CHAPTER 1

INTRODUCTION

Fish is an important source of animal protein which is highly perishable and spoils very rapidly (Barile *et al.*, 1985). It is a common, widely distributed and cheapest source of protein in the world and has been consumed by human since the dawn of civilization. They also constitute a rich source of polyunsaturated fatty acids, essential minerals, and vitamins, and therefore play an important role in human nutrition. Fish and fish products accounts for over 20% of the world's daily animal protein intake (Zang *et al*., 2018; Tacon and Metian, 2017). In South East Asia, people receive up to 60-70% of their protein from fish and fish products (Saisithi, 1994).

The increasing demand of fish and fish products has led to many modern preservation techniques and technologies developed to address the issues such as availability and putrefaction (Gassem, 2019; Xu *et al.,* 2021). However, the traditional ways of fermentation are still extensively used globally apart from the process of drying, smoking, salting and freezing (Sivamaruthi *et al.,* 2021). Fermented fish products have for many years been considered a south Asian product (Huss and Valdimarson, 1990). Fermented fish items are traditionally manufactured on a cottage industry or domestic scale and are widely consumed in east and south-east Asia (Borgstorm, 1962; Adams, 1998). All types of freshwater and marine fishes including mollusks and crustaceans that are processed with salt to create fermentation and avoid decomposition are considered as fermented fish (Ishige, 1993). Essuman (1992) defined “fermented fish” as fish that have undergone degenerative changes as a result of enzymatic or microbial activity with or without the process of salting. However, the term "fermented fish" is used to describe both enzyme-hydrolyzed and microbially fermented fish products, and no clear differentiation is drawn between them (Huss *et al.,* 2003). The process of fermentation has been in practice for thousands of years. It is considered to be as one of the oldest methods used to produce and preserve foods (Salampessy *et al*., 2010).

Fermentation provides ways to preserve food products, destroy undesirable factors, improve sensory quality (flavour, aroma, texture, and colour), increase nutritive value by emphasising the bioavailability of proteins (amino acids and peptides), save the quality of raw materials that would otherwise be unfit for human consumption, and reduce the amount of energy used in the cooking process (Peredes-Lopez and Harry, 1988; Jamas *et al.*, 2020; Isola *et al.,* 2021).

Fermented foods, according to Campbell-Platt (1987) are foods that have been produced through regulated microbial growth and enzymatic action, resulting in essential biochemical changes and substantial modification of the food. Microbiologists use the term "fermentation" to describe a sort of energy-producing chemical breakdown by bacteria, yeasts, and other organisms under anaerobic conditions in which an organic molecule acts as the final electron acceptor (Adams, 1986). Whichever definition used; fermented foods are those that have been exposed to bacteria that produce lactic acid (Sahlin, 1999).

In South-East Asia, the traditional process for fish preservation includes fermentation (Zang *et al*., 2019), salting (Tamang *et al.,* 2016), drying (Tamang *et al.,* 2016), and smoking without refrigeration (Olaleye and Abegunde, 2015; Cooke *et al.*, 1993). There are two types of traditional fermented fish products: fish with salt formulations, such as fish sauce/paste, and fish, salt and carbohydrate mixtures, such as *pla-ra* of Thailand and *burong-isda*, *burong dalag* of Philippines (Adams, 1998; Adams *et al*., 1985; Orillo and Pederson, 1968). Fermented fish products contribute significant protein intake to a larger population of the world (Beddows, 1985).

Food quality and the nutritional value is quicky lost due to the microbial contamination (Machado-Moreira *et al.,* 2019). Bacteria, yeasts, and moulds are the primary causes of food spoilage because they can easily grow and multiply in food products (Lorenzo *et*

al., 2018). While cold storage reduces the problem of extreme perishability in fish in developed countries, fermentation continues to play an important role in tropical regions in providing a variety of high-quality foods for human and domestic stock consumption. Traditional methods for preserving fresh fish, particularly in West Africa, include drying, salting, smoking, fermenting, and mixtures of these treatments (FAO, 1981). Even in many industrialised countries, the use of fermentation to improve the flavour and/or mask the taste of tainted fish products has resulted in higher food acceptance, resulting in increasing consumption and production (Saisithi, 1994). Fermented fish is also a low-cost preservation method for developing countries, particularly Asian countries, as an alternative to excessively salted fish products. In Asia, fish fermentation technology is a traditional home-based technique that produces numerous fermented fish products which are utilized as staple foods, condiments, side dishes, and sauces (Lee *et a*l., 1993). In Asia, traditionally processed fish is prepared for human consumption by fermenting, drying, and salting in large quantities (Hesseltine, 1979; Steinkraus, 1983; Campbell-Platt, 1987). Some of these traditional fish products are *budu* of Malaysia (Merican, 1977), *patis* of the Philippines (Arroyo *et al*., 1978; Sakai *et al*., 1983a, b), *plaa-raa* and *nam-pla* of Thailand (Phithakpol, 1993), *pindang* of Indonesia (Putro, 1993), *jeot* of Korea (Lee, 1993; Mheen, 1993), *shiokara* and *shottsuru* of Japan (Itoh *et al*., 1993) and *nga-pi* of Myanmar (Tyn, 1993).

