those relevant to human health. These data and information, when considered in total, should provide assurance that the food is unlikely to have an adverse effect on human health. The assessment of unintended effects takes into account the biochemical, and physiological characteristics of the microorganism that are typically selected for improving strains for commercial food or beverage uses. These determinations provide a first screen for microorganisms that exhibit unintended traits. Recombinant-DNA microorganisms that pass this screen are subjected to safety assessment as described in Section 4.

Framework of Food Safety Assessment

22. The safety assessment of a food produced using a recombinant-DNA microorganism is based on determining the safety of using the microorganism, which follows a stepwise process of addressing relevant factors that include:

    A) Description of the recombinant-DNA microorganism;

    B) Description of the recipient microorganism and its use in food production;

    C) Description of the donor organism(s);

    D) Description of the genetic modification(s) including vector and construct;

    E) Characterization of the genetic modification(s);

    F) Safety assessment:

        a. expressed substances: assessment of potential toxicity and other traits related to pathogenicity;

        b. compositional analyses of key components;

        c. evaluation of metabolites;

        d. effects of food processing;

        e. assessment of immunological effects;

        f. assessment of viability and residence of microorganisms in the human gastrointestinal tract;

        g. antibiotic resistance and gene transfer; and

        h. nutritional modification.

23. In certain cases, the characteristics of the microorganisms and/or the foods produced/processed using these microorganisms may necessitate generation of additional data and information to address issues that are unique to the microorganisms and/or food products under review.

24. Experiments intended to develop data for safety assessments should be designed and conducted in accordance with sound scientific concepts and principles, as well as, where appropriate, Good Laboratory Practice. Primary data should be made available to regulatory authorities upon request. Data should be obtained using sound scientific methods and analysed using appropriate statistical techniques. The sensitivity of all analytical methods should be documented.

25. The goal of each safety assessment is to provide assurance, in the light of the best available scientific knowledge, that the food will not cause harm when prepared or consumed according to its intended use, nor should the organism itself cause harm when viable organisms remain in the food. Safety assessments should address the health aspects for the whole population, including immuno-compromised individuals,

infants, and the elderly. The expected endpoint of such an assessment will be a conclusion regarding whether the new food and/or microorganisms are as safe as the conventional counterparts taking into account dietary impact of any changes in nutritional content or value. Where the microorganism is likely to be viable upon ingestion, its safety should be compared to a conventional counterpart taking into account residence of the recombinant-DNA microorganism in the gastrointestinal tract, and where appropriate, interactions between it and the gastrointestinal flora of mammals (especially humans) and impacts of the recombinant-DNA microorganism on the immune system. In essence, the outcome of the safety assessment process is to define the product under consideration in such a way as to enable risk managers to determine whether any measures are needed to protect the health of consumers and if so to make well-informed and appropriate decisions in this regard.

SECTION 4- GENERAL CONSIDERATIONS

Description of the Recombinant-DNA Microorganism

26. A description of the bacterial, yeast, or fungal strain and the food being presented for safety assessment should be provided. This description should be sufficient to aid in understanding the nature of the organism or food produced using the organism being submitted for safety assessment. Recombinant-DNA microorganisms used in food production or contained in food, should be conserved as stock cultures with appropriate identification using molecular methods, and preferably, in established culture collections. This may facilitate the review of the original safety assessment. Such stock cultures should be made available to regulatory authorities upon request.

Description of the Recipient Microorganism and its Use in Food Production

27. A comprehensive description of the recipient microorganism or microorganism subjected to the modification should be provided. Recipient microorganisms should have a history of safe use in food production or safe consumption in foods. Organisms that produce toxins, antibiotics or other substances that should not be present in food, or that bear genetic elements that could lead to genetic instability, antibiotic resistance or that are likely to contain genes conferring functions associated with pathogenicity (i.e., also known as pathogenicity islands or virulence factors) should not be considered for use as recipients. The necessary data and information should include, but need not be restricted to:

    A) identity: scientific name, common name or other name(s) used to reference the microorganism, strain designation, information about the strain and its source, or accession numbers or other information from a recognized culture repository from which the organism or its antecedents may be obtained, if applicable, information supporting its taxonomical assignment;

    B) history of use and cultivation, known information about strain development (including isolation of mutations or antecedent strains used in strain construction); in particular, identifying traits that may adversely impact human health;

    C) information on the recipient microorganism's genotype and phenotype relevant to its safety, including any known toxins, antibiotics, antibiotic resistance factors or other factors related to pathogenicity, or immunological impact, and information about the genetic stability of the microorganism;

    D) history of safe use in food production or safe consumption in food; and

    E) information on the relevant production parameters used to culture the recipient microorganism.

