant DNA technology and its capability to produce large amounts of the RF precursors inosine and guanosine (20 - 40 g L⁻¹) [12], which could subsequently be converted metabolically into RF, making *B. subtilis* a good candidate for developing a bacterial process of RF production by fermentation.

*B. subtilis* was first subjected to biotechnological production of RF by using classical genetic mutant selection i.e. exposure of the bacteria to RF analogs, most especially roseoflavin. Mutant strains that are resistant to this analog were able to produce more RF due to the deregulation of the RF derivatives synthesis. The use of roseoflavin, which is an analogue of riboflavin to induce overproduction of the riboflavin, has been well established by Burgess, *et al.* [13] in which it was analyzed that RF-consumming strains in food can be replaced by riboflavin - producing strains and in turn improves or increase the RF bioavailability in the food product and adding value to health benefits. Roseoflavin is a riboflavin analogue, and from previous work in *B. subtilis* it is known that exposure to this compound leads to spontaneous mutants that are constitutive RF over-producers [14]. Exposure to roseoflavin cause a deregulation of the rib operon due to the mutations in *ribC* or *ribO*. Use of roseoflavin for overproduction of RF has been found to be linked with nucleotide changes and deletions in the RFN regulatory element [4,13]. This is a less expensive method resulting in a product of superior quality which uses renewable sources of biotechnological approach [13].

The purpose of this method is not to generate an alternative production strains, but to replace the RF consuming strains that is used in traditional food fermentation processes with RF-producing counterparts, which then helps to increase the RF bioavailability in the food product and introducing an added health benefit [13]. Researchers have reported the production of RF bio-enriched yoghurt [4], pasta and bread [15], bread [16] and soymilk [17] at pilot scale using selected spontaneous roseoflavin resistant strains from different types of bacteria. However, there is a need to start applying this biotechnological method in the fermentation of fermented vegetable proteins of the fermented food consumed in developing countries. This idea of *in situ* biofortification will help to enhance both the nutritional value and commercial value of food products.

**BIBLIOGRAPHY**


Biotechnological Approach to Obtaining Riboflavin Overproducing *Bacillus subtilis*


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