Fermented fish products are deeply embedded in the cultures of many Southeast Asian countries, particularly those used by a diverse range of ethnic groups (Majumdar *et al.,* 2016; Ruddle and Ishige, 2010; Tamang *et al.*, 2020). In India the practice of fermentation and consumption is widely reported from many parts of North Eastern states, Sikkim and Darjeeling hills popular within different ethnic groups. The traditional processed fish products of North Eastern states and Darjeeling hills include

bordia and *karati* (Thapa *et al.,* 2007; Thapa, 2016) and *namsing* from Assam (Chowdhury *et al.,* 2019); *hentak* (Thapa *et al*., 2004; Singh *et* al., 2018) and *ngari* of Manipur (Sarojnalini, 1987); *tungtap* of Meghalaya (Thapa *et al.,* 2004); *lashim*, *gnuchi* and *sukako maacha* of Sikkim and Darjeeling hills, *sidra* and *sukuti* of Sikkim and Darjeeling hills (Thapa, 2002), *phuti shidal, phasha shidal, lona ilish* of Tripura (Muzaddadi, 2013; Suchitra *et al*., 2015). The fermented fish products of North Eastern states are frequently sold in the local markets whereas the fish products of Sikkim and Darjeeling hills are not sold in the local market and are entirely prepared for domestic consumption only.

During fermentation the microorganisms through the action of enzymes produces biochemical changes in the organic substrates such as enrichment of human diet with acceptable flavour, texture and aroma, bio preservation of perishable foods, bio enrichment of substrates with vitamins, protein and essential amino acids, and detoxification of undesirable components (Campbell-Platt, 1994; Steinkraus, 1994; Stiles and Holzapfel, 1997). Bacteria, predominantly lactic acid bacteria, yeasts, and filamentous fungi, which are present in the ingredients, utensils, and surroundings are selected as a result of adaptation to the substrate (Hesseltine, 1983; Tamang, 1998). The use of biological systems for food preservation is growing popularity, with the focus being on lactic acid bacteria although not been widely used in fish products (Jeppesen and Huss, 1993). The fermentative process can be either natural, with desired bacteria growing preferentially, or controlled, with inoculation of characterized fermentative microorganisms added to the raw material under regulated circumstances in the form of a starting culture. To limit water activity (a_w) and eliminate the growth of proteolytic and putrefying microorganisms, fermentation is frequently supplemented with the addition of salt or drying (Salampessy *et al.,* 2010). The process can be partial, lasting

a few hours to several weeks, as in the fermentation of fish foods in Africa and the preparation of *balao balao* in the Philippines, or lasting several months, as in the preparation of fish paste and sauce (Salampessy *et al*., 2010).

Fermentation can improve flavour, digestibility, nutritional content, and medicinal activity in addition to preservation (Jeyram *et al.,* 2009). Fermented fish products serve an important role in supplying protein, adding flavour, and diversity to rice-based diets in hot nations, particularly in rural regions (Campbell-Platt, 1987). Apart from being a significant source of protein, fermented fish also include fish lipids, which are a good source of highly unsaturated fatty acids (Abrami *et al.,* 1992). Due to its great nutritional value and organoleptic features, it has also become quite popular in developed countries (Soni *et al.,* 1990). The organoleptic properties however are unique to geographical locations, food preferences and culture and also in the environment in which they are produced (Tamang and Samuel, 2010; Waisundara *et al.,* 2016).

The Teesta, Balasan and Rangit river systems, as well as their tributaries in the Darjeeling hills and North Bengal, have a wide range of altitudinal gradients and are home to nearly 65 species of fish (Acharjee and Barat, 2013).

Seafood is uncommon to the Eastern Himalayan regions (Thapa, 2002). The people of Darjeeling hills and plains of North Bengal mainly catch fishes from hill rivers, rivulet, streams and ponds, and majority of fish is consumed as fresh. For later consumption some of these fishes are also preserved and processed using indigenous knowledge of fermentation, drying and smoking. Preparation of traditional foods has been mainly influenced by the agro-climatic conditions, ethnic preference, socio-economy and cultural ethos in the Eastern Himalayas. Modern methods of preservation are still uncommon to majority of people, traditional way of preservation of locally available fish by fermentation, smoking, drying is the common practice (Thapa, 2002).

Traditionally processed fish products are being consumed by various ethnic groups of people living in the regions of Nepal, Bhutan, Darjeeling hills and Northeast regions of India. The literature on traditionally processed fish products for human consumption in North Bengal has been very little documented. Documentation on production statistics of some common and lesser-known traditional fish products of the North Bengal and information on microbiology and safety are not available. The proposed dissertation is aimed to study in depth the microbial diversity associated with some traditionally (locally) processed fish products of North Bengal. Study of microbial diversity in the lesser-studied fish products of North Bengal, may contribute a significant insight to the unknown microbial gene pool, which should be preserved.

1.2. OBJECTIVES

The main objectives of the present study are:

1. To document the various types of fish products (fermented, smoke dried, sundried) within Darjeeling and plains of North Bengal.
2. To study the indigenous ways of preparation of locally available fish products.
3. Isolation, characterization and identification of bacteria and other microbes from different fish products using conventional and modern techniques.
4. Enumeration of pathogenic contaminants in the fish products.