28. Relevant phenotypic and genotypic information should be provided not only for the recipient microorganism, but also for related species and for any extrachromosomal genetic elements that contribute to the functions of the recipient strain, particularly if the related species are used in foods or

involved in pathogenic effects in humans or other animals. Information on the genetic stability of the recipient microorganism should be considered including, as appropriate, the presence of mobile DNA elements, i.e. insertion sequences, transposons, plasmids, and prophages.

29. The history of use may include information on how the recipient microorganism is typically grown, transported and stored, quality assurance measures typically employed, including those to verify strain identity and production specifications for microorganisms and foods, and whether these organisms remain viable in the processed food or are removed or rendered non-viable as a consequence of processing.

Description of the Donor Organism(s)

30. Information should be provided on the donor organism(s) and any intermediate organisms, when applicable, and, when relevant, related organisms. It is particularly important to determine if the donor or intermediate organism(s) or other closely related species naturally exhibit characteristics of pathogenicity or toxin production, or have other traits that affect human health. The description of the donor or intermediate organism(s) should include:

A) identity: scientific name, common name or other name(s) used to reference the organism, strain designation, information about the strain and its source, or accession numbers or other information from a recognized culture repository from which the organism or its antecedents may be obtained, if applicable, and information supporting its taxonomic assignment;

B) information about the organism or related organisms that concerns food safety;

C) information on the organism's genotype and phenotype relevant to its safety including any known toxins, antibiotics, antibiotic resistance factors or other factors related to pathogenicity, or immunological impact; and

D) information on the past and present use, if any, in the food supply and exposure route(s) other than intended food use (*e.g.*, possible presence as contaminants).

Description of the Genetic Modification(s) Including Vector and Construct

31. Sufficient information should be provided on the genetic modification(s) to allow for the identification of all genetic material potentially delivered to or modified in the recipient microorganism and to provide the necessary information for the analysis of the data supporting the characterization of the DNA added to, inserted into, modified in, or deleted from the microbial genome.

32. The description of the strain construction process should include:

A) information on the specific method(s) used for genetic modification;

B) information on the DNA used to modify the microorganism, including the source (*e.g.*, plant, microbial, viral, synthetic), identity and expected function in the recombinant-DNA microorganism, and copy number for plasmids; and

C) intermediate recipient organisms including the organisms (*e.g.*, other bacteria or fungi) used to produce or process DNA prior to introduction into the final recipient organism.

33. Information should be provided on the DNA added, inserted, deleted, or modified, including:

A) the characterization of all genetic components including marker genes, vector genes, regulatory and other elements affecting the function of the DNA;

B) the size and identity;

C) the location and orientation of the sequence in the final vector/construct; and

D) the function.

Characterization of the Genetic Modification(s)

34. In order to provide clear understanding of the impact of the genetic modification on the composition and safety of foods produced using recombinant-DNA microorganisms, a comprehensive molecular and biochemical characterization of the genetic modification should be carried out. To facilitate the safety assessment, the DNA to be inserted should be preferably limited to the sequences necessary to perform the intended functions.

35. Information should be provided on the DNA modifications in the recombinant DNA microorganism; this should include:

A) the characterization and description of the added, inserted, deleted, or otherwise modified genetic materials, including plasmids or other carrier DNA used to transfer desired genetic sequences. This should include an analysis of the potential for mobilization of any plasmids or other genetic elements used, the locations of the added, inserted, deleted, or otherwise modified genetic materials (site on a chromosomal or extrachromosomal location); if located on a multicopy plasmid, the copy number of the plasmid;

B) the number of insertion sites;

C) the organisation of the modified genetic material at each insertion site including the copy number and sequence data of the inserted, modified, or deleted material, plasmids or carrier DNA used to transfer the desired genetic sequences, and the surrounding sequences. This will enable the identification of any substances expressed as a consequence of the inserted, modified or deleted material;

D) identification of any open reading frames within inserted DNA, or created by the modifications to contiguous DNA in the chromosome or in a plasmid, including those that could result in fusion proteins; and

E) particular reference to any sequences known to encode, or to influence the expression of, potentially harmful functions.

36. Information should be provided on any expressed substances in the recombinant-DNA microorganism; this should include:

A) the gene product(s) (*e.g.*, a protein or an untranslated RNA) or other information such as analysis of transcripts or expression products to identify any new substances that may be present in the food;

B) the gene product's function;

C) the phenotypic description of the new trait(s);

D) the level and site of expression (intracellular, periplasmic - for Gram-negative bacteria, organellar - in eukaryotic microorganisms, secreted) in the microorganism of the expressed gene product(s), and, when applicable, the levels of its metabolites in the organism;

E) the amount of the inserted gene product(s) if the function of the expressed sequence(s)/gene(s) is to alter the level of a specific endogenous mRNA or protein; and

F) the absence of a gene product, or alterations in metabolites related to gene products, if applicable to the intended function(s) of the genetic modification(s).